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A rapid laboratory test to demonstrate thermal inactivation of exotic Newcastle disease virus in turkey breast

Robert Marshall Davis
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Iowa State University, 1987

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A rapid laboratory test to demonstrate thermal inactivation of exotic Newcastle disease virus in turkey breast

by

Robert Marshall Davis

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
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DOCTOR OF PHILOSOPHY

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GENERAL INTRODUCTION

Overview

Thermal processing requirements for foods are typically defined by regulatory agencies as the minimum internal temperature which must be reached, but they do not consider the combined effects of temperature and time. Food processors, on the other hand, have traditionally calculated equivalent processing conditions for spoilage organisms or human pathogens. A classic example is 12D processing (12 decimal reduction time) for Clostridium botulinum, which can be effected by a variety of temperature and time combinations.

Pathogens of veterinary interest can be transmitted through food when refuse is fed to animals. The implications for domestic livestock are especially important when considering imported foods from countries that have exotic diseases. However, very little work has been done to define equivalent thermal processes for inactivating pathogens of veterinary significance.

The analytical laboratory is in a difficult position. The true thermal processing of a sample is unknown. Yet the laboratory is charged with determining after the fact whether processing reached a given temperature and

satisfied regulations, keeping in mind that different processes can have equivalent effects.

The purpose of this research was twofold. The first purpose was to define thermal inactivation characteristics of viscerotropic velogenic Newcastle disease (VVND) virus in turkey breast so that calculation of equivalent thermal processing conditions is possible. And the second purpose was to correlate thermal processing conditions which inactivate VVND with the inactivation of acid phosphatase, which could then be used as a rapid laboratory test to demonstrate adequate processing.

Newcastle disease virus

History A new disease of fowl was first recognized in 1926 as described by Kraneveld (1926) in Java, Doyle (1927) in England and by Konno (Konno et al., 1929) in Korea. Various workers proposed such names as pseudo-fowlpest (Pickard, 1928), Ranikhat disease (Edwards, 1928) and Philippine fowl disease (Rodier, 1928). Doyle (1927) had first observed the disease on a poultry farm near Newcastle-on-Tyne, England, and gave it the provisional name Newcastle disease until a more appropriate name was found. Although similar to fowl plague, by 1938 it was shown to be a separate entity and the causative agent of outbreaks which had occurred in England, Dutch East Indies,

Philippine Islands, India, Ceylon, Korea, Japan and Australia (Doyle, 1935). Both the disease and the name had become established worldwide. Shortly thereafter, an inapparent milder form of Newcastle disease virus (NDV) appeared in California but was not recognized as such for almost nine years (Beach, 1943; Beach, 1944). Speculation by Shope (1964) has suggested in retrospect that this may have occurred by virus attenuation in an intermediate host.

Investigations by many workers following the original appearance of NDV addressed incidence, etiology, pathogenesis, epizootiology, diagnosis, prevention and control. Most work was done in chickens. When other species were examined, it was usually to determine if they were susceptible. Several comprehensive reviews have been written (Beaudette, 1943, 1949, 1950, 1951; Brandly et al., 1946a, 1946b; Hanson and Brandly, 1958; Hanson, 1964, 1978).

The first outbreak of the exotic form of NDV in the United States occurred in Contra Costa, California, in 1950. It was attributed to chickens and pheasants imported from Hong Kong, and was eradicated without spreading. An outbreak in 1972 in southern California required the destruction of nearly 12 million infected or exposed birds in a two-year effort that cost $56 million (USDA, 1978).

The outbreak was thought to have originated from infected parrots imported from South America (Hanson, 1973; Lancaster and Alexander, 1975). Surveillance of both legally and illegally imported birds remains of major concern to the USDA as the economic risk of NDV and the vulnerability to introduction have been ranked higher than most other foreign animal diseases (Pilchard and McDaniel, 1983).

Etiology Newcastle disease virus is a paramyxovirus (Andrews, 1962). Along with parainfluenza and mumps of humans, and Yucaipa virus of avians, NDV shares several common characteristics (Dinter, 1964; Ginsberg, 1980). The virions are spherical enveloped particles of about 180 nm. The envelope is about 10 nm thick and is disrupted by lipid solvents. Projecting from the envelope are 8 nm spikes of glycoprotein of two different types: one (HN) has both hemagglutinin and neuraminidase activities, and the other (F) has hemolytic and cell fusion functions. The envelope contains the antigenic components that stimulate the host to produce hemagglutinin-inhibition (HI) and virus-neutralizing (VN) antibodies (Rott, 1964), and many humans with mumps develop antibodies to NDV (Ginsberg, 1980). Within the envelope is a structural matrix (M) protein. Disrupting the envelope reveals a

helical nucleocapsid consisting of a single species of protein (NP) and one molecule of unsegmented single-strand RNA having negative polarity. An RNA-dependent RNA polymerase is present for virus multiplication, which is not affected by actinomycin D. Replication of virus components occurs in the cytoplasm with assembly and release at the cell membrane. Recent areas of research on NDV have included production of monoclonal antibodies against strain specific antigens (Srinivasappa et al., 1986) and cloning complementary DNA to the NDV genome (Chambers et al., 1986).

Four general types of NDV have been recognized. Viscerotropic, velogenic Newcastle disease (VVND), sometimes called exotic or Asiatic NDV, is the acute lethal infection originally described by Doyle (1927). The incubation period in chickens is two to four days and hemorrhagic lesions of the digestive tract are common. Morbidity and mortality often reach 100 percent. Neurotropic, velogenic is the acute, often lethal infection described by Beach (1943). Also called avian pneumoencephalitis, characteristic lesions are found in the respiratory tract and nervous system of chickens but not the digestive tract. Mesogenic NDV was recognized by Beaudette and Black (1946). This type produces acute

respiratory and sometimes lethal nervous infections in young chickens, but is usually inapparent in adults. Some mesogenic strains have been used as vaccines. The final type, and least virulent, was described by Hitchner and Johnson (1948). This lentogenic type produces mild, often inapparent, respiratory infections in birds of all ages. It is rarely lethal, and widely used for vaccines. A summary of characteristics of the four NDV types is presented in Table 1.

Thermal stability of NDV has been often studied. However, comparing results from different workers is difficult due to wide variation in strains tested, sample matrix [amniotic-alantoic fluid (AAF), diluted AAF, tissue], method of heating (wet, dry, come-up lag) and method of demonstrating inactivation [hemagglutination (HA), infectivity]. Foster and Thompson (1957) observed that the time required for inactivation was dependent upon initial titer, analogous to the trailing of heat inactivation curves of bacteria. However, titer usually has not been mentioned for NDV inactivation studies.

Much thermal inactivation work has been done with AAF or diluted AAF. Thermal inactivation times as demonstrated by loss of infectivity have ranged from 45 to 105 min at 55 to 56^{o}C, and from 5 to 60 minutes at 60^{o}C (Asplin, 1949;

Brandly et al., 1946b; DiGioia et al., 1970; Foster and Thompson, 1957). A review by Hanson and Brandly (1958) indicates 31 strains were infective after 15 min at 56°C, with 3 strains retaining infectivity after 180 min. Heat resistant mutants have been selected from stock virus (Iinuma et al., 1979).

Thermal inactivation of NDV in AAF as demonstrated by HA has been used for strain identification (Hanson and Brandly, 1955; Hanson et al., 1949). Inactivation of 24 strains at 56°C showed wide variability as some strains lost activity at 15 min while others remained active at 360 min (Hanson et al., 1949). A later repetition by the same investigator, including an expanded number of strains, revealed discrepancies with the previous work (Hanson, 1964) and emphasizes the care which must be taken to control the many variables in heat inactivation studies (Picken, 1964). DiGioia et al. (1970) concluded from energy of activation and entropy of activation studies that loss of HA is due to denaturation of protein in the outer coat.

Infectivity and HA sensitivity to heating are independent of each other. One strain may lose its ability to hemagglutinate before it loses infectivity, while another strain may behave in the opposite manner (Hanson et al., 1949).

Thermal effects of storage conditions for vaccines have also been studied (Rhoades, 1958; Hofstad, 1963). Strain variation under identical conditions were noted, as well as effects due to temperature, humidity, storage pressure and storage atmosphere.

In summarizing the extensive literature which addresses many aspects of NDV thermostability, the words of Picken (1964) should be heeded: "Marked differences in the thermostabilities of various strains of NDV exist.... Identical virus populations placed in different environments will not be inactivated at the same rates by a given heat stress." Although sample matrix affects thermal stability, and carcass tissues of infected birds contain NDV, virus inactivation in turkey breast has not been addressed.

Stability of NDV is also affected by chemical and physical agents. Hofstad (1964) has reviewed inactivation by formalin, β-propiolactone, urethane and irradiation, and their affects on immunogenicity for producting vaccines. Lowering pH to 4 did not affect NDV (Brandly et al., 1946b) and it apparently can withstand pH as low as 2 and as high as 10 (Moses et al., 1947). NDV sensitivity to ultraviolet light is somewhat greater than that of fowl plague virus (Brandley et al., 1946b) but the mechanism of inactivation

is similar (Levinson et al., 1944). Disinfectants which inactivate other viruses also destroy NDV (Doyle, 1927; Beaudette, 1943; Cunningham, 1948).

Epizootiology Newcastle disease has been reported in more than thirty species of domestic and wild birds (Doyle, 1927; Picard, 1928; Farinas, 1930; Brandly, 1959). Erickson (1976) reviewed pet bird species and listed forty from which NDV had been recovered. Apparently few, if any, are resistant with the exception of carniverous birds (Placidi and Santucci, 1956). At least fifteen mammalian species, including man, have shown responses ranging from local to generalized infection (Brandly, 1964). Conjunctivitis is a typical human response. Genetics (Albiston and Gorrie, 1942; Cole and Hutt, 1961) and age (Brandly, 1959) have been shown to influence response in birds. There is a general decrease both in incubation time and severity with increased maturity. The wide variety of both species and responses, together with the different types of NDV itself, makes identification of carriers difficult.

Transmission of NDV between birds in a flock is by aerosol (Hanson, 1978). Infected birds liberate virus in expired air (Sinha et al., 1954) which accumulates and is circulated by normal activity of the birds (Lancaster and Alexander, 1975). Vaccines are routinely administered by

spray (Hitchner and Reising, 1953) and as dust (Price et al., 1955). Vaccines administered in drinking water are thought to enter the bird as a drinking-induced aerosol (Hanson, 1978). Transmission of NDV between separated flocks has usually occurred by movement of live birds, especially through markets (Beaudette, 1943; Jungherr and Terrell, 1946). Contaminated feed, water, offal, clothing, crates and even vaccinating crews have also been responsible (Doyle, 1935; Beaudette, 1943; Hanson, 1978; Utterback and Schwartz, 1973). Contaminated objects, depending upon environmental conditions, have retained infectivity for months (Asplin, 1949; Olesiuk, 1951; Boyd and Hanson, 1958). Eggs laid by infected birds can contain NDV (which kills the embryo), but virus has not been demonstrated in chicks hatched from eggs laid by recovering hens (Hofstad, 1949; Bivins et al., 1950). Transmission of NDV to distant geographical areas has often been noted to affect coastal cities and therefore implicates overseas traffic (Beaudette, 1943).

Pathogenesis Once birds have been infected, incubation in various avian species is about 2 to 15 days (Pickard, 1928; Beaudette, 1943). After virus multiplication at the site of introduction, virus liberated into the bloodstream is distributed to secondary sites of

multiplication (Asdell and Hanson, 1960). Jungherr (1964) states that both virulent and avirulent strains multiply in the visceral organs at about the same rate, but virulent strains pass the blood-brain barrier more quickly. Also, virulent strains destroy cells while avirulent strains permit cell regeneration. Slower release of avirulent strains may give time for formation of antibody.

Clinical signs of NDV vary with the type of virus, species and age of birds. The following summary of clinical and pathological descriptions comes from the American Association of Avian Pathologists (Whiteman and Bickford, 1983) and reviews by Jungherr (1964) and Hanson (1978). In adult chickens infected with non-exotic types, mild to inapparent respiratory signs usually occur suddenly accompanied by a drop in egg production. Production may resume slowly or not at all. A few birds may show signs of central nervous system (CNS) disease. Young chickens infected with non-exotic types also show rapid onset of respiratory signs. Up to 25 percent develop CNS signs. Prostration, paralysis and death of 50 to 95 percent is common. Adult and young chickens infected with exotic NDV develop dyspnea, virulent diarrhea, conjunctivitis, paralysis and death in two to three days. Birds surviving

longer may exhibit signs of CNS involvement. Morbidity and mortality reach 100 percent.

Pathological examination of young or old birds with non-exotic NDV seldom reveals lesions except occasional mild air sacculitis or tracheitis. Exotic NDV produces severe inflammation of the trachea and air sacs. The digestive system contains hemorrhagic or necrotic focal lesions often involving lymphoid tissue in the mucosa. Cecal tonsils are often involved. Hemorrhages occasionally occur in the proventriculus, gizzard or mucosa of the esophagus. Histopathology reveals neuronal degeneration, perivascular cuffing with lymphocytic cells and endothelial hypertrophy in the CNS. Turkeys exhibit milder lesions than chickens, and wild or pet birds often reveal no lesions.

Diagnosis Because clinical signs and pathology of NDV are not unique, it is essential to demonstrate virus and/or seroconversion in the animal. Other diseases with similar effect are infectious bronchitis, infectious larynogotracheitis, chronic respiratory disease and infectious coryza.

Control Control of NDV requires isolation or vaccination of healthy animals, and slaughter and sanitation of infected animals and premises (Robertson, 1964).

All phases of production from stocking to feeding to cleanliness of workers must be considered. A large variety of vaccines and recommended inoculation procedures exist (Hitchner, 1964; Hanson, 1978). The USDA enforces regulations requiring quarantine of imported livestock and pet birds.

This review will now consider acid phosphatase and its application to food analysis.

Acid phosphatase in food analysis

Background Phosphatase is a general term encompassing a variety of enzymes which hydrolyze single phosphate groups from substrates. Phosphate compounds serve many important functions throughout all plants and animals. They are found in phospholipids, nucleic acids and as intermediates in biosynthetic pathways. Degradation of phosphate compounds is usually not specific, and phosphatases act on a variety of substrates. Alkaline phosphatases (ALP) have optimum pH above 7, and acid phosphatases (ACP) below pH 7. The International Union of Biochemistry (IUB) Enzyme Commission has assigned ACP the systematic name orthophosphoric monoester phosphohydrolase, and the number E.C. 3.1.3.1 (IUB, 1964). Generally, phosphatases are located within cells, with ACP in lysosomes (deDuve, 1969) and ALP on plasma membranes (Kaplan, 1972).

In some cases tissue specific phosphatases have been demonstrated. Their presence in serum has been used to aid diagnosis of prostatic malignancy, Gauchers disease and hematopoetic, liver, and bone disorders (Kiefer, 1977). Semen is identified in forensic analysis by its specific ACP (Riisfeldt, 1946). Purified phosphatases enjoy wide popularity as in vitro markers covalently linked to other molecules (such as antibody or nucleic acid probes) which are then quantitated or located histochemically by adding substrate for the phosphatase (Charan and Gautam, 1984; Blaedel and Boguslaski, 1978; Sauls and Caskey, 1985).

History Phosphatase enzymes remain present in dormant form in foods. At harvest, post mortem biochemical reactions occur until equilibrium conditions are reached. Stability of the dormant enzyme during storage depends upon storage conditions, and enzymes themselves can become substrates for proteases. Much research has been directed towards finding optimum storage conditions for foods (Desrosier and Desrosier, 1977; Cheftel, 1977; DeMan, 1980).

Thermal processing induces physico-chemical reactions which denature proteins and result in enzyme inactivation (Stearn, 1949; Whitaker, 1977). Laboratory tests which demonstrate alkaline phosphatase inactivation have become

official methods of the Association of Official Analytical Chemists for milk, cream, butter, margarine, cheese and ice cream (AOAC, 1975). The tests are based on the works of Sanders and Sager (1946 and 1947), Sanders (1948), Kleyn and Lin (1968) and Campbell and McFarren (1961). Alfalfa meal processing has also been determined by this method (Sanders et al., 1956). Richardson et al. (1964) reported reactivation of ALP, which is more pronounced for higher temperature shorter time processing.

Acid phosphatase is more heat stable than ALP (Mullen, 1950) and therefore can be used to demonstrate more extensive heat processing. The USDA Food Safety Inspection Service (FSIS, 1986) test for processing of canned hams is based on the ACP inactivation studies of Lind (1965) and Cohen (1969). Animal swill (Czech and Sunseri, 1968) and citrus juice heating (Axelrod, 1947) have also been demonstrated by inactivation of ACP. Reactivation of ACP in meat reportedly does not occur (Kormendy and Gantner, 1960).

In general, phosphatase is present in all foods, and thermal processing inactivates phosphatase. While alkaline phosphatase levels of a variety of processed dairy foods have been determined for establishing official testing requirements, analogous information about acid phosphatase

is notably lacking. Specifically, normal values for raw or cooked chicken or turkey breast are not available.

Laboratory tests to demonstrate thermal processing by phosphatase inactivation do not universally apply. Factors which influence the level of residual activity include initial activity, additives, thermal conductivity, size of container, shape of container, heating protocol and cooling protocol. Changing any variable will affect the resultant level of phosphatase. Research can define combinations of variables which produce equivalent processing, but their effects on phosphatase inactivation must be addressed.

Acid phosphatase methods The FSIS method for ACP in canned ham is colorimetric, with phenyl disodium phosphate (PDSP) substrate in pH 6.5 buffer. The liberated free phenol is reacted with 2,6 dibromoquinone chlorimide to produce a blue indophenol which is measured by absorbance at 610 nm (FSIS, 1986). The method was modified from early dairy analysis for ALP (Sanders and Sager, 1947) by Kormendy and Gantner (1960) who altered the pH of the incubation and color development buffers but retained the color reaction described by Gibbs (1927).

Acid phosphatase methodology in foods apparently evolved from clinical chemistry work (Kay, 1930; King and Armstrong, 1934). Various substrates, each with an optimum

pH for colorimetric analysis of ACP, have been described. Czech and Sunseri (1968) incubated sample with phenolphthalein monophosphate at pH 4.6 to 5.0 and then added alkaline buffer to demonstrate a red color. Andersch and Szczypinski (1947) used ρ-nitrophenyl phosphate at pH 4.8 and then added alkaline buffer to demonstrate a 410 nm yellow color. Powell and Smith (1954) and Kind and King (1954) used phenyl disodium phosphate at pH 5.0 then measured phenol by first condensing with 4-aminoantipyrine and then oxidizing with potassium ferricyanide, and measuring absorbance at 505 nm. The method ran on an automated analyzer. An automated method using α-naphthyl phosphate at pH 5.2 to 5.9 and developing 405 nm color with Fast Red TR has been described (Bais and Edwards, 1976; Shaw et al., 1977). Roy et al. (1971) used thymolphthalein monophosphate at pH 5.4 and added alkaline buffer to measure color at 590 nm.

In addition to the various colorimetric methods of analysis, other non-colorimetric methods for ACP have been described. Fluorometry after incubation with 4-methylumbelliferyl phosphate was developed by Chambers et al. (1977). Electrophoresis and counterimmunoelectrophoresis have been used to detect ACP and ALP isoenzymes (Lee et al., 1974; Yam, 1974; Foti et al., 1978). Potentiometric

analysis using a flow-through phosphate ion-selective electrode was described by Hara et al. (1981). And Foti et al. (1975) developed a radioimmunoassay for acid phosphatase.

The following review applies to thermal processing of meat and methods of demonstrating heat processing.

Thermal processing of meat

History Nicholas Appert (1752-1841) is recognized as the individual who had the most to do with the development of preserving food by canning. The work of Pasteur (1822-1895) provided scientific understanding of why thermal processing was effective. By the turn of the twentieth century, S.C. Prescott and W.L. Underwood had combined the science and technology of thermal processing to lay the foundation of the modern canning industry. A very interesting historical account is presented by Goldblith (1971, 1972).

Bigelow et al. (1920) published the first major work on thermal processing. Called the graphical method, it outlines the approach still used today of defining thermal lability characteristics of the organism of interest, defining heat penetration for the product and container size, and integrating the variables to demonstrate equivalent effects of different processing conditions.

Specifically, a thermal death time curve is constructed by plotting temperature/time combinations which inactivate the specified organism. New curves must be determined for each organism. From the curve, the z value (degrees corresponding to a tenfold change in inactivation time) permits calculating the lethal rate at any given temperature. By plotting lethal rate vs time during processing, and integrating the area under the curve, the total lethal effect of any given process is known. Equivalency can be demonstrated as illustrated in Figure 1.

Subsequent work in thermal processing produced the formula method of process calculation by Ball (1923) and the nomograph method of Olson and Stevens (1939). While research continues in the field of thermal processing, these original works continue as the basic tenets. Recent work has been directed towards refinement and incorporation of new technology, for example by applying computers to correcting thermal death kinetic graphs (Perkin et al., 1977), modeling of heat penetration to precisely define the critical area of spore survival within a container (Flambert and Deltour, 1972) and calculating processing parameters (Hayakawa, 1977).

While techniques for determining equivalent processing have been widely applied to spoilage organisms and human

pathogens such as Clostridium botulinum (Esty and Meyer, 1922), they have not been applied to eliminating veterinary disease agents which may be transmitted through food. Thus, pork products are cooked to 58.3°C to eliminate trichina, and imported meats are cooked to 68.8°C to eliminate exotic disease agents, without time specification. Two problems arise from specifying fixed endpoint temperatures. Equivalent processing at a lower temperature for a longer time (e.g., for organoleptic qualities) is not an option to the processor. And the analytical laboratory must be able to differentiate between equivalent processes which do not meet the required temperature.

Heating effects on meat As meat is heated to higher temperatures, a series of physico-chemical changes take place. At 30 to 50°C myofibrillar proteins first unfold and then form loose crosslinkages which result in increased rigidity (Hamm and Deatherage, 1960). The pH and isoelectric point both increase (Hamm, 1960) as do free Ca^{++} and Mg^{++} ions (Wierbicki et al., 1957). ATPase is inactivated (Nemitz and Partmann, 1959). At 50 to 55°C myofibrillar proteins rearrange and stable crosslinkages form. Changes in pH, water holding capacity and free Ca^{++} and Mg^{++} are delayed in this range (Hamm, 1966). At 55 to

80°C most of the changes occurring at lower temperatures continue. Most myofibrillar, sarcoplasmic and globular proteins are coagulated by 70°C. Collagen shrinks at about 63°C and transforms to gelatin at higher temperatures (Hamm and Deatherage, 1960). Above 80°C, sulfhydryl groups of actomyosin are oxidized and form disulfide bonds. Above 90°C hydrogen sulfide is liberated and Maillard browning reactions occur (Hamm and Hofmann, 1965; Pearson et al., 1962).

Microscopic changes in meat during heating have also been studied (Schmidt and Parrish, 1971; Hegarty and Allen, 1972, 1975). Endomysial connective tissue shrinkage is initiated at 50°C and completed at about 70°C, where perimysial connective tissue shrinkage begins. Sarcomeres shorten at 50°C. At 60°C, M-line structure, myofibrillar protein shrinkage and the disintegration and coagulation of thin and thick filaments begin. Additional heating continues the disintegration and coagulation until at 90°C an amorphous structure results. The maximum tenderness reported at 60°C (Schmidt et al., 1970), which decreases at higher temperatures, has been correlated with the solubilization of collagen (Goll et al., 1964) combined with loss of endomysial connective tissue.

Extensive reviews of meat biochemistry including thermal processing effects have been prepared by Hamm (1966) and Asghar and Pearson (1980).

Laboratory methods to demonstrate thermal processing of meat have made use of the various changes which take place. Measuring residual acid and alkaline phosphatase has been tried with varying degrees of success. Some workers reported poor correlation (Suvakov et al., 1967; Visacki et al., 1967) while others found semi-quantitative relationships (Wielkowska and Monikowski, 1956; Kormendy and Gantner, 1960; Czech and Sunseri, 1968). Lind (1965) found correlations that permitted calculation of equations for canned hams, and his work was adapted by USDA for official laboratory testing (FSIS, 1986).

The importance of considering the combined effect of temperature and time is apparent from examining the experimental data of Cohen (1969). Two canned ham plants provided results which were pooled to calculate an equation to predict residual ACP. Some of the hams were processed to identical temperatures using different heating programs as shown in Table 2. In every case the sample with the longer processing time had lower residual ACP. However the effect of time was not considered.

Other laboratory procedures that have been proposed for demonstrating cooking temperature include electrophoresis (Lee et al., 1974; Cheng and Parrish, 1979), isoelectric focusing (King, 1978) and residual water-extractable muscle proteins (Cohen, 1966; Davis and Anderson, 1983; Davis et al., 1985). An undocumented coagulation procedure is used by FSIS for pork products up to 65.6°C (FSIS, 1986).

Turkey processing The Poultry Inspectors Handbook (FSIS, 1986) indicates that a minimum internal temperature of 71.1°C must be reached in a heat processed poultry product which is not smoked or cured. Smoked poultry must reach a minimum internal temperature of 68.3°C. However, consumers prefer poultry cooked to higher temperatures. Smoked turkey processed to 68.3°C was found to be inedible without further cooking (Hale et al., 1977). Optimum endpoint internal temperature of smoked turkey is in the 72 to 79°C range (Gardner et al., 1980; Murphy and Goodwin, 1980). Consumers prefer unsmoked, uncured turkey to be cooked to an internal breast temperature of 80 to 95°C (Goodwin et al., 1962; Goertz et al., 1960). Cooking to 71 to 82°C was judged as underdone in a report by Alexander et al. (1951). The National Turkey Federation recommends cooking to 82.2 to 85°C, which is slightly lower than the

86 to 88°C optimum for chicken and presumably reflects the effects of slower come-up and cool-down for the larger bird.

The combined effect of temperature and time of processing on consumer acceptability is emphasized in a report by Stone and May (1969). Chicken cooked to 88°C in open kettles at 100°C was satisfactory while chicken cooked to 88°C at 121°C and 20 p.s.i. appeared undercooked. The authors concluded that the undercooked appearance was due to the rapid cooking rate, and found that lengthening the cooking time eliminated the undercooked appearance. Poultry formed into rolls containing muscle and skin is processed to 71 to 74°C (Wilkinson et al., 1965; Breclaw and Dawson, 1970), as is mechanically deboned turkey meat when mixed with beef and pork to make frankfurters (Froning et al., 1971). While latitude in processing temperatures is apparent, 70°C is the lower end of the range.

Explanation of thesis/dissertation format

The alternate format of thesis organization is used here. The research is separated into sections I, II and III. Each section is intended to stand alone for submission to the Journal of Food Science or the Journal of Food Protection. Minor changes within sections, such as

footnote or reference format, are anticipated at time of submission for conformance to journal requirements.

Joint authorship is indicated for those individuals who provided guidance, advice and methods training. All research was original.

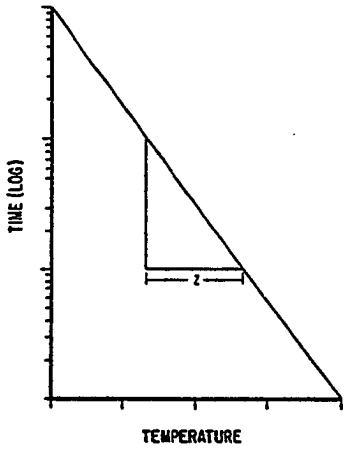
Preceding the sections of research is a general introduction section, and following the sections of research is a general summary which ties together the thesis into a unified whole.

Table 1. Characteristics of the four types of Newcastle disease virus. General characteristics are indicated for the example strains noted. Specific strain characteristics will vary (Hanson and Brandly, 1955; Hanson, 1964; Hanson, 1978; Schloer, 1965; Spalatin et al., 1970)

	Viscerotropic velogenic (e.g., Herts)	Neurotropic velogenic (e.g., Texas GB)	Mesogenic (e.g., Roakin)	Lentogenic (e.g., LaSota)
Tropism[a]	D/N/R	N/R	R	R
Embryo death (d)	2	2	2.5-3.5	>4
Chick intracerebral index[b]	1.7	1.8	0.8	0.1
Chicken lethality	99%	70%	0%	0%
HA Elution[c] (hr)	48	86	34	120
HA Stability at 56°C[d] (min)	30	60	5	5
Plaque type[e]	C/R	6	6	Mg
Plaque size (mm dia)	0.5-4	1-2	1	<0.5-1.5

[a]D=digestive tract; N=central nervous sysem; R=respiratory tract.
[b]Day-old chick, intracerebrally inoculated; 0=low, 2=high.
[c]Hemagglutination elution. Varies widely.
[d]Virus heated at 56°C then tested for HA.
[e]C=clear plaque, R=red plaque, Mg=plaque only if Mg and DEAE present.

Figure 1. Steps involved in determining equivalent processing. (A) thermal death time curve for specific organism. (B) sample temperature and corresponding lethal rate during commercial processing. (C) lethality curves, each with equal area and therefore equal processing. Other organisms must be treated independently. Adapted from Bigelow et al. (1920) and Goldblith et al. (1961)

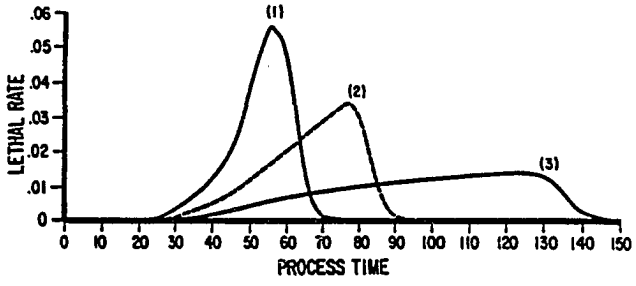


(A) Thermal death time curve

Process 1 (high temperature)			Process 2 (intermediate)			Process 3 (low temperature)		
Time	Temp.	Lethal Rate	Time	Temp.	Lethal Rate	Time	Temp.	Lethal Rate
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-
		-	-	-	-	-	-	-
		-	-	-	-	-	-	-
					-	-	-	-
					-	-	-	-
					-	-	-	-
					-	-	-	-

(B) Processing data.

$$\text{Lethal rate} = \frac{1}{\text{antilog}\left(\frac{121^{o}C - {}^{o}C}{z}\right)}$$



(C) Lethality curves

Table 2. Data from Cohen (1969) for canned hams cooked to the same internal temperature by different heat programs

Plant I			Plant II		
Internal Temp°C	ACP[a]	Heat Program[b]	Internal Temp°C	ACP	Heat Program
62.8	178	G (84.4°C 205 min)	62.8	268	C (78.9°C 200 min)
			62.8	234	C (")
64.4	168	G (84.4°C 205 min)	64.4	100	D (79.4°C 235 min)
			64.4	127	D (")
65.6	94	G (84.4°C 205 min)	65.6	103	C (78.9°C 200 min)
65.6	47	G (")			
65.6	33	B (74.4°C 285 min)			
65.6	24	B (")			

[a]Acid phosphatase (ACP) activity as μ moles phenol per kg sample.

[b]Equilibrated waterbath temperature, and time required to reach internal temperature.

SECTION I. SPICE INTERFERENCE WITH PHOSPHATASE ANALYSIS OF FOODS USING 2,6 DIBROMOQUINONE CHLORIMIDE FOR COLORIMETRY

Spice interference with phosphatase analysis of foods using 2,6 dibromoquinone chlorimide for colorimetry

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ABSTRACT

Estimating heat treatment of foods by phosphatase enzyme analysis using phenyl disodium phosphate (PDSP) as substrate and 2,6 dibromoquinone chlorimide (2,6 DBQ) for color development is subject to interference by some common spices. Both false high and false low reactions have been demonstrated. Phenolics, which are common in spices and flavorings, may react with 2,6 DBQ to form the characteristic blue indophenol color indicating enzyme activity in samples with no phosphatase, or may interfere with indophenol formation in samples containing phosphatase, depending upon their concentration. The possibility of false high or false low results and therefore implied over or under processing should be considered when using 2,6 DBQ in phosphatase enzyme analysis of foods.

INTRODUCTION

Phosphatase inactivation is widely used to indicate adequacy of heat treatment of a variety of foods including dairy products (Sanders, 1948; McGugan and Howson, 1964), citrus juices (Axelrod, 1947) and meat (Lind, 1965). Under United States Department of Agriculture (USDA) regulations, imported canned hams are routinely tested for residual acid phosphatase activity to estimate their processing temperature. The method used by the Food Safety and Inspection Service (FSIS, 1986) specifies incubation of the sample with phenyl disodium phosphate (PDSP) substrate, and the enzymatically liberated free phenol is reacted with 2,6 dibromoquinone chlorimide (2,6 DBQ) to produce a blue indophenol color which is quantitated spectrophotometrically at 610 nm (FSIS, 1986; Gibbs, 1927). The possibility of reaction of phenolic compounds present in spices and flavorings with 2,6 DBQ was recognized as a potential source of interference when this test was applied to other products. This report examines the effect of spices and flavoring components on phosphatase analysis by the 2,6 DBQ method.

MATERIALS AND METHODS

FSIS acid phosphatase procedure

A 2.5 g sample of ground meat was incubated with 10 ml citrate buffer (0.15 M, pH 6.5) and 5 ml PDSP (0.01 M) for 60 minutes at 37°C. The reaction was stopped and proteins precipitated by adding 5 ml of 20% trichloracetic acid (TCA), leaving any free phenol in solution. After filtration, 3 ml of the filtrate was added to 3 ml sodium carbonate (0.5 M), then 0.1 ml of 2,6 DBQ (0.13 mM) was added to start color development. Thirty minutes later absorbance at 610nm was read and compared to known phenol standards treated in a like manner.

Interference by phenolics

Various phenolic compounds purchased commercially were subjected to the phosphatase test. Each compound was equilibrated with citrate buffer, and then TCA, based on visible dissolution. Finally, carbonate and 2,6 DBQ were added to duplicate the sequence of the test procedure. Quantitation of the various phenolics was not possible due to lack of analytical methodology for samples prepared in this matter. Analysis was qualitative based on whether or not color formation occurred. Because eugenol was soluble in this system, and a solution of known purity was

available, a semi-quantitative analysis of its effects was performed by adding known dilutions in citrate buffer to the test system.

RESULTS

The effects of reacting a series of phenolic compounds that could be present in spices or flavorings with 2,6 DBQ is shown in Table 1. Several phenolics produced the blue color characteristic of indophenol and therefore would give false high phosphatase values for foods in which these compounds were present. Most of the compounds, both solid and liquid, had low solubility (visual estimation) in this system. However, when color formation occurred, it was very obvious.

Eugenol, depending upon concentration, demonstrated both color formation and inhibition of color formation as shown in Figure 1. Maximum A_{610} occurred with a 10 ppm solution. At higher concentrations, color formation was decreased and would therefore give false low phosphatase values for foods containing eugenol at these concentrations. While the concentration of eugenol in citrate buffer was known, the true concentration in the final reaction mixture was not determined as mentioned earlier.

CONCLUSIONS

The reactivities with 2,6 DBQ of the various phenolics reported here are based on solubility in citrate buffer. The solubilities of phenolics in food products may be quite different as the presence of lipids or emulsifiers could enhance solubility. Therefore each food product must be treated as a unique case. The group of substances tested in this study is not exhaustive, but includes a variety of chemical structures of varying complexity.

The concentration dependence of eugenol interference with the 2,6 DBQ reaction finds precedence in work done by Harwood and Huyser (1970). They observed that the concentration of reactants affected indophenol color formation when analyzing water soluble ammonia.

In general, these results agree with work done on the related Bethelot reaction in which indophenol formation is used to quantitate ammonia or nitrogen digested and reduced to ammonia (for an excellent review see Searle, 1984). The reaction involves ammonia forming an imine which then reacts with phenol to form the blue indophenol (Tarugi and Lenci, 1911). Modifications to the Berthelot reaction have used the substituted phenols thymol (Hansen, 1930) and salicylic acid (Lapin and Hein, 1934) to improve sensitivity or solubility, with subsequent reactivity

depending on position of substitution and stearic hindrance. In the present phosphatase method, it is phenol, not ammonia, that is quantitated although the final indophenol reaction is the same. Here the imine, 2,6 DBQ, is purchased commercially and added to a solution to detect the presence of phenol by formation of the blue indophenol. Substituted phenols which may be present in spices and flavorings can also react and cause interference.

Both false high and false low phosphatase values can result from interference with the 2,6 DBQ reaction. When the interfering substance contributes to color formation, a false high phosphatase value is implied which could lead to further processing and product degradation. An appropriate control to determine false high values is to process a sample without adding substrate to determine non-phosphatase A_{610}. On the other hand, interference may inhibit the 2,6 DBQ reaction. A false low phosphatase value is implied which could lead to inadequate processing and spoilage loss. An appropriate control to determine false low values is to add a known amount of phenol to a sample and verify that the expected additional A_{610} is obtained.

REFERENCES

Axelrod, B. 1947. Phosphatase activity as an index of pasteurization in citrus juices. Fruit Prod. J. 26:132.

FSIS (Food Safety Inspection Service). 1986. Chemistry laboratory guidebook. U.S. Government Printing Office, Washington, D.C.

Gibbs, H. D. 1927. Phenol tests. III. The indophenol test. J. Biol. Chem. 72:649.

Hansen, P. A. 1930. The detection of ammonia production by bacteria in agar slants. J. Bacteriol. 19:223.

Harwood, J. E, and D. J. Huyser. 1970. Some aspects of the phenol-hypochlorite reaction as applied to ammonia analysis. Water Research 4:501.

Lapin, L., and W. Hein. 1934. Eine neue farbenreaktion des ammoniaks. Z. Anal. Chem. 98:236.

Lind, J. 1965. The determination of the acid phosphatase activity in canned hams. Danish Meat Products Laboratory, The Royal Veterinary and Agricultural College, September 23.

McGugan, W. A., and G. Howson. 1964. Alkaline phosphatase in cheddar cheese made from raw and heat treated milk. J. Dairy Sci. 47:139.

Sanders, G. P. 1948. Report on the phosphatase test in pasteurization of dairy products. J. Assoc. Off. Agric. Chemists 31:306.

Searle, P. L. 1984. The Berthelot or indophenol reaction and its use in the analytical chemistry of nitrogen. A review. Analyst 109:549.

Tarugi, N., and F. Lenci. 1911. Some color reactions. Boll. Chim. Farm. 50:907.

Table 1. Substances tested for interference with the 2,6 dibromoquinone chlorimide acid phosphatase method

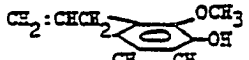
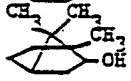
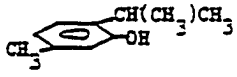
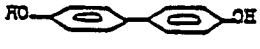
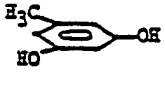
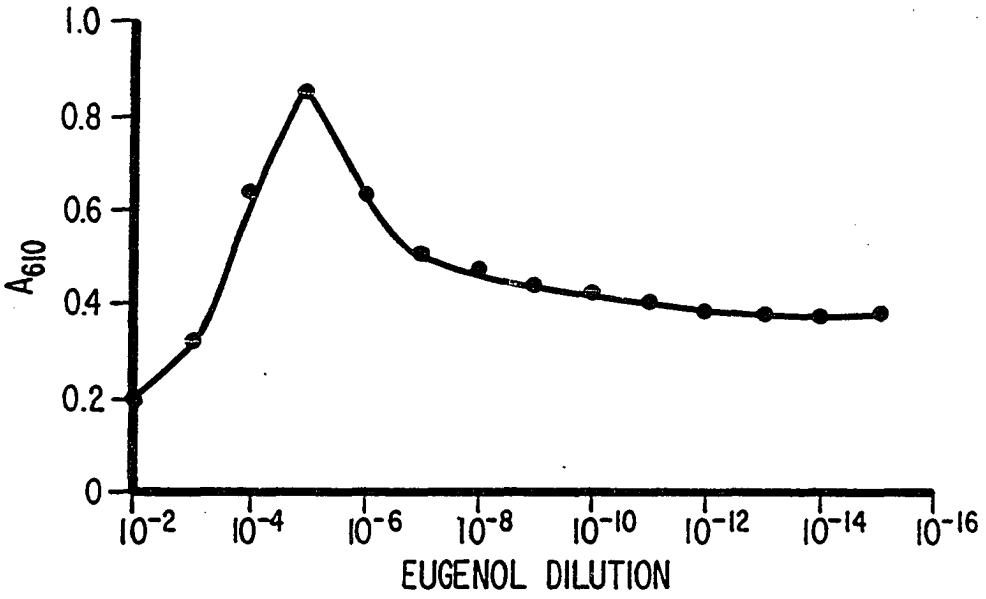
Substance	Structure	2,6 DBQ Reaction
Eugenol		Blue (high conc. inhib)
Isoeugenol		Yellow
Borneol		Yellow
Thymol		Blue
Vanillin		Yellow-green
o-Vanillin		Green-brown
Vanillic acid		Blue
Isovanillic acid		Blue
Benzaldehyde		Blue
Benzoic acid		Yellow
Toluene		Brown
Xylene		Brown
p-Phenyl phenol		Blue
Salicylic acid		Blue
5-Sulfosalicylic acid		Yellow
Orcinol		Blue

Figure 1. Eugenol interference with the standard phenol reaction in the 2,6 DBQ acid phosphatase procedure. Diluted eugenol and 2.63×10^{6} M phenol were mixed 1:1 for indophenol reaction


1.0
0.8
0.6
0.4
0.2
0
A_{610}
10^{-2}
10^{-4}
10^{-6}
10^{-8}
10^{-10}
10^{-12}
10^{-14}
10^{-16}
EUGENOL DILUTION

SECTION II. COMPARISON OF THERMAL INACTIVATION CHARACTERISTICS OF FIVE NEWCASTLE DISEASE VIRUS STRAINS IN SPIKED TURKEY BREAST

Comparison of thermal inactivation characteristics of five Newcastle disease virus strains in spiked turkey breast

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ABSTRACT

Thermal inactivation characteristics of Newcastle disease virus (NDV) have been defined in turkey breast meat spiked with stock virus. One strain of mesogenic and four strains of velogenic NDV were compared for loss of infectivity at 56°C. Roakin and Fontana strains were more stable than Texas GB, San Juan or Parrot Project viruses. The thermal death time curve for Roakin strain NDV was defined over the temperature range of 56 to 68.8°C. Based on time required to inactivate virus at various temperatures, the values $F_{68.8^{\circ}C}$ = 5.0 min and z = 8.9°C were calculated. Implications for demonstrating thermal inactivation of NDV in food products are discussed.

INTRODUCTION

Newcastle disease virus (NDV) was first described by Doyle (1927). Four general types of NDV are now defined by tropism and incubation time (Hanson, 1978). Viscerotropic velogenic Newcastle disease (VVND), also called exotic or Asiatic NDV, is the highly pathogenic type of great economic significance to the poultry industry and therefore of interest to the United States Department of Agriculture (USDA, 1978). The less significant types are neurotropic velogenic, mesogenic, and lentogenic. Lentogenic strains are widely used as vaccines. NDV infectivity has been demonstrated in carcasses, egg shells, feces and contaminated surfaces for up to one year (Asplin, 1949), and outbreaks have been attributed to feeding offal from infected birds (Doyle, 1927; Farinas, 1930). Carcasses and tissues from infected animals must be adequately treated or destroyed to prevent the spread of NDV.

Thermal inactivation of NDV has been reported by several workers (Asplin, 1949; Brandly et al., 1946; DiGioia et al., 1970; Foster and Thompson, 1957; Hanson and Brandly, 1958). However, most of these workers investigated amnionic-allantoic fluid (AAF) from infected chicken eggs, and none of the work was done with muscle tissue. Because food products could reasonably be a transmission

vector of NDV, insight into its stability in muscle tissue would be helpful for defining adequate thermal processing. The present study was undertaken to provide basic information about the thermal inactivation characteristics of NDV in turkey breast.

MATERIALS AND METHODS

The five strains of NDV used in this study were Fontana, San Juan, Parrot Project, Roakin and Texas GB. Stock cultures of virus were obtained from the Diagnostic Virology Laboratory,[1] and they had estimated virus concentrations of 10^8 to 10^9 per ml. They were diluted 1:10,000 with diluent[2] and 0.1 ml of the resulting dilution was injected into 10-day-old embryonated eggs. Dead embryos at 24 hours after inoculation were considered nonspecific deaths. Amnionic-allantoic fluid was harvested from embryos that died after 24 hours. It was pooled and clarified by centrifugation at 1,500 rpm for 15 min at 10°C. The supernatant fluid was used as stock virus, and was divided into aliquots and frozen at -80°C.

Fresh turkey breast was obtained from a local retail source. After discarding skin and connective tissue, the muscle was ground through 5 mm and 2 mm plates, and stock virus was added in the ratio of one part AAF to two parts

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[1]USDA-APHIS-NVSL-DVL, Ames, IA.

[2]Tryptose broth containing per ml: 1.21 mg Tris HCl, 10,000 units penicillin G, 2,000 µg streptomycin sulfate, 650 µg kanamycin sulfate, 1,000 µg gentamicin sulfate and 20 µg amphotericin B.

turkey breast. An adequate supply of ground turkey breast was prepared for use with all five strains. For each strain the spiked turkey breast was dispensed in 2.0 g aliquots into 12x75 mm centrifuge tubes, capped and stored frozen at -80°C. For thermal inactivation, replicate tubes for time treatments at a given temperature were thawed and a sterile 6 mm diameter glass rod was inserted. The sample thereby assumed the geometric configuration of a hollow cylinder with 6 mm i.d. and 8 mm o.d. This configuration permitted rapid, uniform heating. Thermocouple probes were inserted between the glass rod and wall of four tubes in each batch. Temperature was monitored with disinfected thermocouples attached to a datalogger (Fluke model 2240C). All tubes for a given temperature were distributed in a rack to allow circulation, and equilibrated in an ice bath. At time zero the rack was immersed in an agitated waterbath (Blue M "Magni-Whirl"®) previously set at the treatment temperature. At set intervals replicates for that given time were returned to the ice bath for cooling. Temperature was monitored throughout, and the entire cycle was repeated for each temperature treatment.

The five strain comparison was done at 56°C (±0.3). For a given strain, three replicate tubes were heated for varying times. With careful attention to aseptic handling

techniques, the glass rod was removed and 2 ml of diluent was added to each tube. After thorough mixing with a sterile wooden applicator stick to extract virus, the tubes were centrifuged and 0.1 ml of the supernatant was injected without dilution into each of 4 eggs. Thus, a total of 12 eggs representing 3 replicate treatment tubes at each time were inoculated. The entire procedure was repeated on different days for each of the five different strains. All dead embryos were checked by the hemagglutination (HA) plate test.

The thermal death time curve (TDTC) work using the Roakin strain followed the same techniques at 56, 60, 64 and 68.8^{o}C. Undilute supernatant from three to nine replicate tubes for each temperature/time treatment was inoculated into at least four eggs per replicate tube to demonstrate NDV inactivation. A total of 150 tubes at 44 temperature/time treatments were tested.

RESULTS

The titers of the five strains of NDV in frozen tubes of spiked turkey breast were $10^{7.7}$ to $10^{8.7}$ per gram as shown in Table 1.

A typical heating and cooling curve showing the actual sample temperature is presented in Figure 1 for 56°C. At 2.5 min the sample reached 95% of the target temperature. At 3.5 and 4.5 min the sample reached 99 and 100% of the target temperature, respectively. Little variation between tubes was noted. The time required to reach temperature was the same for 56, 60, 64 and 68.8°C, with the come-up curve rising more quickly at higher temperatures. Similarly, the cool-down curves showed analogous shapes. For all temperatures, the treatment time included come-up but not cool-down time.

Thermal inactivation of the five NDV strains at 56°C is shown in Figure 2. Virus infectivity as determined by embryo death was recovered from all strains after 3 hr heating. The Fontana and Roakin strains were more heat stable than the other strains, but they were not significantly different from each other.

The thermal death time curve data for the Roakin strain is summarized in Figure 3. Plotting a line through points representing no recovered virus gave $F_{68.8^{\circ}C} = 5.0$

min and z = 8.9°C. Some eggs inoculated from one of nine tubes at 60°, 60 min and from one of four tubes at 64°, 40 min died and were HA positive. However, samples treated for shorter times at these temperatures did not infect eggs, so the two questionable tubes were not considered when drawing the thermal death line.

CONCLUSIONS

To produce AAF with maximum NDV titer, diluting the inoculum 1:10,000 was more effective than inoculum which was not diluted. Approximately 3 to 5 ml of AAF was harvested from each egg, and only after mixing with turkey breast and freezing were the initial NDV titers determined for the spiked meat. The spiked meat matrix was chosen because it closely resembled natural viremic breast, yet permitted starting with a very high titer. The natural concentration of NDV in turkey or even chicken breast is apparently low as previous workers have not included skeletal muscle in tissues investigated. Hofstad (1951) found virus levels in six tissues of chickens infected with four NDV strains, but muscle was not included. Likewise, a review by Beaudette (1943) indicates the body distribution of NDV in fifteen body tissues and fluids, but muscle was not included. It is also possible that muscle was of secondary interest to offal, which was fed to healthy birds. Maximum NDV titer in blood of infected chickens was about 10^3 per ml (Hofstad, 1951). The titers of spiked samples used here were $10^{7.7}$ to $10^{8.7}$.

Much previous NDV work has been done with AAF or diluted AAF. Reported thermal inactivation times have ranged from 45 to 105 minutes at 55 to 56^{o}C, and from 5 to

60 minutes at 60°C (Asplin, 1949; Brandly et al., 1946; DiGioia et al., 1970; Foster and Thompson, 1957). The review by Hanson and Brandly (1958) indicates 31 NDV strains were infective after 15 min at 56°, with 3 strains retaining infectivity after 180 min. Iinuma et al. (1979) have reported heat-resistant mutants of NDV. Considering the variability of reported thermal inactivation times, the present results with spiked turkey breast do not appear unusual and they indicate that the breast tissue matrix is protective to the virus.

An inconsistency was noted between the thermal stability of Roakin NDV in the five-strain comparison (active at 180 min, 56°C) and the thermal death time study (inactive at 140 min, 56°C). While handing the tubes midway through the first study, a dried film was noted inside the top of some tubes. Apparently portions of some samples had become desiccated during frozen storage and may have become more heat stable than the moist samples (Asplin, 1949). The comparison was not repeated because all tubes were treated similarly, and because the purpose was to select one strain for more detailed study. For the thermal death time work, the exposed inside surface of all tubes were routinely cleaned with a swab dipped in 70% ethanol before the tubes were heated. The Roakin strain was chosen over the Fontana

strain because their heat stabilities were not significantly different and because the Roakin strain did not require high security containment.

To ensure NDV destruction when calculating processing effects from F and z values, three safety margins were included. First, the Roakin strain with more heat resistance was used for determining the TDTC. Second, the NDV titer of $10^{8.6}$ was higher than expected in nature and complete inactivation was required for defining the TDTC. Finally, come-up time was included in the measured time for inactivation. If samples could have been instantaneously brought to temperature, the inactivation times would have been shorter than reported here. At 68.8°C the come-up time was the major portion of the total time. However, attempts to correct for come-up effects were not made so that the margin of safety was retained.

Customarily F values are determined for 250°F (121°C). Here $F_{68.8^{o}C}$ ($F_{156^{o}F}$) is stated because the lower inactivation range of NDV makes $F_{250^{o}F}$ meaningless, and because 68.8°C is a common reference for virus inactivation in processed foods (Blackwell et al., 1985).

The thermal inactivation characteristics of NDV were determined here in spiked turkey breast and measured by loss of infectivity. The method described here, whereby

F and z values are determined for a specific system, should find general application in defining thermal processing requirements. However, each system must be investigated separately. Knowing F and z values permits calculating lethality whether short time-high temperature or long time-low temperature processes are used (Bigelow et al., 1920). Further, it is possible to correlate an easily measured biochemical change to the time and temperature conditions which inactivate NDV. A logical choice would be acid phosphatase inactivation, which is presently used to analyze a variety of food products (Axelrod, 1947; Lind, 1965; Sanders et al., 1956; Sanders, 1948). An acid phosphatase test would provide a rapid, inexpensive laboratory method to demonstrate processing conditions sufficient to destroy NDV in turkey breast.

REFERENCES

Asplin, F. D. 1949. Observations on the viability of Newcastle disease virus. Vet. Rec. 61:159.

Axelrod, B. 1947. Phosphatase activity as an index of pasteurization in citrus juices. Fruit Prod. J. 26:132.

Beaudette, F. R. 1943. A review of the literature on Newcastle disease. Proc. 47th Ann. Mtg., U.S. Livestock Sanit. Assoc. 47:122-177.

Bigelow, W. D., G. S. Bohart, A. C. Richardson, and C. O. Ball. 1920. Heat Penetration in Processing Canned Foods. Bull. No. 16-L. Res. Lab. Natl. Canners Assoc., Wash., D.C.

Blackwell, J. H., D. O. Cliver, J. J. Callis, N. D. Heidelbaugh, E. P. Larkin, P. D. McKercher, and D. W. Thayer. 1985. Foodborne viruses: Their importance and need for research. J. Food Protect. 48:717.

Brandly, C. A., H. E. Moses, E. L. Jungherr, and E. E. Jones. 1946. The isolation and identification of Newcastle disease virus. Am. J. Vet. Res. 7:289.

DiGioia, G. A., J. J. Licciardello, J. T. R. Nickerson, and S. A. Goldblith. 1970. Thermal inactivation of Newcastle disease virus. Appl. Microbiol. 19:451.

Doyle, T. M. 1927. A hitherto unrecorded disease of fowls due to a filter-passing virus. J. Comp. Pathol. Therapeut. 40:144

Farinas, E. C. 1930. Avian pest, a disease of birds hitherto unknown in the Philippine islands. Philippine J. Agric. 1:311.

Foster, N. M., and C. H. Thompson. 1957. The comparative thermolability of four strains of Newcastle disease virus of widely varying virulence. Vet. Med. 52:119.

Hanson, R. P. 1978. Newcastle Disease. Pages 513-535 in M. S. Hofstad, ed. Diseases of poultry. 7th ed. Iowa State University Press, Ames, IA.

Hanson, R. P., and C. A. Brandly. 1958. Newcastle disease. Ann. N.Y. Acad. Sci. 70:585.

Hofstad, M. S. 1951. A quantitative study of Newcastle disease virus in tissues of infected chickens. Am. J. Vet. Res. 12:334.

Iinuma, M., Y. Nishiyama, M. Hamaguchi, T. Yoshida, Y. Nagai, K. Maeno, and T. Matsumoto. 1979. Isolation and characterization of heat-resistant (HR) mutants of Newcastle disease virus. Microbiol. Immunol. 23:1179.

Lind, J. 1965. The determination of the acid phosphatase activity in canned hams. Danish Meat Products Laboratory, The Royal Veterinary and Agricultural College, September 23.

Sanders, G. P. 1948. Report on the phosphatase test in pasteurization of dairy products. J. Assoc. Offic. Agric. Chemists 31:306.

Sanders, G. P., J. A. Hupfer, and H. G. Wiseman. 1956. A phosphatase test for determining heat treatment of alfalfa meal. J. Dairy Sci. 39:561.

United States Department of Agriculture. 1978. Eradication of exotic Newcastle disease in southern California 1971-1974. Animal and Plant Health Inspection Service APHIS-91-34.

Table 1. Titer of NDV per gram of spiked turkey breast in frozen tubes used for strain comparison and thermal death time curve treatments

Strain	Titer
Fontana (viscerotropic, velogenic)	$10^{8.4}$
San Juan (viscerotropic, velogenic)	$10^{7.7}$
Parrot project (viscerotropic, velogenic)	$10^{8.5}$
Roakin (mesogenic)	$10^{8.6}$
Texas GB (neurotropic, velogenic)	$10^{8.7}$

Figure 1. Typical heating and cooling curves for 2.0 g meat in 12 x 75 mm tubes with a 6 mm rod inserted. The temperature range for four replicates is indicated. While this is shown for a 56°C waterbath, the time required for come-up and cool-down was the same at higher temperatures

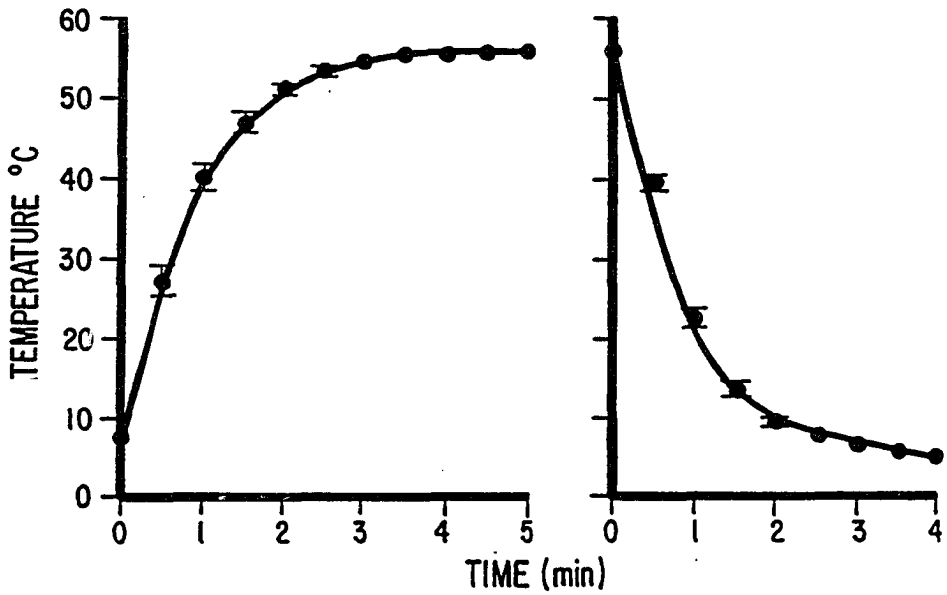

TEMPERATURE °C
60
50
40
30
20
10
0
0
1
2
3
4
5
0
1
2
3
4
TIME (min)

Figure 2. Thermal inactivation of NDV at 56°C as demonstrated by survival of embryonating eggs inoculated with an extract of heated, spiked turkey breast

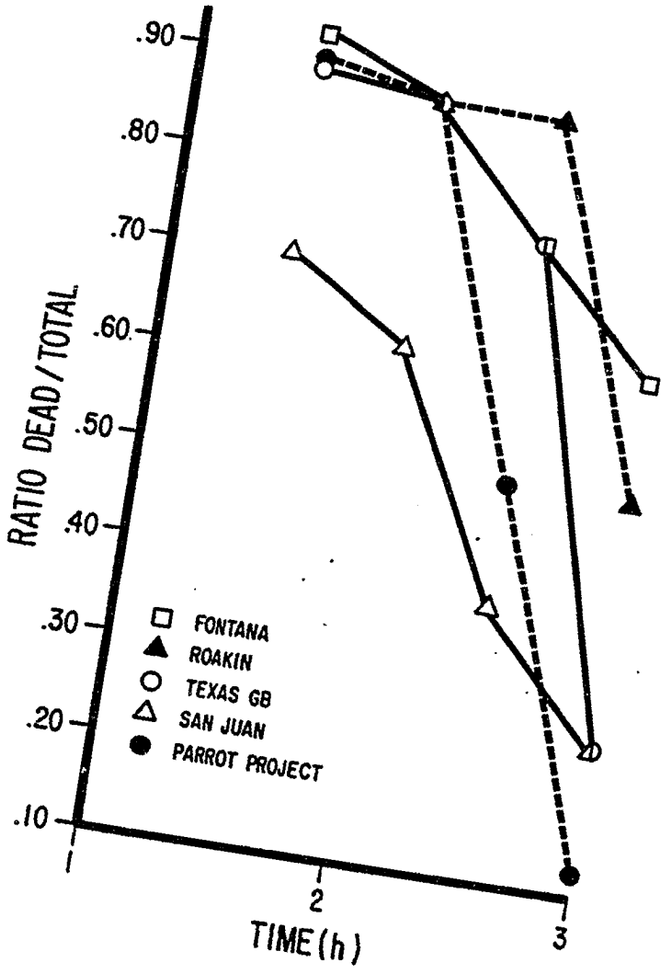
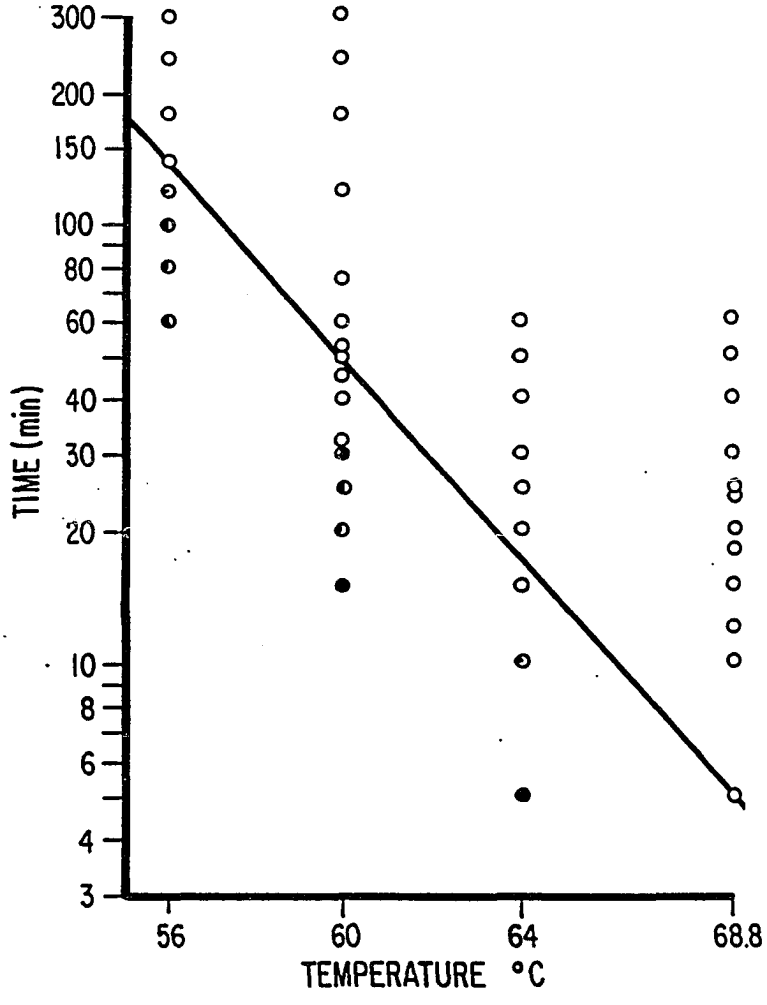

.90
.80
.70
.60
.50
.40
.30
.20
.10
RATIO DEAD / TOTAL
FONTANA
ROAKIN
TEXAS GB
SAN JUAN
PARROT PROJECT
1
2
3
TIME (h)

Figure 3. Thermal death time curve for Roakin strain NDV, in spiked turkey breast (titer $10^{8.6}$ per gram). Extracts from three to nine replicates for each temperature/time treatment were inoculated into at least four eggs per replicate to demonstrate NDV inactivation. All data from replicates at each temperature/time treatment were pooled and the percent of dead eggs (live virus) indicated by shading


300
200
150
100
80
60
40
30
20
10
8
6
4
3
TIME (min)
56
60
64
68.8
TEMPERATURE °C

SECTION III. COOKING OF VIREMIC TURKEY BREAST: THERMAL INACTIVATION OF EXOTIC NEWCASTLE DISEASE VIRUS AND ACID PHOSPHATASE

Cooking of Viremic Turkey Breast: Thermal Inactivation of Exotic Newcastle Disease Virus and Acid Phosphatase

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ABSTRACT

Thirty adult market turkeys of at least 20 lbs each were inoculated with the Fontana strain of viscerotropic velogenic Newcastle disease virus to produce viremic breast tissue. Pre-inoculation hemagglutination inhibition antibody titers did not exceed 1:16. Inoculation of turkeys with virus in amnionic-allantoic fluid was by combinations of intravenous, intraocular and intramuscular routes. Twelve of the thirty birds produced virus as demonstrated by egg inoculation. Maximum response (estimated titer 10^2 to 10^3 per gram of superficial pectoral muscle) occurred when birds were inoculated by all three routes. No virus was recovered from any of the viremic turkey breasts after cooking under proposed commercial processing conditions. Acid phosphatase activity of the cooked turkey breasts decreased to less than 2% of the uncooked activity.

INTRODUCTION

Viscerotropic velogenic Newcastle disease (VVND) is caused by a highly pathogenic paramyxovirus (Andrewes, 1962). The incubation period in various avian species is about 2 to 15 days, with mortality rates of up to 100% (Pickard, 1928; Beaudette, 1943). Because of its fast spread and high mortality, VVND outbreaks have serious economic impact. A southern California outbreak in 1972 required the destruction of nearly 12 million infected and exposed birds in a 2-year effort that cost $56 million (USDA, 1978).

To prevent the spread of Newcastle disease virus (NDV) and other diseases of domestic animals, the USDA restricts importation of products derived from animals originating in countries where such diseases exist. For example, pork products from countries with foot and mouth disease (FMD) are prohibited, however canned hams are specifically permitted because a laboratory test exists which can verify that they have been cooked sufficiently to destroy FMD (FSIS, 1986).

Phosphatase inactivation is a well documented test to demonstrate heat processing. The variety of foods tested by this method include canned ham (Lind, 1965; Cohen, 1969), dairy products (Sanders, 1948; McGugan and Howson,

1964), citrus juices (Axelrod, 1947) and animal feed (Sanders et al., 1956; Czech and Sunseri, 1968). This investigation was undertaken to determine the relationship between VVND inactivation and acid phosphatase (ACP) inactivation in cooked turkey breast.

MATERIALS AND METHODS

Viremic turkey breast

Three groups of 10 adult market turkeys of either sex and weighing at least 20 lbs each were acclimated in the high security isolation room used for this study for one week prior to use. They were not caged nor penned within the room, and were allowed to eat and drink ad libitum. A pre-inoculation serum sample was taken to determine anti-NDV titer by hemagglutination inhibition (HAI) of a standardized NDV reaction with chicken red cells.

The turkey inoculum was Fontana strain VVND virus in amnionic-allantoic fluid (AAF) and was produced in 10-day-old embryonating chicken eggs. It was stored frozen at -20°C or, if used within four days, it was refrigerated at 3°C. One group of 10 turkeys was inoculated with VVND AAF that had been stored frozen and diluted 1:10 with sterile physiological saline (.85% NaCl) for use. A second group of 10 turkeys was inoculated with undiluted VVND AAF that had been stored frozen. And the third group of 10 turkeys was inoculated with undiluted VVND AAF within 4 days of harvest and which had been refrigerated but not frozen.

Inoculation routes were combinations of intravenous (IV, wing vein), intraocular (IO) and intramuscular (IM,

left breast). For each route 1.0 ml was administered. All inoculated groups of birds were kept in the isolation room under high security conditions including air filtration, air flow away from common areas, and clothing change by personnel after showering upon exiting. The turkeys were observed daily for mortality. At natural death or after euthanasia at 3 to 7 days postinoculation approximately 75g of right superficial pectoral muscle was removed, subdivided for subsequent use and frozen at -20°C.

Virus work

For both uncooked and cooked turkey breast, 5g of tissue was placed in 10 ml of tryptose broth diluent[1] and homogenized for 5 to 10 seconds using a Fluke model SDT Tissuemizer. Samples were then centrifuged at 3,000 rpm for 15 min at ambient temperature. Supernatant from uncooked tissue was diluted in serial tenfold dilutions and then inoculated by the allantoic route into four 9-11 day old embryonating chicken eggs per dilution. Supernatant from duplicate cooked tissue samples was inoculated without

[1]Tryptose broth containing per ml: 1.21 mg Tris HCl, 10,000 units penicillin G, 2,000 µg streptomycin sulfate, 650 µg kanamycin sulfate, 1,000 µg gentamicin sulfate and 20 µg amphotericin B.

dilution into 6 eggs for each of the two duplicate samples. All eggs were candled daily, and AAF from dead embryos was checked by the hemagglutination (HA) plate test. Verification that positive HA was due to NDV by checking for HAI was not deemed necessary in this experimental design.

Acid phosphatase activity

The 2,6 dibromoquinone chlorimide (2,6 DBQ) method used by the Food Safety and Inspection Service (FSIS, 1986) was adapted to determine ACP activity. For raw tissue, 0.5g was homogenized in 20 ml of citrate buffer (0.15 M, pH 6.5) and then further diluted with buffer as necessary. For cooked tissue, 2.5g was homogenized in 10 ml of buffer and used without further dilution. Reaction was initiated by adding 5 ml phenyl disodium phosphate (PDSP) substrate (0.01 M) to 10 ml homogenate, and the mixture was incubated for 60 minutes at 37^{o}C. The reaction was stopped and proteins precipitated by adding 5 ml of 20% trichloracetic acid (TCA) leaving any free phenol in solution. A blank, in which TCA was added before substrate, was included to determine non-enzyme background. After filtration, 3 ml of the filtrate was added to 3 ml sodium carbonate (0.5 M), then 0.1 ml of 2,6 DBQ (0.13 mM) added to start color development. Thirty minutes later absorbance at 610 nm was

read and compared to known phenol standards treated in like manner.

Thermal processing

Two separate thermal processing procedures were used. One was to establish the acid phosphatase inactivation for temperature/time treatments defined by Davis et al. (1987) which inactivated NDV in turkey breast. The other was to mimic a proposed commercial heating process for turkey breast. Both were done with an agitated waterbath (Blue M "Magni-Whirl"®) and temperature was monitored with thermocouple probes attached to a datalogger (Fluke model 2240C).

The procedure to establish acid phosphatase inactivation was to heat turkey breast to given temperatures for a given time, and then to cool the sample. Fresh turkey breast was obtained from a local retail source, ground through a 5 mm plate, mixed, ground through a 2 mm plate and mixed again. A stock supply of treatment tubes was produced by weighing 2.0g of ground turkey breast into 12x75 mm centrifuge tubes, capping, and storing frozen at -80^{o}C until needed. For each treatment, replicate tubes were thawed and a 6mm diameter glass rod was inserted. The sample thereby assumed the geometrical configuration of a hollow cylinder with 6 mm i.d. and 8 mm o.d. This

configuration permitted rapid, uniform heating. Thermocouple probes were inserted in the meat between the glass rod and wall of four tubes in each batch to monitor temperature. All tubes for a given treatment were spaced in a rack to allow circulation and then equilibrated in an icewater bath. At time zero the rack was immersed in the waterbath set at the treatment temperature, and at the given time it was returned to the icebath for cooling. Temperature was monitored throughout, and the entire cycle repeated for each given temperature. Acid phosphatase activity was determined for each combination of temperature and time.

The procedure for heating turkey breast to mimic a proposed commercial heating process was to heat blocks of tissue of approximately 2.5x2.5x4 cm and weighing approximately 23g. The samples were put into 7x16 cm plastic bags, the air expelled manually, and the bags were folded and sealed. Disinfected thermocouple probes were inserted into the center of four samples in each group of ten, and the entry puncture sealed with tape and clamped to hold both the tape and the probe in place. Groups of 10 samples were held submerged in the waterbath, and the temperature adjusted manually such that the sample temperature duplicated a commercial processing curve

whereby temperature was monitored at the geometric center of whole breasts. A differential of less than 2°C between the waterbath temperature and the center of the samples was maintained, and each group of 10 was processed in an identical manner. Following heating, the samples were removed from the waterbath and air cooled at ambient temperature, then refrigerated at 3°C.

RESULTS

A summary of results is listed in Table 1. Pre-inoculation hemagglutination titers for 28 of 30 turkeys did not exceed 1:8, while the remaining 2 had titers of 1:16. Inoculation of the first two groups of 10 turkeys was done immediately after the pre-inoculation bleeding, while the third group was inoculated one week after the pre-inoculation bleeding. Inoculation of turkeys in the first group of 10 by various routes with VVND AAF diluted 1:10 produced recoverable virus in two birds. Both of the viremic birds had been inoculated by a combination of IO, IV and IM. Neither of the two died naturally, but were euthanized 3 days postinoculation. The titer of the inoculum for the first group was not established, but to improve response in the second group the same VVND AAF inoculum was used undilute and was administered to all 10 turkeys by the combined IO, IV and IM routes. The titer of the inoculum for the second group was found to be $10^{4.5}$, and breast tissue from three of the ten birds had virus. None of the turkeys in this group died naturally, but were euthanized four days post inoculation. New inoculum was produced for the third group of 10 turkeys by harvesting VVND AAF from infected eggs and refrigerating until use. Titer of the new inoculum was $10^{7.5}$, and all birds were

inoculated by the combined IO, IV and IM routes. Breast tissue from seven of the ten turkeys contained virus, and all seven of the viremic turkeys died naturally 2 to 4 days postinoculation. Postmortem examination revealed a few duodenal and tracheal hemorrhagic foci, but otherwise nothing specifically characteristic of NDV.

NDV was receovered from 12 of 30 turkey breasts. Only a crude estimation of titer in the tissue was possible due to limited egg supply and incubator capacity in the isolation building. The titers were estimated to be 10^2 - 10^3 per gram. For cooked breast samples all eggs were inoculated with undilute homogenate and no NDV was demonstrated.

Acid phosphatase activity as indicated by absorbance at 610 nm is shown in Table 1. The activity in uncooked turkey breast ranged from 7.0 to 24.0, while cooking reduced the activity of all samples to less than 2% of the uncooked value, or an absolute A_{610} value of 0.04 to 0.19.

Acid phosphatase activity of breast tissue heated to temperature/time treatments which inactivated NDV is shown in Table 2. Activity remaining after heating varied from 3% to 9%, or absolute A_{610} values of 0.6 to 1.9.

The laboratory and proposed commercial heating curves are shown in Figure 1. At 60°C and above, the two curves

coincided and reached 70°C in 3.5 hours. Come-up time to 60°C was about 1 hour. Within each group of 10, the internal temperatures of four samples varied about 0.5°C and were within 1.5°C of the waterbath temperature.

CONCLUSIONS

The significance of pre-inoculation HAI titers of up to 1:16 that were found here in adult turkeys is not clear. While it is tempting to compare these titers to titers sometimes exceeding 1:1,000 in young vaccinated chickens (Levine and Fabricant, 1952; Winterfield et al., 1957) and conclude that 1:16 is not significant, some workers have reported inducing neutralizing antibody immunity lasting 12 months with little HAI response (Doyle and Wright, 1950). The vaccination history of the turkeys used in this study could not be verified, and therefore their resistance to infection is unknown. Reports on the natural susceptibility of turkeys conflict (Beaudette, 1943; Box et al., 1970).

Dividing the turkeys into three groups was necessary due to limited availability of both isolation space and 10-day-old embryonated eggs for virus work. This facilitated some trial and error, especially in optimizing the inoculum concentration and route. Because inoculation by the combined IO plus IV plus IM routes produced the only viremic turkey breasts in the first group, the remaining turkeys were inoculated the same way.

The Fontana strain of NDV was chosen for two important reasons. First, it is a viscerotropic velogenic strain and

thereby more likely to produce viremic breast tissue, and secondly the highly pathogenic strains are of the greatest concern to the USDA. Sacrificing turkeys that did not die naturally by four days postinoculation was intended to give maximum viremia (Hofstad, 1951). However, virus concentration did not exceed approximately 10^3 per gram where up to 10^5 was hoped for. Precise titers were difficult to establish due to limited capacity for egg incubation. It is doubtful that postmortem physiological changes within the muscle, such as lactic acid increase, would inactivate the NDV as it has been reported to be stable to pH 4 (Brandly et al., 1946), and carcasses held at $2^{o}C$ have been shown to remain infective for more than 6 months (Asplin, 1949). Finding only minimal pathological effects of VVND in the turkey is apparently common (Taylor, 1987).

Some of the cooked samples had pink interiors indicating that cooking conditions were not severe enough to denature the hemichrome of the pigment (Fox, 1966).

Maintaining a small temperature differential between the center of the sample and the waterbath mimicked the heat exposure of a similar intact piece of meat at the center of a breast. The laboratory heating curve was matched to the proposed commercial heat processing curve

only above 60°C because the lethal effects at lower temperatures were less significant, and because matching the entire curve would require 8.25 hours. Although the total heating effect of the laboratory heating was thereby less than the commercial process, it was still sufficient to kill the virus.

Very little information about turkey breast acid phosphatase is available in the literature. Baxter and Suelter (1984) reported that ACP is elevated in muscle from one-month-old dystrophic chickens. Degenerative pig muscle also exhibits increased ACP (Dutson et al., 1971). Investigation of the ACP levels of normal and NDV infected turkey breast would be appropriate.

The acid phosphatase activity of the breasts cooked following the proposed commercial protocol was 0.2 to 1.6% of the uncooked breast values. This level was lower than the acid phosphatase activity remaining in breast tissue after heating to temperature/time treatments which inactivated NDV as shown in Table 2. Therefore, demonstrating acid phosphatase inactivation in turkey breasts heated by the proposed commercial protocol described here could be an alternative to direct virus recovery for verifying processing sufficient to destroy exotic Newcastle disease virus.

REFERENCES

Andrewes, C. H. 1962. Classification of viruses of vertebrates. Pages 271-296 in K. M. Smith and M. A. Lauffer, eds. Advances in virus research. Vol. 9. Academic Press, New York.

Asplin, F. D. 1949. Observations on the viability of Newcastle disease virus. Vet. Rec. 61:159.

Axelrod, B. 1947. Phosphatase activity as an index of pasteurization in citrus juices. Fruit Prod. J. 26:132.

Baxter, J. H., and C. H. Suelter. 1984. Multiple acid phosphatases in avian pectoral muscle. The postmicrosomal supernatant acid phosphatase is elevated in avian dystrophic muscle. Arch. Biochem. Biophys. 228:397.

Beaudette, F. R. 1943. A review of the literature on Newcastle disease. Proc. 47th Ann. Mtg., U.S. Livestock Sanit. Assoc. 47:122-177.

Box, P. G., B. I. Helliwell, and P. H. Helliwell. 1970. Newcastle disease in turkeys. Vet. Rec. 86:524.

Brandly, C. A., H. E. Moses, E. L. Jungherr, and E. E. Jones. 1946. The isolation and identification of Newcastle disease virus. Am. J. Vet. Res. 7:289.

Cohen, E. H. 1969. Determination of acid phosphatase activity in canned hams as an indicator of temperatures attained during cooking. Food Technol. 23:101.

Czech, F. P., and A. P. Sunseri. 1968. Field test to determine proper heat treatment of animal swill. J. Assoc. Off. Anal. Chemists 51:1184.

Davis, R. M., D. A. Senne, A. A. Kraft, and J. G. Sebranek. 1987. Comparison of thermal inactivation characteristics of five Newcastle disease virus strains in spiked turkey breast. Prepared for submission to J. Food Protect.

Doyle, T. M. and E. C. Wright. 1950. An inactivated vaccine against Newcastle disease. Brit. Vet. J. 106:139.

Dutson, T. R., A. M. Pearson, and R. A. Fennell. 1971. Histochemical demonstration of acid phosphatase activity in pig skeletal muscle fibers. J. Food Sci. 36:710.

FSIS (Food Safety Inspection Service). 1986. Chemistry laboratory guidebook. U.S. Government Printing Office, Washington, D.C.

Fox, J. B., Jr. 1966. The chemistry of meat pigments. J. Agric. Food Chem. 14:207.

Hofstad, M. S. 1951. A quantitative study of Newcastle disease virus in tissues of infected chickens. Am. J. Vet. Res. 12:334.

Levine, P. P., and J. Fabricant. 1952. Efficacy of Newcastle disease vaccines under controlled conditions. Cornell Vet. 42:449.

Lind, J. 1965. The determination of the acid phosphatase activity in canned hams. Danish Meat Products Laboratory, The Royal Veterinary and Agricultural College, September 23.

McGugan, W. A., and G. Howson. 1964. Alkaline phosphatase in cheddar cheese made from raw and heat treated milk. J. Dairy Sci. 47:139.

Pickard, W. K. 1928. Pseudo-fowlpest. Dept. of Agric., Industry and Commerce in the Dutch East Indies. Veterinary Bulletin No. 65:1-46.

Sanders, G. P. 1948. Report on the phosphatase test in pasteurization of dairy products. J. Assoc. Off. Agric. Chemists 31:306.

Sanders, G. P., J. A. Hupfer, and H. G. Wiseman. 1956. A phosphatase test for determining heat treatment of alfalfa meal. J. Dairy Sci. 39:561.

Taylor, W. D. 1987. USDA, APHIS, Ames, Iowa, personal communication.

United States Department of Agriculture. 1978. Eradication of exotic Newcastle disease in southern California 1971-1974. Animal and Plant Health Inspection Service APHIS-91-34.

Winterfield, R. W., C. L. Goldman, and E. H. Seadale. 1957. Newcastle disease immunization studies. 4. Vaccination of chickens with B_1, F and LaSota strains of Newcastle disease virus (NDV) administered through drinking water. Poult. Sci. 36:1076.

Table 1. Summary of responses and breast acid phosphatase (ACP) activity of 30 turkeys inoculated with viscerotropic velogenic Newcastle disease virus

Turkey	HAI Pre-inoc	(Inoculum)[a] and route	Death[b]	Breast NDV demonstrated		ACP (A_{610})		
				Raw	Cooked	Raw	Cooked	% Inact.
I-1	1:8	(1) IO	E(3)	-	-	10.2	.079	99.2
I-2	1:8	"	E(3)	-	-	9.09	.109	98.8
I-3	1:8	"	E(7)	-	-	10.7	.050	99.5
I-4	<1:8	"	E(7)	-	-	8.5	.057	99.3
I-5	1:8	(1) IO+IV	E(3)	-	-	9.2	.079	99.1
I-6	<1:8	"	E(3)	-	-	9.7	.080	99.2
I-7	1:8	"	E(7)	-	-	10.4	.066	99.4
I-8	<1:8	"	E(7)	-	-	9.3	.047	99.5
I-9	<1:8	(1) IO+IV+IM	E(3)	+	-	7.0	.114	98.4
I-10	1:8	"	E(3)	+	-	10.3	.105	99.0
II-1	1:8	(2) IO+IV+IM	E(4)	+	-	15.9	.069	99.6
II-2	<1:8	"	E(4)	-	-	15.2	.135	99.1
II-3	<1:8	"	E(4)	-	-	15.1	.190	98.8
II-4	<1:8	"	E(4)	-	-	14.9	.089	99.4
II-5	<1:8	"	E(4)	-	-	14.7	.128	99.1
II-6	<1:8	"	E(4)	+	-	15.6	.118	99.2
II-7	<1:8	"	E(4)	-	-	17.3	.124	99.3
II-8	<1:8	"	E(4)	-	-	17.9	.194	98.9
II-9	1:8	"	E(4)	+	-	20.5	.135	99.3
II-10	1:8	"	E(4)	-	-	15.7	.159	99.0

III-1	1:8	(3) IO+IV+IM	N(2)	+	-	19.7	.080	99.6
III-2	1:8	"	E(4)	-	-	9.8	.038	99.6
III-3	<1:8	"	N(4)	+	-	15.5	.034	99.8
III-4	1:8	"	E(4)	-	-	11.9	.100	99.2
III-5	1:16	"	N(3)	+	-	21.2	.057	99.3
III-6	<1:8	"	N(4)	+	-	24.0	.063	99.3
III-7	1:8	"	N(2)	+	-	7.5	.057	99.2
III-8	1:16	"	N(4)	+	-	16.2	.036	99.8
III-9	1:8	"	E(4)	-	-	16.6	.107	99.4
III-10	1:8	"	N(3)	+	-	15.8	.036	99.8

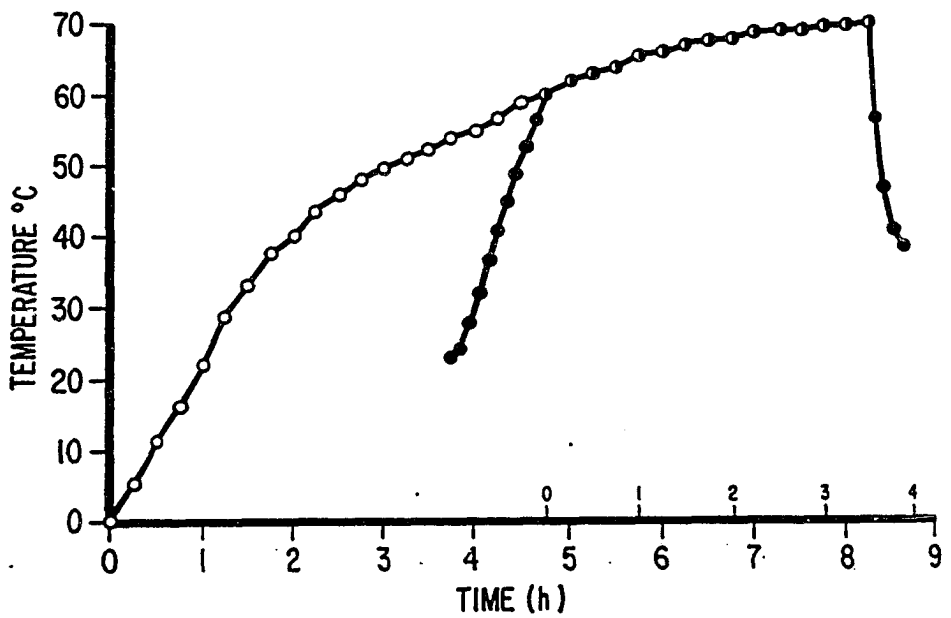
[a]Inoculums were (1) frozen AAF 1:10 titer unknown, (2) frozen AAF undilute titer ~$10^{4.5}$ (3) fresh AAP undilute titer ~$10.^{7.6}$.

[b]E if by euthanasia. N if natural. (Days post inoculation).

Table 2. Turkey breast acid phosphatase (ACP) inactivation at temperature/time combinations which inactivate Newcastle disease virus (average of six replicates)

	ACP (A_{610})	% Inact.
unheated	21.7	0
56°C/140 min	1.94	91.1
60°C/49 min	0.806	96.3
64°C/17.2 min	0.585	97.3
68.8°C/5.0 min	0.608	97.2

Figure 1. Proposed commercial thermal processing curve (open circles) recorded at the geometric center of intact turkey breast, and its laboratory reproduction above 60°C with 23 g tissue blocks in a manually adjusted waterbath (shaded circles)


TEMPERATURE °C
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60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
9
TIME (h)
0
1
2
3
4

GENERAL SUMMARY AND DISCUSSION

The work presented here, when considered as a whole, forms the basis for a rapid laboratory test to demonstrate thermal inactivation of exotic Newcastle disease virus in turkey breast. Further, adequate processing can be demonstrated regardless of the actual time and temperature parameters used to effect the process.

The overall approach to this project was designed to minimize the possibility of erroneously determining that turkey breast is adequately processed if in fact it is not. For the virology part of this research, a comparatively heat stable NDV strain, as measured by infectivity, was picked for defining thermal inactivation characteristics. Inactivation was demonstrated in actual turkey breast spiked to artificially high NDV titer. Complete loss of infectivity was required for processing to be considered as adequate. And come-up lag time was included when inactivation time was measured at various temperatures.

Lethality of industrial processing can be calculated from the thermal death time curve (TDTC) z and F constants established in Section II. With $z = 8.9^{\circ}C$, the lethal rate at any temperature on the industrial heating curve can be determined from the equation:

$$\text{lethal rate} = \frac{1}{\text{antilog } \left(\frac{68.8^{\circ}C - \text{observed } ^{\circ}C}{z}\right)}$$

Plotting lethal rate vs. time and knowing $F_{68.8^{\circ}C} = 5.0$ min. gives the area underneath the lethal rate curve which represents lethal processing. For the proposed commercial processing in Section III, the processing was the equivalent of three lethal processes summed together as shown here in Figure 1. Calculation of process lethality by using z and F values applies to any heating curve regardless of shape. It should be remembered that the z and F values reported in Section II of this study are based on inactivating $10^{8.6}$ Roakin NDV per gram of spiked turkey breast tissue.

Correlation of ACP inactivation with NDV inactivation was determined. Temperature/time combinations from the TDTC, each of which equally inactivated NDV, were checked for ACP inactivation in Section III. Figure 2 here illustrates that the ACP was not inactivated equally. At lower temperatures ACP is more heat stable compared to NDV than at higher temperatures, whereas for an ideal correlation ACP and NDV would be inactivated equally at all temperatures. In terms of laboratory analysis, the lowest level of residual ACP activity must be selected as the

threshold to indicate adequate processing. All unknown samples with residual ACP activity less than the threshold would then be adequately processed. In terms of processing, this means that lower temperature processes must actually be more extensive than required to inactivate NDV so that ACP will be inactivated below the threshold test level. In reality, it is doubtful that processors would choose a low-temperature long-time process with endpoint temperature less than 64°C due to poor consumer acceptance. At 64°C and above there is good correlation between ACP and NDV inactivation. The residual ACP activity after the proposed commercial process investigated here was below the level corresponding to NDV inactivation and reflects the extensive processing shown in Figure 1. In Section III, Table 1, the 30 cooked breast ACP values are all shown to be below the level corresponding to NDV inactivation.

Several areas of future work present themselves as logical extensions of this research. The 2,6 DBQ colorimetric method of ACP analysis is effective and does not require sophisticated instruments. However, it is time and labor intensive. Therefore, a study showing correlation with an updated method for turkey breast samples would improve laboratory efficiency. Also, the natural range of

ACP activity in market turkeys, and its variation with breed, sex and age, was not investigated here. Residual ACP after thermal processing would be influenced by the initial level, therefore information about the natural variability of ACP activity would be essential if this model system was applied to actual commercial processing. And a study to find whether reactivation of ACP occurs in cooked turkey breast would also seem appropriate. Reactivation was not detected in cooked pork, but has not been investigated in turkey.

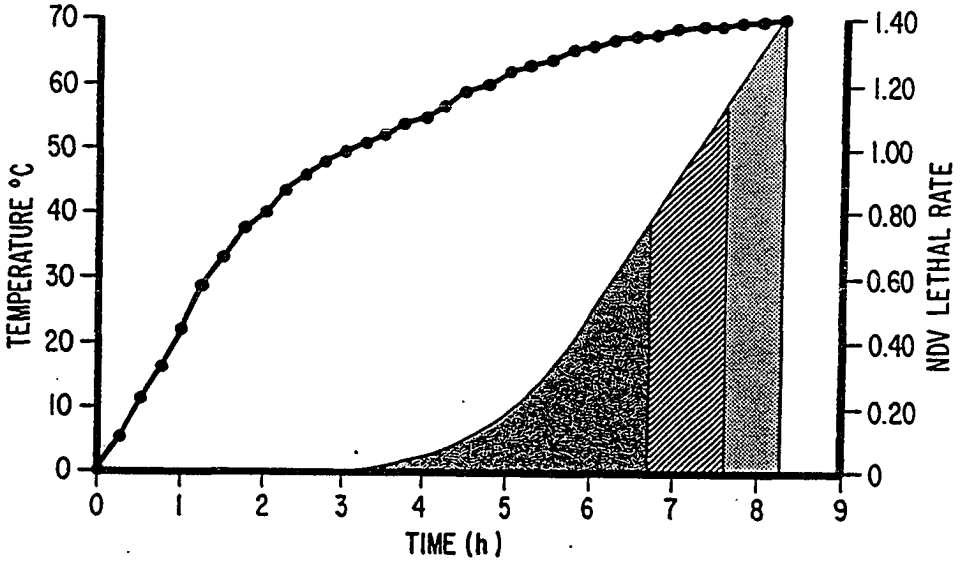
The use of this work for regulatory purposes permits flexibility, and at the same time requires certain cautions. In addition to the already mentioned margins of safety built into the experimental design, this work permits finding laboratory ACP test levels which correspond to set processing requirements. For example, suppose processing sufficient to give the equivalent of two lethal units was desired. Residual ACP levels at various temperature/time combinations that correspond to two lethal units would be determined and set as the threshold laboratory test value for adequate processing. The inherent cautions include the natural variability of ACP, the possibility of NDV strains that are more heat stable than those investigated here, the overprocessing required for low-

temperature long-time heating, the possibility of NDV contamination after processing, and the need to repeat the steps outlined in this work for each different type of turkey product.

While in general foodborne viruses of veterinary regulatory significance are of little concern to human health, the implications for domestic livestock are real. This appears to be the first report of the application of classical equivalent thermal processing considerations to the inactivation of foodborne viral agents. The steps outlined in this model system with turkey breast can also be used to develop laboratory tests for other tissues, avian species or product formulations.

The information in this dissertation should not be extrapolated as to exact criteria for evaluating the safety of any product. Rather, it should serve as a model of how information needs to be developed for each individual type of product.

Figure 1. Lethal effect of proposed commercial processing on NDV in turkey breast. Each of the three shaded areas under the lethal rate curve represents lethal processing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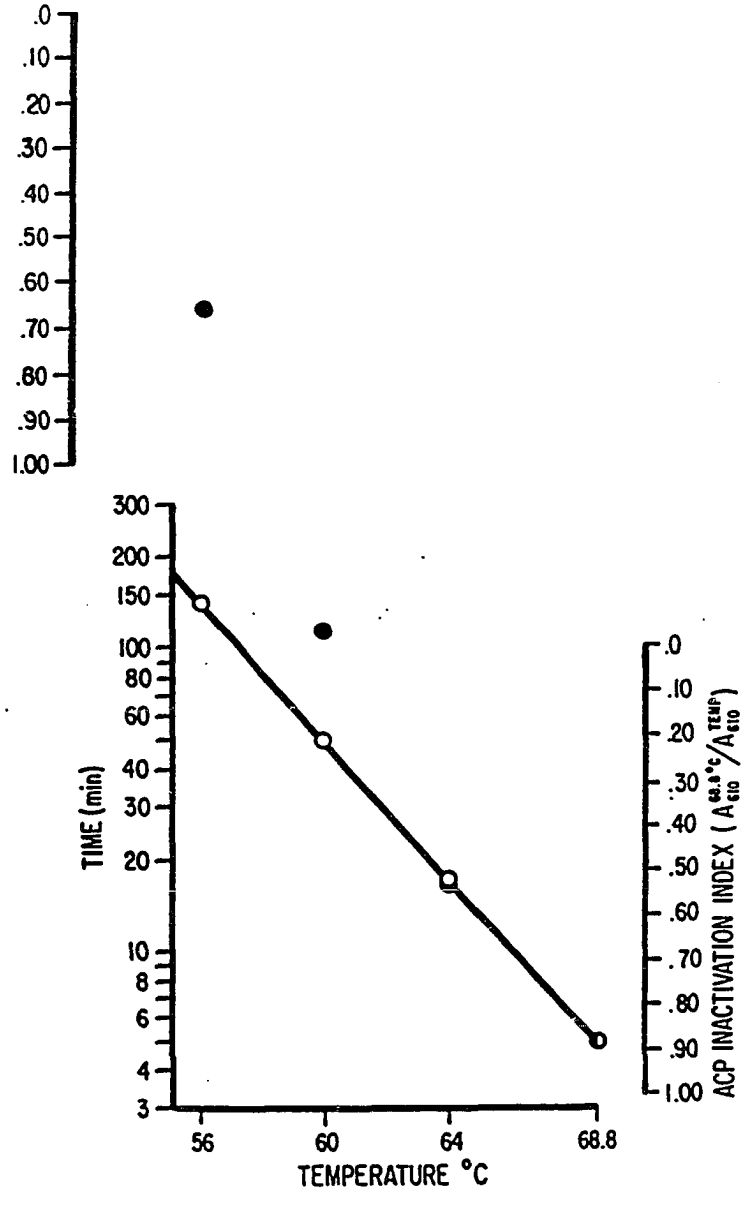
60
50
40
30
20
10
0
TEMPERATURE °C
0
1
2
3
4
5
6
7
8
9
TIME (h)
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0
NDV LETHAL RATE

Figure 2. NDV thermal inactivation temperature/time treatments (circles) and the corresponding acid phosphatase (ACP) inactivation normalized to 68.8°C (dots)


.0
.10
.20
.30
.40
.50
.60
.70
.80
.90
1.00
300
200
150
100
80
60
40
30
20
10
8
6
4
3
TIME (min)
56
60
64
68.8
TEMPERATURE °C
ACP INACTIVATION INDEX ($A_{610}^{68.8°C}/A_{610}^{TEMP}$)

REFERENCES

Albiston, H. E., and J. R. Gorrie. 1942. Newcastle disease in Victoria. Australian Vet. J. 18:75.

Alexander, L. M., G. E. Schopmeyer, J. C. Lamb, and S. J. Marsden. 1951. Turkey temperature as the endpoint in roasting. Poultry Sci. 30:520.

Andersch, M. A., and A. J. Szczypinski. 1947. Use of ρ-nitrophenyl phosphate as the substrate in determination of serum acid phosphatase. Am. J. Clin. Pathol. 17:571.

Andrewes, C. H. 1962. Classification of viruses of vertebrates. Pages 271-296 in K. M. Smith and M. A. Lauffer, eds. Advances in virus research. Vol. 9. Academic Press, New York.

Asdell, M. K. and R. P. Hanson. 1960. Sequential changes in the titer of Newcastle disease virus in tissues - a measure of the defense mechanism of the chicken. Am. J. Vet. Res. 21:128.

Asghar, A., and A. M. Pearson. 1980. Influence of ante- and postmortem treatments upon muscle composition and meat quality. Pages 53-213 in C. O. Chichester, E. M. Mrak, and G. F. Stewart, eds. Advances in food research. Vol. 26. Academic Press, New York.

Asplin, F. D. 1949. Observations on the viability of Newcastle disease virus. Vet. Rec. 61:159.

Association of Official Analytical Chemists. 1975. Official Methods of Analysis. Twelfth Edition. AOAC, Washington, D.C.

Axelrod, B. 1947. Phosphatase activity as an index of pasteurization in citrus juices. Fruit Prod. J. 26:132.

Bais, R., and J. B. Edwards. 1976. An optimized continuous-monitoring procedure for semiautomated determination of serum acid phosphatase activity. Clin. Chem. 22:2025.

Ball, C. O. 1923. Thermal process time for canned food. Bull., Nat. Research Council 7-1 (37).

Beach, J. R. 1943. Avian pneumoencephalitis. Proc. 46th Ann. Mtg. U.S. Livestock Sanit. Assoc. 46:203.

Beach, J. R. 1944. The neutralization in vitro of avian pneumoencephalitis virus by Newcastle disease immune serum. Science 100:361.

Beaudette, F. R. 1943. A review of the literature on Newcastle disease. Proc. 47th Ann. Mtg., U.S. Livestock Sanit. Assoc. 47:122-177.

Beaudette, F. R. 1949. An addendum to a review of the literature on Newcastle disease. Proc. 53rd Ann. Mtg., U.S. Livestock Sanit. Assoc. 53:202-220.

Beaudette, F. R. 1950. Recent literature on Newcastle disease. Proc. 54th Ann. Mtg., U.S. Livestock Sanit. Assoc. 54:132-153.

Beaudette, F. R. 1951. Current literature on Newcastle disease. Proc. 55th Ann. Mtg., U.S. Livestock Sanit. Assoc. 55:108-174.

Beaudette, F. R., and J. J. Black. 1946. Newcastle disease in New Jersey. Proc. 49th Ann. Mtg., U.S. Livestock Sanit. Assoc. 49:49-58.

Bigelow, W. D., G. S. Bohart, A. C. Richardson, and C. O. Ball. 1920. Heat penetration in processing canned foods. Bull. No. 16-L. Res. Lab. Natl. Canners Assoc., Wash., D.C.

Bivins, J. A., B. R. Miller, and F. R. Beaudette. 1950. Search for virus in eggs laid during recovery post-inoculation with Newcastle disease virus. Am. J. Vet. Res. 11:426.

Blaedel, W. J., and R. C. Boguslaski. 1978. Chemical amplification in analysis. A review. Anal. Chem. 50:1026.

Boyd, R. J., and R. P. Hanson. 1958. Survival of Newcastle disease in nature. Avian Dis. 2:82.

Brandly, C. A. 1959. Newcastle disease. Pages 464-503 in H. E. Biester and L. H. Schwarte, eds. Diseases of poultry. Iowa Sate University Press, Ames, Iowa.

Brandly, C. A. 1964. Recognition of Newcastle disease as a new disease. Pages 53-64 in R. P. Hanson, ed. Newcastle disease virus. Univ. Wisc. Press, Madison.

Brandly, C. A., H. E. Moses, E. E. Jones, and E. L. Jungherr. 1946a. Epizootiology of Newcastle disease of poultry. Am. J. Vet. Res. 7:243.

Brandly, C. A., H. E. Moses, E. L. Jungherr, and E. E. Jones. 1946b. The isolation and identification of Newcastle disease virus. Am. J. Vet. Res. 7:289.

Breclaw, E. W., and L. E. Dawson. 1970. Smoke-flavored chicken rolls. J. Food Sci. 35:379.

Campbell, J. E., and E. F. McFarren. 1961. Collaborative study of a differential test for reactivated and residual phosphatase in dairy products. J. Assoc. Off. Anal. Chemists 44:444.

Chambers, J. P., L. Aquino, R. H. Glew, R. E. Lee, and L. R. McCafferty. 1977. Determination of serum acid phosphatase in Gaucher's disease using 4-methyl-umbelliferyl phosphate. Clin. Chim. Acta 80:67.

Chambers, P., N. S. Millar, R. W. Bingham, and P. T. Emmerson. 1986. Molecular cloning of complementary DNA to Newcastle disease virus, and nucleotide sequence analysis of the junction between the genes encoding the haemagglutinin-neuraminidase and the large protein. J. Gen. Virol. 67:475.

Charan, S., and D. P. Gautam. 1978. Applications of enzyme-linked immunosorbent assay in veterinary medicine: A bibliography. Vet. Res. Commun. 8:255.

Cheftel, J. C. 1977. Chemical and nutritional modifications of food proteins due to processing and storage. Pages 401-445 in J. R. Whitaker and S. R. Tannenbarum, eds. Food proteins. AVI Pub. Co., Westport, Connecticut.

Cheng, C., and F. C. Parrish, Jr. 1979. Heat-induced changes in myofibrillar proteins of bovine longissimus muscle. J. Food Sci. 44:22.

Cohen, E. H. 1966. Protein changes related to ham processing temperatures. I. Effect of time-temperature on amount and composition of soluble proteins. J. Food Sci. 31:746.

Cohen, E. H. 1969. Determination of acid phosphatase activity in canned hams as an indicator of temperatures attained during cooking. Food Technol. 23:101.

Cole, R. K., and F. B. Hutt. 1961. Genetic differences in resistance to Newcastle disease. Avian Dis. 5:205.

Cunningham, C. H. 1948. The effect of certain chemical agents on the virus of Newcastle disease of chickens. Am. J. Vet. Res. 9:195.

Czech, F. P., and A. P. Sunseri. 1968. Field test to determine proper heat treatment of animal swill. J. Assoc. Off. Anal. Chemists 51:1184.

Davis, C. E., and J. B. Anderson. 1983. Effect of heat on biuret-positive water-extractable porcine muscle proteins. J. Food Protect. 46:947.

Davis, C. E., A. J. Bracewell, J. B. Anderson, and J. O. Reagan. 1985. Time temperature heating effect on biuret-positive water-extractable porcine and bovine muscle proteins. J. Food Protect. 48:215.

deDuve, C. 1969. The lysosome in retrospect. Pages 3-30 in L. J. Dingle and H. B. Fell, eds. Lysosomes in biology and pathology. North Holland Pub. Co., Amsterdam.

deMan, J. M. 1980. Principles of food chemistry. AVI Pub. Co., Westport, Conn.

Desrosier, N. W., and J. N. Desrosier. 1977. The technology of food preservation. Fourth edition. AVI Pub. Co., Westport, Conn.

DiGioia, G. A., J. J. Licciardello, J. T. R. Nickerson, and S. A. Goldblith. 1970. Thermal inactivation of Newcastle disease virus. Appl. Microbiol. 19:451.

Dinter, Z. 1964. Avian myxoviruses. Pages 299-312 in R. P. Hanson, ed. Newcastle disease virus. Univ. Wisc. Press, Madison, Wisconsin.

Doyle, T. M. 1927. A hitherto unrecorded disease of fowls due to a filter-passing virus. J. Comp. Pathol. Therapeut. 40:144.

Doyle, T. M. 1935. Newcastle disease of fowls. J. Comp. Pathol. Therapeut. 48:1.

Edwards, J. T. 1928. A new fowl disease. Ann. Report of the Imperial Institute of Veterinary Research, Muktesar, India for the year ending 31 March.

Erickson, G. A. 1976. Viscerotropic velogenic newcastle disease in six pet bird species: Clinical response and virus-host interactions. Ph.D. thesis, Iowa State University, Ames, Iowa.

Esty, J. R., and K. F. Meyer. 1922. The heat resistance of the spores of B. botulinus and allied anaerobes. XI. J. Infect. Dis. 31:434.

Farinas, E. C. 1930. Avian pest, a disease of birds hitherto unknown in the Philippine Islands. Philippine J. Agric. 1:311.

Flambert, F., and J. Deltour. 1972. Localization of the critical area in thermally-processed conduction heated canned food. Lebensm.-Wiss. U. Technol. 5:7.

Foster, N. M., and C. H. Thompson. 1957. The comparative thermolability of four strains of Newcastle disease virus of widely varying virulence. Vet. Med. 52:119.

Foti, A. G., H. Herschman, and J. F. Cooper. 1975. A solid-phase radioimmunoassay for human prostatic acid phosphatase. Cancer Res. 35:2446.

Foti, A. G., J. F. Cooper, and H. Herschman. 1978. Counterimmunoelectrophoresis in determination of prostatic acid phosphatase in human serum. Clin. Chem. 24:140.

Froning, G. W., R. G. Arnold, R. W. Mandigo, C. E. Neth, and T. E. Hartung. 1971. Quality and storage stability of frankfurters containing 15% mechanically deboned turkey meat. J. Food Sci. 36:974.

FSIS (Food Safety Inspection Service). 1986. Chemistry laboratory guidebook. U.S. Government Printing Office, Wash., D.C.

Gardner, F. A., J. H. Denton, and D. B. Mellor. 1980. The effects of curing and cooking methods on the yield of smoked chicken broilers. Poult. Sci. 59:1612.

Gibbs, H. D. 1927. Phenol tests. III. The indophenol test. J. Biol. Chem. 72:649.

Ginsberg, H. S. 1980. Paramyxoviruses. Pages 1139-1159 in B. D. Davis, R. Dulbecco, H. N. Eisen, and H. S. Ginsberg, eds. Microbiology: Including immunology and molecular genetics. Third Edition. Harper and Row, Philadelphia.

Goertz, G. E., K. Cooley, M. C. Ferguson, and D. L. Harrison. 1960. Doneness of frozen, defrosted turkey halves roasted to several end point temperatures. Food Technol. 14:135.

Goldblith, S. A. 1971. A condensed history of the science and technology of thermal processing. Part 1. Food Technol. 25:44.

Goldblith, S. A. 1972. A condensed history of the science and technology of thermal processing. Part 2. Food Technol. 26:64.

Goldblith, S. A., M. A. Joslyn, and J. T. R. Nickerson. 1961. Introduction to thermal processing of foods. AVI Pub. Co., Westport, Conn.

Goll, D. E., W. G. Hoekstra, and R. W. Bray. 1964. Age associated changes in bovine muscle connective tissue. 2. Exposure to increasing temperatures. J. Food Sci. 29:615.

Goodwin, T. L., V. D. Bramblett, G. E. Vail, and W. J. Stadelman. 1962. Effects of end-point temperature and cooking rate on turkey meat tenderness. Food Technol. 16:101.

Hale, K. K., Jr., D. D. Cohn, and J. D. Stubblefield. 1977. Effects of finishing diet and cure procedures on quality of smoked poultry. Poultry Sci. 56:211.

Hamm, R. 1960. Biochemistry of meat hydration. Adv. Food Res. 10:355.

Hamm, R. 1966. Heating of muscle systems. Pages 363-385 in E. J. Briskey, R. G. Cassens, and J. C. Trautman, eds. The physiology and biochemistry of muscle as a food. Univ. Wisc. Press, Madison, Wisconsin.

Hamm, R., and F. E. Deatherage. 1960. Changes in hydration, solubility and changes of muscle proteins during heating of meat. Food Res. 25:587.

Hamm, R., and K. Hofmann. 1965. Changes in the sulfhydryl and disulfide groups in beef muscle proteins during heating. Nature 207:1269.

Hanson, R. P., ed. 1964. Newcastle disease virus. Univ. Wisc. Press, Madison, Wisconsin.

Hanson, R. P. 1973. Worldwide spread of viscerotropic Newcastle disease. Proc. 76th Ann. Mtg., U.S. Animal Health Assoc. 76:276-279.

Hanson, R. P. 1978. Newcastle disease. Pages 513-535 in M. S. Hofstad, ed. Diseases of poultry. Iowa State University Press, Ames, Iowa.

Hanson, R. P., and C. A. Brandly. 1955. Identification of vaccine strains of Newcastle disease virus. Science 122:156.

Hanson, R. P., and C. A. Brandly. 1958. Newcastle disease. Ann. N.Y. Acad. Sci. 70:585.

Hanson, R. P., E. Upton, C. A. Brandly, and N. S. Winslow. 1949. Heat stability of hemagglutinin of various strains of Newcastle disease virus. Proc. Soc. Exp. Biol. Med. 70:283.

Hara, T., M. Imaki, and M. Toriyama. 1981. The determination of alkaline phosphatase activity in serum by means of flow-through analysis with an ion-selective electrode. Bull. Chem. Soc. Jpn. 54:1396.

Hayakawa, K. 1977. Mathematical methods for estimating proper thermal processes and their computer implementation. Adv. Food Res. 23:75.

Hegarty, P. V. J., and C. E. Allen. 1972. Rigor-stretched turkey muscles. Effects of heat on fiber dimensions and shear values. J. Food Sci. 37:652.

Hegarty, P. V. J., and C. E. Allen. 1975. Thermal effects on the length of sarcomeres in muscles held at different tensions. J. Food Sci. 40:24.

Hitchner, S. B. 1964. Control of Newcastle disease in the United States by vaccination. Pages 85-98 in R. P. Hanson, ed. Newcastle disease virus. Univ. Wisc. Press, Madison, Wisconsin.

Hitchner, S. B., and E. P. Johnson. 1948. A virus of low virulence for immunizing fowls against Newcastle disease (avian pneumoencephalitis). Vet. Med. 43:525.

Hitchner, S. B., and G. Reising. 1953. Results of field tests on spraying a commercially prepared Newcastle disease vaccine. Proc. 90th Ann. Mtg. Am. Vet. Med. Assoc. 90:350.

Hofstad, M. S. 1949. A study on the epizootiology of Newcastle disease (pneumoencephalitis). Poultry Sci. 28:530.

Hofstad, M. S. 1964. Immunogenicity of Newcastle disease virus. Pages 189-204 in R. P. Hanson, ed. Newcastle disease virus. Univ. Wisc. Press, Madison.

Hofstad, M. S., and H. W. Yoder, Jr. 1963. Inactivation rates of some lyophilized poultry viruses at 37 and 3°C. Avian Dis. 7:170.

International Union of Biochemistry. 1964. Enzyme Nomenclature. Elsevier Pub. Co., New York.

Iinuma, M., Y. Nishiyama, M. Hamaguchi, T. Yoshida, Y. Nagai, K. Maero, and T. Matsumoto. 1979. Isolation and characterization of heat-resistant (HR) mutants of Newcastle disease virus. Microbiol. Immunol. 23:1179.

Jungherr, E. L. 1964. Pathogenicity of the Newcastle disease virus for the chicken. Pages 257-272 in R. P. Hanson, ed. Newcastle disease virus. Univ. Wisc. Press, Madison.

Jungherr, E. L., and N. Terrell. 1946. Observations on the spread of Newcastle disease. Proc. 50th Ann. Mtg., U.S. Livestock Sanit. Assoc. 50:158.

Kaplan, M. M. 1972. Alkaline phosphatase. Gastroenterology 62:452.

Kay, H. D. 1930. Plasma phosphatase. I. Method of determination. Some properties of the enzyme. J. Biol. Chem. 89:235.

Kiefer, H. C. 1977. Measurement of phosphatases in biological fluids. Annals Clin. Lab. Sci. 7:500.

Kind, P. R. N., and E. J. King. 1954. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J. Clin. Path. 7:322.

King, E. J., and A. R. Armstrong. 1934. A convenient method for determining serum and bile phosphatase activity. Can. Med. Assoc. J. 31:376.

King, N. L. R. 1978. Isoelectric variants of actin in raw and cooked meat. Meat Sci. 2:313.

Kleyn, D. H., and S. H. C. Lin. 1968. Collaborative study of a new alkaline phosphatase assay system for milk. J. Assoc. Off. Anal. Chemists 51:802.

Konno, T., Y. Ochi, and K. Hashimoto. 1929. Neue geflügelseuche in Korea. Dtsch. Tieraerztl. Wochenschr. 37:515.

Kormendy, L., and Gy. Gantner. 1960. The acid phosphatase of meat. Z. Lebensm.-Untersuch. u. -Forsch. 113:13.

Kraneveld, F. C. 1926. Over een in Ned.-Indië heerschende ziekte onder het pluimvee. Tijdschr. Diergeneesk. 38:448.

Lancaster, J. E., and D. J. Alexander. 1975. Newcastle disease virus and spread, a review of some of the literature. Canada (Ottawa) Dept. Agric. Monograph No. 11.

Lee, Y. B., D. A. Rickansrud, E. C. Hagberg, and E. J. Brisker. 1974. Application of SDS-acrylamide gel electrophoresis for determination of the maximum temperature to which bovine muscles have been cooked. J. Food Sci. 39:428.

Levinson, S. O., A. Milzer, H. Shaughnessy, J. Neal, and F. Oppenheimer. 1944. Production of potent inactivated vaccines with ultraviolet irradiation. J. Am. Med. Assoc. 125:531.

Lind, J. 1965. The determination of the acid phosphatase activity in canned hams. Danish Meat Products Laboratory, The Royal Veterinary and Agricultural College, September 23.

Moses, H. E., C. A. Brandly, and E. E. Jones. 1947. The pH stability of viruses of Newcastle disease and fowl plague. Science 105:477.

Mullen, J. E. C. 1950. The acid phosphatase of cows' milk. J. Dairy Res. 17:288.

Murphy, B. D., and T. L. Goodwin. 1980. The effect of raw carcass storage and final internal temperature on the yield and quality of smoked turkey. Poultry Sci. 59:1642.

Nemitz, G., and W. Partmann. 1959. Über die Hitzeinaktivierung des Apyrasesystems der Muskulatur. Z. Lebensm. Untersuch. Forsch. 109:121.

Olesiuk, O. M. 1951. Influence of environmental factors on viability of Newcastle disease virus. Am. J. Vet. Res. 12:152.

Olson, F. C. W., and H. P. Stevens. 1939. Nomograms for graphic calculation of thermal processes for non-acid canned foods exhibiting straight line semi-logarithmic heating curves. Food Res. 4:1.

Pearson, A. M., G. Harrington, R. G. West, and M. E. Spooner. 1962. The browning produced by heating fresh pork. I. The relation of browning intensity to chemical constituents and pH. J. Food Sci. 27:177.

Perkin, A. G., H. Burton, H. M. Underwood, and F. L. Davies. 1977. Thermal death kinetics of Bacillus stearothermophilus spores at ultra high temperatures. II. Effect of heating period on experimental results. J. Food Technol. 12:131.

Picard, W. K. 1928. Pseudo-fowlpest. Dept. of Agric., Industry and Commerce in the Dutch East Indies. Veterinary Bulletin No. 65:1-46.

Picken, J. C., Jr. 1964. Thermostability of Newcastle disease virus. Pages 167-188 in R. P. Hanson, ed. Newcastle disease virus. Univ. Wisc. Press, Madison.

Pilchard, E. I., and H. A. McDaniel. 1983. Foreign diseases and arthopod pests of livestock and poultry. Proc. 87th Ann. Mtg., U.S. Anim. Health Assoc. 87:11-23.

Placidi, L., and J. Santucci. 1956. Agglutination comparée des hematies de la poule, du chameau et des équides par les virus de la maladie de Newcastle et de la peste aviaire. Ann. Inst. Pastuer 90:528.

Powell, M. E. A., and M. J. H. Smith. 1954. The determination of serum acid and alkaline phosphatase activity with 4-aminoantipyrine (A.A.P.). J. Clin. Path. 7:245.

Price, R. J., C. A. Bottorff, K. Seeger, A. W. Sylstra, and F. S. Markham. 1955. Vaccination against Newcastle disease and infectious bronchitis. 2. Field trials in mass vaccination with live virus dust vaccines. Poult. Sci. 34:449.

Rhoades, H. E. 1958. The effect of storage on viability of lyophilized cultures of bacteria, viruses, yeasts and molds. Am. J. Vet. Res. 19:765.

Richardson, L. A., E. F. McFarren, and J. E. Campbell. 1964. Phosphatase reactivation. J. Dairy Sci. 47:205.

Riisfeldt, O. 1946. Acid phosphatases employed as a new method of demonstrating seminal spots in forensic medicine. Acta Pathol. Microbiol. Scand. Supplement 58.

Robertson, A. 1964. Methods of control of Newcastle disease and their limitations. Pages 71-84 in R. P. Hanson, ed. Newcastle disease virus. Univ. Wisc. Press, Madison.

Rodier, E. A. 1928. Philippine fowl disease. Proc. Soc. Exp. Biol. Med. 25:781.

Rott, R. 1964. Antigenicity of Newcastle disease virus. Pages 133-146 in R. P. Hanson, ed. Newcastle disease virus. Univ. Wisc. Press, Madison.

Roy, A. V., M. E. Brower, and J. E. Hayden. 1971. Sodium thymolphthalein monophosphate: A new acid phosphate substrate with greater specificity for the prostatic enzyme in serum. Clin. Chem. 17:1093.

Sanders, G. P. 1948. Report on the phosphatase test in pasteurization of dairy products. J. Assoc. Off. Agric. Chemists 31:306.

Sanders, G. P., J. A. Hupfer, and H. G. Wiseman. 1956. A phosphatase test for determining heat treatment of alfalfa meal. J. Dairy Sci. 39:561.

Sanders, G. P., and O. S. Sager. 1946. Modification of the phosphatase test as applied to cheddar cheese and application of the test to fluid milk. J. Dairy Sci. 29:737.

Sanders, G. P., and O. S. Sager. 1947. Phosphatase test for various dairy products. J. Dairy Sci. 30:909.

Sauls, C. D., and C. T. Caskey. 1985. Applications of recombinant DNA to pathologic diagnosis. Clin. Chem. 31:804.

Schloer, G. 1965. Plaque characteristics of Newcastle disease virus strains. Ph.D. Thesis. Univ. Wisc. (Libr. Congr. Card No. Mic. 65-4839). University Microfilms, Ann Arbor, Michigan.

Schmidt, J. G., and F. C. Parrish, Jr. 1971. Molecular properties of postmortem muscle. 10. Effect of internal temperature and carcass maturity on structure of bovine longissimus. J. Food Sci. 36:110.

Schmidt, J. G., E. A. Kline, and F. C. Parrish, Jr. 1970. Effects of carcass maturity and internal temperature on bovine longissimus attributes. J. Anim. Sci. 31:861.

Shaw, L. M., W. Brummund, and R. J. Dorio. 1977. An evaluation of a kinetic acid phosphatase method. Am. J. Clin. Path. 68:57.

Shope, R. E. 1964. The birth of a new disease. Pages 3-22 in R. P. Hanson, ed. Newcastle disease virus. Univ. Wisc. Press, Madison.

Sinha, S. K., R. P. Hanson, and C. A. Brandly. 1954. Aërosol transmission of Newcastle disease in chickens. Am. J. Vet. Res. 15:287.

Spalatin, J., R. P. Hanson, and P. D. Beard. 1970. The hemagglutination-elution pattern as a marker in characterizing Newcastle disease virus. Avian Dis. 14:542.

Srinivasappa, G. B., D. B. Snyder, W. W. Marquardt, and D. J. King. 1986. Isolation of a monoclonal antibody with specificity for commonly employed vaccine strains of Newcastle disease virus. Avian Dis. 30:562.

Stearn, A. E. 1949. Kinetics of biological reactions with special reference to enzymic processes. Pages 25-74 in F. F. Nord, ed. Advances in enzymology and related subjects of biochemistry. Interscience Pub. Inc., New York.

Stone, E. W., and K. N. May. 1969. The effect of time and temperature of cooking on quality of freeze-dehydrated chicken. Poultry Sci. 48:813.

Suvakov, M., V. Visacki, M. Marinkov, and S. Korolija. 1967. Relation of acid phosphatase content to heat treatment of cured meat. Technologija Mesa 8:306.

United States Department of Agriculture. 1978. Eradication of exotic Newcastle disease in southern California 1971-1974. Animal and Plant Health Inspection Service, APHIS-91-34.

Utterback, W. W., and J. H. Schwartz. 1973. Epizootiology of velogenic viscerotropic Newcastle disease in southern California, 1971-1973. J. Am. Vet. Med. Assoc. 163:1080.

Visacki, V., S. Korolija, M. Suvakov, and M. Marinkov. 1967. Comparison and application of various methods for determination of cooking rate of meat products. Technologija Mesa 8:274.

Whitaker, J. R. 1977. Denaturation and renaturation of proteins. Pages 14-49 in J. R. Whitaker and S. R. Tannenbaum, eds. Food Proteins. AVI Pub. Co., Westport, Conn.

Whiteman, C. E., and A. A. Bickford. 1983. Avian disease manual. 2nd ed. Am. Assoc. Avian Pathol., Univ. Pennsylvania, Kennett Square, Philadelphia.

Wielkowska, E., and K. Monikowski. 1956. Phosphatase test for meat and meat products. Roczniki Pansuvowego Zakladu Hig. 7:79.

Wierbicki, E., L. E. Kunkle, and F. E. Deatherage. 1957. Changes in water-holding capacity and cationic shifts during the heating and freezing and thawing of meat as revealed by a simple centrifugal method for measuring shrinking. Food Technol. 11:69.

Wilkinson, R. J., W. L. Mallmann, L. E. Dawson, T. F. Irmiter, and J. A. Davidson. 1965. Effective heat processing for the destruction of pathogenic bacteria in turkey rolls. Poultry Sci. 44:131.

Yam, L. Y. 1974. Clinical significance of the human acid phosphatases. Am. J. Med. 56:604.

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