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Chin-Gin Low

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Alden Booren

Major professor
Alden M. Booren

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THE EFFECT OF LIQUID SMOKE PREPARATIONS ON THE FUNCTIONALITY OF MEAT PROTEINS

By

Chin-Gin Low

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ABSTRACT

The EFFECT OF LIQUID SMOKE PREPARATIONS ON THE FUNCTIONALITY OF MEAT PROTEINS

By

Chin-Gin Low

Effects of direct addition of 0.5% w/w liquid smoke (LS) or smoke fractions to 4% chicken breast salt-soluble proteins (SSP) and SSP gels were determined. Three types of commercial LS (F, G and H) were investigated. Liquid smoke high in carbonyls and/or acids decreased SSP solubility dramatically. This resulted in lower moisture retention, higher gel strength and produced a non-homogenous gel network. Salt-soluble proteins treated with LS high in phenols decreased slightly in solubility; produced protein aggregates or high molecular weight proteins (HMW) and a homogenous, denser microstructure. Superior gel strengths were measured in SSP gels treated with LS high in phenols. Most functional properties of SSP were changed within 6 hr of LS addition. Lower fat and moisture retention resulted in low fat frankfurters containing LS. Results from this study suggest that LS with variation of smoke constituents from lot to lot may influence the functional properties of processed meat.

Dedicated to my parents

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INTRODUCTION

During the past 10 years, the variety of liquid smoke (LS) as well as consumption of meat processed with LS has increased dramatically (Hollenbeck, 1977, 1979). This may be due to the fact that traditional commercial methods for preparing smoked meat face many environmental and safety concerns and that several advantages are offered by LS (Govbatov et al., 1971). The major advantages of LS include lower risks of possible carcinogenesis compared to wood smoke in foods; better control of smoke component level as related to flavor and color; and decrease in smoke effluent from the smokehouse as an environmental concern.

Liquid smoke is used to provide a smoke flavor and develop a characteristic color to processed meat products (Hollenbeck, 1977). It may be applied to meat products by direct addition, dipping, spraying, vaporization or atomization (Ruzek, 1981). Of these methods, atomization has most frequently been used. However, the simplest method of LS application is by direct addition, which offers several advantages. These include: (1) uniform distribution of smoke flavor to the products; (2) incorporation of smoke flavor into products which use cook-in pouches or impermeable casings; and (3) reduced costs associated with

smoke generation such as equipment and space.

The variability in LS constituents can be traced to types of wood, processing methods and conditions of production. Therefore, a great deal of work has been done on determining the constituents of smoke, and their relationship to flavor and color. The flavor and color of smoked foods are related to the acid, carbonyl and phenol content in smoke. Few researchers have reported the chemical effects of smoke on meat proteins. Sink and Hsu (1977, 1979) reported that LS incorporated into a meat emulsion resulted in low juiciness and tenderness scores for frankfurters. Fewer studies have addressed how LS affects the functional properties of meat proteins, especially when LS is directly incorporated into meat emulsions or mixtures. More extensive chemical reactions between smoke components and proteins may occur when smoke compounds are distributed evenly throughout the product than when applied to the surface. Therefore, further investigations are necessary in this area to understand the effect of LS preparations on the functionality of meat proteins.

Salt-soluble proteins (SSP) are the principle functional components in meat products. Therefore, SSP gels were used as a model system to study the effects of direct addition of LS on meat proteins. Three brands (F, G and H) of commercial LS were chosen in this study.

The specific objectives of this study were:

(1) To characterize SSP before and after LS addition using

sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

(2) To study the effect of LS on SSP and SSP gels using scanning electron microscopy, rheological and functional analyses.

(3) To characterize the effect of LS constituents (phenols and carbonyls) on SSP and SSP gels.

(4) To observe the effect of LS in a frankfurter meat system.

LITERATURE REVIEW

2.1 THE CHEMISTRY OF SMOKE

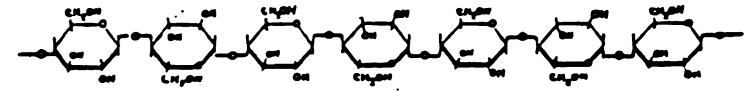
Smoke is a complex mixture. Smoke generation is strictly defined as the destructive anaerobic distillation of wood followed by partial oxidation (Wistreich, 1979). The composition of smoke is influenced by several generation parameters such as type of wood, humidity of the wood, smoke production conditions, such as generation temperature and amount of oxygen available.

2.1.1 Smoke Production

2.1.1.1 The composition of wood and its thermal degradation

Smoke normally is produced from wood. The knowledge the chemical composition of wood and the pyrolysis of wood are important factors in the formation of flavor components in smoke. Wood is composed of approximately 50% cellulose, 25% hemicellulose and 25% lignin. The structures of wood components are illustrated in Fig. 1. Different types of wood may vary in the relative proportions of these components. For example, the structure of lignin in soft wood (e.g. pine, fir, spruce) is different from that of hard wood (e.g. beech, oak, hickory). Lignin from hard wood contains more methoxy groups ($-OCH_3$) than soft wood. The

variability of wood composition exerts great influence on the flavor and color of smoked products (Tóth and Potthast, 1984).



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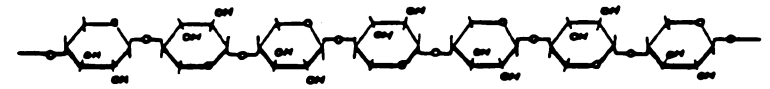

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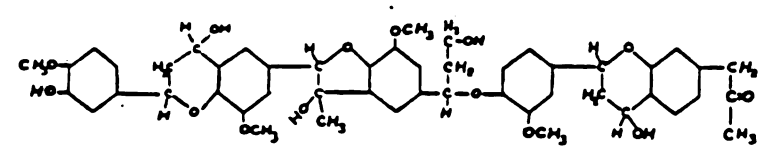


LIGNIN

Figure 1. Principle components of hard wood (adapted from Wistreich, 1979).

During thermal degradation of wood, various products are obtained by pyrolysing proportional amounts of cellulose, hemicellulose and lignin (Goos, 1952). Therefore, it seems reasonable to deal with the pyrolysis of each wood component individually.

Pyrolysis of cellulose.

The initial reaction of pyrolysis appears to be an acid-catalyzed hydrolysis to glucose followed by dehydration to 1,6-anhydroglucose (β-glucosan). Secondary pyrolysis occurs immediately producing acetic acid and its homologies, water and occasionally small amounts of phenols and furans

(Goos, 1952). The latter compounds are more characteristic degradation products of hemicellulose and lignin (Gilbert and Knowles, 1975). The pyrolysis temperature of cellulose is between 260° and 310°C (Tóth and Potthast, 1984).

Pyrolysis of hemicellulose

The exact polymeric structure assigned to hemicellulose depends on its source; hardwoods are richer in pentosans, while hexosans may or may not predominate in softwoods. Hemicellulose is the least heat-stable component of wood. At a temperature between 200° and 260°C, the decomposition of hemicellulose will yield furan and its derivatives together with a range of aliphatic carboxylic acids. Hardwoods, containing greater quantities of pentosans yield appreciably higher amounts of acids. Hexosans decompose in a similar manner to cellulose (Gilbert and Knowles, 1975).

Pyrolysis of lignin

Lignin is a macromolecule consisting of phenol units having three-carbon side chains. The pyrolysis temperature of lignin is between 310° and 500°C (Tóth and Potthast, 1984). The compounds formed are reputed to be the most important fraction in producing smoke flavoring. They are phenols and phenolic ethers, typified by guaiacol (2-methoxyphenol), syringol (2,6-dimethoxyphenol), and their homologies and derivatives. The substitute groups are largely methyl, ethyl, propyl, vinyl, allyl and propenyl.

It is generally accepted that the side chains do not exceed three carbon atoms in length, and occur almost exclusively in the para-position of the phenolic hydroxyl (Goos, 1952).

2.1.1.2. Smoke generation

Smoke is generated commercially in the United States by three methods: burning dampened sawdust, burning dry sawdust continuously, and by friction (Draudt, 1963). Burning dampened hardwood sawdust is a batch operation. This operation usually comprises a box or drum with a mechanical agitator and a blower. In a continuous generator, dry sawdust is fed by a screw to a small electrically heated chamber where it is burned, giving a relative high yield of smoke for the amount of sawdust used. In the friction generator, smoke is produced by pressing the end grain of a hardwood block against a rotating carbide-tipped disk (Draudt, 1963).

For better control of smoke production, water content of sawdust, amount of air supply, temperature and heat supply are main points of consideration. The smoke composition depends upon temperature variations within the glowing zone (pyrolysis), which relies on the amount of air and the water content of the sawdust (Tóth and Potthast, 1984). Differences in amount and composition of smoke should be virtually eliminated by precise temperature and humidity controls in the smoke generator (Tóth and Potthast, 1982).

The method to produce more uniform composition and

quality of smoke utilizes external heating of wood materials to allow better temperature control during pyrolysis. Wood is heated to a temperature between 300° and 400°C to allow pyrolysis in the absence of oxygen. Then the pyrolytic compounds will react with oxygen in reaction chambers connected to the smoke generator. These secondary reactions take place at lower temperatures and have a positive influence on the flavor contributions of smoke (Tóth and Potthast, 1984).

2.1.2. The Chemical Composition of Smoke

The pyrolysis of wood at temperatures of 200° to 500°C under anaerobic conditions includes three principle phases of smoke. These three phases are the particulate phase, the non-condensible phase, and the condensible phase (Wistreich, 1979). The particulate phase consists of particles of fly ash, charcoal and condensed long chain tars. The non-condensible phase consists of air, combustion gases, methane, nitrogen oxides, and other gaseous compounds. The condensible phase is a water-soluble organic compound fraction, which contains high and low molecular weight compounds. These are responsible for most of the desirable effects on smoke flavored foods. This phase can be classified into three classes; acid substances, carbonyl compounds and phenolic substances (Hollenbeck, 1977).

2.1.3. Problems Associated with The Use of Wood Smoke

During the past quarter century, the meat industry has been under constant pressure to find suitable alternatives to the conventional use of solid (wood-based) vapor smoking systems. Environmental concerns are an issue because of obvious air pollution caused by smoke emissions (Sink, 1981). There is no direct evidence that the consumption of smoked or barbecued meat products containing polycyclic aromatic hydrocarbons (PAHs) cause cancer in humans. However, it has been speculated that these compounds are responsible for the high incidence of stomach and colon cancer in Iceland where heavily smoked meats (up to many weeks) containing high concentrations of PAHs are consumed (Fritz and Sóos, 1980).

The PAHs found in hardwood sawdust smoke include naphthalene, fluorene, phenanthrene, anthracene, pyrene, fluoranthene, 1,2-benzanthracene, chrysene, 3,4-benzopyrene and 1,2-benzopyrene (Rhee and Bratzler, 1968). One of the best known PAHs is benzo[*a*]pyrene (B[*a*]p), which was demonstrated to be mutagenic and carcinogenic in animal experiments. The total carcinogenicity of PAH exposure is estimated to be ten times that due to B[a]p alone (Tóth and Potthast, 1984). The concentrations of PAHs deposited on smoked products is affected by the method of smoke generation, the form of the smoke application, and the temperature of the smoking process (Potthast, 1978). A linear increase in the production of PAHs was shown to occur

as the smoke generation temperature was increased from 400° to 1000°C (Tóth and Potthast, 1984). Therefore, by maintaining the combustion temperature of the sawdust below 350°C, the production of these derivatives will be minimized. The maximum B[a]p content in smoked meat products has been limited by regulation to 1 ppb (1ug/kg) in the Federal Republic of Germany (Tóth and Potthast, 1984). However, there is no regulation for B[a]p concentration in smoked meat in the U.S.A. and other countries.

2.2 LIQUID SMOKE (LS)

2.2.1 The Production of Liquid Smoke

Liquid smoke (LS) has been widely accepted and used during the past twenty years. The most prevalent method for producing LS in the U.S.A. is by smoldering sawdust under controlled oxidation conditions and absorbing the smoke constituents in water (Hollenbeck, 1977). Other methods for producing LS include smoldering of sawdust under controlled conditions and condensing smoke in a condenser, or treating the finely divided wood chips with super-heated steam and condensing the steam distillate (Fessmann, 1976).

The production of smoke from smoldering sawdust can be divided into two steps (Miler, 1962): (1) Pyrolysis of wood as a primary reaction, and (2) changes in products of pyrolysis by secondary reactions in the presence of oxygen. Maximum exposure of the smoke to water is achieved by countercurrent flow through an absorption tower (Hollenbeck,

1977). The smoke solution is recycled until the desired concentration of smoke constituents is reached. The smoke solution is aged for a given period of time to allow for polymerization and precipitation of the tar to occur. The LS is then filtered through cellulose pulp to remove particulate.

2.2.2 Liquid Smoke Constituents

Liquid smoke is used to provide a smoke flavor and characteristic cured color to food products. Smoke constituents: acids, carbonyls and phenols are believed to play a major role in these areas (Draudt, 1963; Gorbatov et al., 1971; Gilbert and Knowles, 1975; Baryłko-Pikielna, 1977; and Hollenbeck, 1977).

2.2.2.1 Acids

The carboxylic acid fraction of smoke has a strong influence on preservation in addition to flavoring properties which affect the overall quality of smoked food products (Tóth and Potthast, 1984; Ikins, 1986). The main component of the acid fraction is acetic acid (3.7g/100g wood), which constitutes 40% to 70%. Acetic acid is one of the primary factors responsible for the bacteriostatic properties of smoke inhibiting, in particular *E. coli* (Wendorff, 1981). Another component of the acid fraction is formic acid (approximately 0.8g/100g wood). The amounts of carboxylic acids in LS preparations result in a pH range

from 2 to 4 (Tóth and Potthast, 1984).

2.2.2.2. Carbonyls

Carbonyl content of liquid smoke preparations

Tóth and Potthast (1984) reported that thirteen aldehydes, seventeen ketones, glycolaldehyde, and methylgloxyal have been detected in the carbonyl fraction. Major components of the carbonyl fraction in LS are acetaldehyde, formaldehyde and acetone. They have been detected up to 1150 mg, 200 mg, and 740 mg per 100g of combusted wood, respectively (Love and Bratzler, 1966; Tóth and Potthast, 1984).

Functionality of carbonyls in smoked food

The carbonyl fraction contributes to the mahogany brown color in smoked products. In addition, it contributes to the sharpness in the smoke flavor (Fiddler et al., 1970; Hollenbeck, 1977). The most important color contribution of smoke is due to browning or Maillard-like reactions between the active carbonyls and the amino groups of the meat protein (Ruiter, 1970, 1979; Chen and Issenberg, 1972; Hollenbeck, 1977).

Both glycolaldehyde and methylgloxyal are reported to be the most active browners (Ruiter, 1971). Formaldehyde reacts rapidly with proteins without giving rise to brown color (Chen and Issenberg, 1972). Chen and Issenberg (1972) examined a casein-formaldehyde model system and observed a

rapid decrease of available lysine without color development. However, in model experiments with glycolaldehyde, methylgloxyal, and an amine, formaldehyde accelerated browning rather than retarded it (Ruiter, 1979). Color formation does not directly involve phenolic compounds (Chen and Issenberg, 1972).

The factors that are involved in color formation include temperature in the smokehouse, concentration of carbonyls in the smoke or LS, amine reactants as a substrate, and amount of moisture on the surface of the product (Daun, 1979). Ideal color is obtained at a moisture level between 6% and 10% on the smoked products exterior (Daun, 1979). At this level, the surface is sufficiently moist to allow adsorption of the smoke constituents but is dry enough to allow penetration of the smoke constituents into the product (Hollenbeck, 1977). Other factors involved in characteristic cured color formation include uptake of colored smoked constituents, oxidation and/or polymerization of smoke components (Tóth and Potthast, 1984).

The reaction between the carbonyl fraction of smoke and amines of food cause a loss of lysine (Ruiter, 1970; Chen and Issenberg, 1972; Tang, 1978). Losses of serine, threonine, and sulfur-containing amino acids have also been reported as a result of smoking foods (Mauron, 1970). Amino acid losses decrease the nutritional value of smoked products. However, a nutritional deficiency is not apparent, except for a remarkable losses of lysine from the

surface layers of some heavily smoked meat products (Tóth and Potthast, 1984).

2.2.2.3. Phenols

The phenolic substances contribute mainly to the flavor of smoked foods. Many researchers have investigated the phenolic components in wood smoke in relation to flavor functional attributes and quality control in LS production.

The chemical and physical properties of phenols

Phenols are aromatic hydrocarbons with one or more hydroxyl groups directly connected to the benzene ring. They are designated as mono-, di-, tri-, or polyhydroxyphenols, depending on the number of hydroxyl groups. When dissolved in water they have an acid pH. In alkaline solutions phenols become soluble by dissociation as phenolates. They are sensitive to light and oxygen and form complexes with metals. The boiling point of the typical phenol is 183°C. A second hydroxy group increases the boiling point by about 100°C. Resorcinol, for example, boils at 270°C. Chemical and physical properties of phenols may be further influenced by functional groups derived from alcohols, aldehydes, ketones, and acids (Tóth and Potthast, 1984).

Classification of phenols

Phenols are classified as mono-, di-, tri- and polyhydroxyphenols which includes phenolic compound with

additional functional groups (Tóth and Potthast, 1984). The monohydroxyphenols in smoke condensate and smoked food include phenol, *o*-cresol, *m*-cresol and *p*-cresol (Lustre and Issenberg, 1969, 1970 ; Kornreich and Issenberg, 1972; Fujimaki, et al., 1974). The predominant dihydroxyphenol compounds in smoke are guaiacol and its derivatives (Lustre and Issenberg, 1969, 1970; Wittkowski and Baltes, 1990). The major smoke compounds that have been detected in the trihydroxyphenols class is syringol and its derivatives (Lustre and Issenberg, 1969, 1970; Tóth and Potthast, 1984). Phenol alcohols, aldehydes, ketones, acids, and esters are also present in smoke and can be identified using gas chromatography (GC), infrared spectrum (IR) and mass spectrum (MS) analysis (Lustre and Issenberg, 1969, 1970).

The formation of phenols in smoke

Phenols originate mainly from the pyrolysis of lignin. Small amounts of phenols are also produced from pyrolysis of cellulose. The destruction of lignin during pyrolysis leads to the formation of ferulic acid (guaiacol derivatives) and/or sinapinic acid (syringol derivatives), which are the primary thermal decomposition products (Fiddler, *et. al.*, 1966; Tóth and Potthast, 1984). The ferulic acid and sinapic acid can decompose to 4-vinylguaiacol and vinylsyringol, respectively, which in turn appear to be the source of other phenolic products (Steinke and Paulson, 1964).

The type of wood, smoke generation temperature and amount of oxygen supplied are three main factors for better quality control of the formation of phenols. According to Tóth (1982), syringol and syringol derivatives, as well as guaiacol and guaiacol derivatives, may be found in both hardwood and softwood, but in differing amounts. In smoke from hardwoods syringol is predominant; while in softwoods guaiacol prevails. The different phenolic compounds produced by hardwoods and softwoods influence the taste of the products. Tilgner (1958) stated that smoke from various hardwoods or softwoods leads to different but comparable smoke flavor and taste.

Potthast (1976) demonstrated that the formation of phenol was temperature dependent, but the optimum temperature for high density smoke generation varied. Phenols concentrations develop at generation temperatures from about 600° to 700°C. Fenner and Lephardt (1981) reported 380°C as the generation temperature resulting in the highest phenols formation. Fretheim et al., (1980), however, indicate a slight decrease in phenol formation between 350° and 500°C. According to Tóth (1980), the smoldering temperature not only influences the total amount of phenolic compounds, but also leads to different proportions of individual phenols. For example, syringol from smoke generated between 450° and 850°C is the predominant phenols, but the relative amount of pyrocatechol increases with temperatures exceeding 650°C.

The functionality of phenolic fraction

Although non-phenolic compounds contribute to smoke flavor development (Fujimaki et al., 1974; Tóth and Potthast, 1984), the phenolic fraction of smoke contains the most important flavor and aroma compounds (Wasserman, 1966; Bratzler et al., 1969; Fiddler et al., 1970; Lustre and Issenberg, 1970; Fujimaki et al., 1974; Daun, 1979). Kurko (1959) found that most of the phenols present in smoke had excellent antioxidative properties. Fractions with lower boiling points containing phenol, cresol and guaiacol as main constituent were less antioxidative than fractions of higher boiling point containing mainly eugenol, syringol and their derivatives (Wendorff, 1981). Phenol fractions of smoke also proved to have partial bactericidal and fungicidal effects in meat products (Kurko and Perova, 1961; Kersken, 1973; Baryłko-Pikielna, 1977).

2.2.3. The Application of Liquid Smoke

Hollenbeck (1977) estimated that 65% of the smoked meat produced in the U.S. and Canada is treated with LS. The treatment of meat and meat batters with LS is becoming more common. The methods of application of LS can be divided into four categories. These categories include direct addition, drenching, regenerating (vaporization of LS) and atomizing (Wistreich, 1979; Ruzek,1981; Tóth and Potthast, 1984; Ikins, 1986).

The simplest method of LS application is by direct

addition of smoke flavoring into the food product such as a meat batter, cheese spread and barbecue sauce when surface treatment is either not desired or impossible. In the drenching method, the meat products are either sprayed, showered or dipped with a solution of LS. Spraying or showering is used in continuous wiener lines. The wieners are showered at the beginning of the smoke/cook tunnel with a heated smoke solution. As they are heat processed, the characteristic smoke flavor develops along with the desired cured meat flavor profile.

In vaporization, or smoke regeneration, LS is atomized into a stream of air withdrawn from the smokehouse. The cloud of LS droplets is then heated by a series of electric coils and the liquid is transformed into smoke vapor at approximately 104°C. The smoke vapor is introduced into the smokehouse where it contacts the meat products. The smoke regenerator recirculates smoke from the oven and at each pass the concentration of smoke components becomes greater, until an extremely dense smoke is achieved. The reaction of the smoke constituents with the food product occurs at an accelerated rate, to the point that a 30 min static step is equivalent to an eight hour continuous traditional smoke (Wistreich, 1979). Operating expenses have been the limiting factor to this concept (Ruzek, 1981).

Of the generally accepted methods for applying LS, atomization is most frequently used for imparting smoke flavor and characteristic cured color to meat products.

Liquified smoke is atomized using air pressure into the smokehouses to develop smoke flavor and color. Fine droplets of LS deposit on the meat product surface.

2.2.4. Benefits of Using Liquid Smoke

Use of LS flavoring is expanding in both the United States and other countries. The major reasons for this are that LS can perform all the desired functions of wood smoke, and it has several advantages over conventional wood smoke (Hollenbeck, 1977). Draudt (1963) and Hollenbeck (1977) reviewed the advantages of using LS in food systems. These include: (1) desire for better control of the levels of smoke flavor and characteristic color in the smoked food; (2) ability to incorporate smoke flavor into the body of the product; (3) increased variety of smoked foods that can be produced; (4) lower cost of producing smoked foods due to the reduced amounts of equipment required; (5) environmental factors of decreasing the level of smoke effluent into the environment from the smokehouse; and (6) methodologies for producing LS have decreased or eliminated the compounds that are carcinogenic in nature. These carcinogenic compounds include PAHs that are associated with the crude tar fraction of wood smoke (Daun, 1979; Hollenbeck, 1977; Wistreich, 1979). The aging of LS allows the PAHs to precipitate out of solution with the tars; PAHs in LS can be further removed by filtering LS through cellulose filters (Hollenbeck, 1977).

2.2.5. The Influence of Liquid Smoke on Formation of Toxic Compounds

The addition of sodium nitrite to cured meat can perform several vital functions. The most important function of nitrite is to inhibit the outgrowth of *Clostridium botulinum* in vacuum packaged, smoked meat products (Tompkin et al., 1978). *Clostrium botulinum* is a bacterium that produces a highly potent toxin capable of causing nervous disorder and death. It is generally believed that nitrite does not inhibit the true spore germination process. Rather, the nitric oxides will react with an essential iron-containing compound within the germinated botulinal cell and prevent outgrowth. Thus, the presence of sodium nitrite insures that these products will be free of the deadly toxin produced by this bacteria (Tompkin et al., 1978). Unbuffered LS are very acidic because they contain substantial amounts of acids and phenols. The inclusion of an unbuffered LS in a curing brine can result in low levels of residual nitrite in the cured products (Sleeth et al., 1982). Nitrite can be reduced to nitric oxides in acidic conditions which may then volatilize. The solution to this problem is to buffer the LS, however, solubilizing agents must be included to keep the phenolic compounds in solution because they have higher solubility in acidic conditions (Sleeth et al., 1982).

Liquid smoke may also play a role in contributing to N-nitrosamine formation. The addition of LS to the curing

brine or the atomizing of LS on the surface of cured meat products has the potential to influence N-nitrosamine formation in several ways. The phenols in LS can act as either catalysts or inhibitors on N-nitrosamine formation (Massey, et al., 1978; Theiler, et al., 1984). In addition, the presence of formaldehyde in LS may lead to formation N-nitrosothiazolidine (Ikins, 1986).

Phenolic compounds which can potentially form nitro- and nitroso- derivatives (eg. nitrosophenols) by reaction with nitrite can also act as catalysts on the formation of N-nitrosamines from nitrite and secondary amines (eg. cysteamine from aged meat) (Knowles et al., 1975a; Davies and McWeeny, 1977; Walker et al., 1979, 1982). Nitrophenols themselves show a mutagenic effect (Gilbert et al., 1980). Whether nitro- and nitrosophenols are present in smoked meat products in an amount dangerous to human health is not yet known. Further investigation in this area is needed.

2.3. MUSCLE PROTEINS

Muscle proteins are generally classified as sarcoplasmic, myofibrillar or stromal proteins on the basis of their solubility in aqueous solutions (Goll et al., 1970). The sarcoplasmic proteins account for approximately 30% of the total protein and are soluble in low salt solutions (ionic strength 0.1 or less) at neutral pH (Morrissey, 1987; Lawrie, 1988). The myofibrillar proteins contribute about 60% of the total protein and are soluble in

moderate to high salt solutions (ionic strength range from 0.4 to 1.5)(Goll et al., 1970; Lawrie, 1988). The stroma protein fraction, makes up approximately 10% of muscle proteins, and may be defined as that fraction of the muscle protein which is insoluble in neutral aqueous solvents (Goll et al., 1970; Lawrie, 1988). Of these three general classes, the myofibrillar proteins are generally considered to contribute the most to functionality of processed meat products. The most important functional properties of meat proteins include gelation, solubility, water binding and emulsifying capacity (Kinsella, 1982).

2.3.1. Myofibrillar Proteins

The myofibrillar proteins, termed salt-soluble proteins (SSP), contain myosin and actin as contractile proteins; and tropomyosin, troponin, C-protein, M-protein and F-protein as regulatory proteins. Myosin (~MW 460,000) is the major myofibrillar protein (Goll et al., 1977). It accounts for 50-55% of myofibrillar protein (Lawrie, 1988). The myosin molecule consists of a long rod (fibrous protein) with two globular heads at one end (Fig. 2). Vertebrate skeletal myosin consists of two large polypeptides called heavy chains (~MW 200,000) and four small subunits called light chains (~MW 20,000) (Lowey et al., 1969). The myosin molecule contains regions in its polypeptide chains which are susceptible to mild proteolytic cleavage (Mihályi and Szent-Györgyi, 1953). Trypsin cleaves myosin heavy chain

into two major fragments, heavy meromyosin (HMM) and light meromyosin (LMM). Heavy meromyosin can be further hydrolyzed by trypsin or chymotrypsin. It yields two globular heads (HMM-S1) and a fibrous part (helical structure region) of HMM (HMM-S2). The molecular weight of HMM-S1 and HMM-S2 are 115,000 and 60,000, respectively (Goll, et al., 1977). The two pairs of light chains include the 5,5'-dinitrobis-2-nitrobenzoic acid (DTNB) and the alkali light chains (Morel and Pinset-Härström, 1975). The two DTNB chains are regulatory light chains which affect the calcium binding activity of myosin (Holt and Lowey, 1975). The alkali light chains are essential for ATPase activity (Kominz et al., 1959).

Actin (~MW 42,000) represents 20-25% of the myofibrillar protein (Lawrie, 1988). It can exist in either of two forms, globular (G-actin) and fibrous (F-actin), depending upon the ionic strength of the environment. The G-actin monomer can polymerize to form filaments or F-actin at high ionic strength (> 0.1M) plus Mg and ATP (Morrissey et al., 1987).

Tropomyosin (~MW 67,000) and troponin (~MW 70,000) together account for 10% of the myofibrillar proteins and are major regulatory proteins which impart Ca^{2+} sensitivity to the contractile protein system (Morrissey et al., 1987). Both of these proteins are present in the groove of actin filaments (Morrissey et al., 1987).

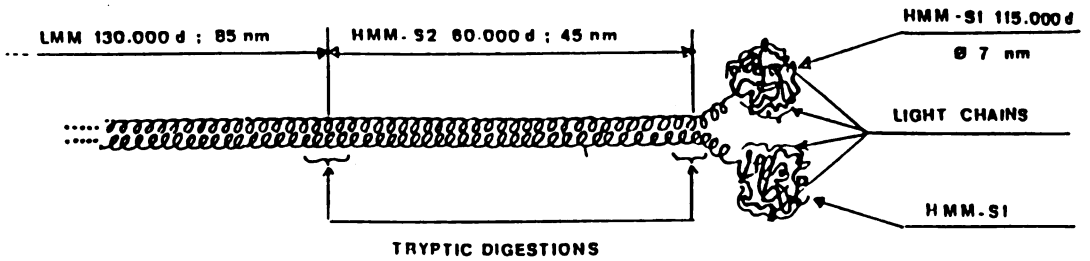


Figure 2. Schematic representation of the myosin molecule. (adapted from Morel and Pinset-Härström, 1975).

2.3.2. Gelation of Proteins

The protein gels formed by heat induced gelation may be defined as three-dimensional matrices or networks in which polymer-polymer and polymer-solvent interactions occur in an ordered manner resulting in the immobilization of large amounts of water by small amounts of protein (Tombs, 1974; Hermansson, 1979; Schmidt, 1981). The mechanism underlying the formation of the gel matrices is not fully understood, however, the mechanism suggested by Ferry (1948) is still the most generally accepted theoretical heat-induced protein gelation mechanism. This two-step mechanism involves partial unfolding of proteins (denaturation) followed by aggregation into a three dimensional, well-ordered network under the appropriate conditions:

$$xPn \xrightarrow{\text{heat}} xPd \xrightarrow{\text{heat and/or cooling}} (Pd)x$$

where x is the number of protein molecules P, with n

denoting the native state and d the denatured state. For the formation of a highly ordered gel matrix, it is imperative that the aggregation step proceed at a slower rate than the unfolding step (Hermansson, 1978, 1979). The term protein denaturation is defined by Kauzmann (1959) as "a process in which the spatial arrangement of the polypeptide chains within the molecule is changed from that typical of the native protein to a more disordered arrangement."

In general, excessive attractive forces result in coagulation, while excessive repulsion forces lead to a solution (Morrissey et al., 1987). In the denaturation and aggregation steps, the attractive forces may include disulfide crosslinks or thiol-disulfide interchange, hydrogen bonding, hydrophobic and electrostatic interactions (Schmidt, 1981; Morrissey et al., 1987). The characteristics of the gel network are affected by this intra- and inter-strand crosslinking. This crosslinking combined with the fluidity of the immobilized solvent give gels their characteristic strength, elasticity and flow behavior. Degree of crosslinking must be optimal. Insufficient crosslinking results in an undesirable gel structure. The general type of protein bonding, their characteristics and the proposed role in gels have been summarized in Table 1.

Table 1. Groups involved in crosslinking of protein gel structures and their properties. [1]

Type	Energy (Kcal/mole)	Interaction Distance	Groups Involved	Role in Gel Matrix
Covalent Bonding	80-90	1-2 A	-S-S-	Bridging; Ordering
Hydrogen Bonding	2-10	2-3 A	-NH···O=C- -OH···O=C-	Bridging; Stabilizing
Hydrophobic and Related Interaction	1-3	3-5 A	Nonspecific	Strand thickening; Strengthening; Stabilizing
Ionic Bonding and Interaction	10-20	2-3 A	$-NH_3^+$, $-COO^-$, etc.	Solvent interaction; Salt linking

1: Adapted from Schmidt (1981).

The gelation mechanism of SSP has been intensively studied through the physico-chemical properties of protein which includes surface charge, sulfhydryl content, hydrophobicity, molecular weight, conformational stability and association/dissociation behavior (Kinsella, 1982; Wilding et al., 1984; Smith, 1988). Solubility is used as an indication of protein denaturation (Hermansson and Åkesson, 1975; Li-Chan et al., 1984). Turbidity is used as an indication of protein aggregation (Deng et al., 1976; Shimada and Matsushita, 1980a, 1980b, 1981; Acton et al., 1982; Liu et al., 1982; Ziegler and Acton, 1984a). The formation of intermolecular disulfide (S-S) bonds by oxidation of sulfhydryl (SH) groups to S-S bonds and/or SH-induced disulfide interchange reactions may be involved in protein aggregation (Stracher, 1964; Jacobson and Henderson,

1973; Ishioroshi et al., 1980; Shimada and Matsushita, 1980a, 1980b, 1981; Schmidt, 1981; Yamagishi et al., 1982; Nakai, 1983; Samejima et al., 1988; Shimada and Cheftel, 1988, 1989). Hydrophobicity, which is related to protein functionality, is measured by fluorescence (Nakai, 1983; Voutsinas et al., 1983a, 1983b; Li-Chan et al., 1984; Wicker et al., 1986). Scanning electron microscopy (SEM) is a valuable tool for evaluating the microstructure of protein gels in meat or model systems at small distances (Ishiroshi et al., 1979; Yasui et al., 1979, 1980, 1982; Carrol and Lee, 1981; Samejima et al., 1981, 1982; Montejano et al., 1984; Schmidt, 1984; Hermansson et al., 1986; Hermansson and Langton, 1988; Gordon and Barbut, 1989, 1990b; Wang, 1990).

2.3.3. Gelation of Muscle Protein

The heat-induced gelation of myofibrillar proteins either singly or in combination, has been extensively studied (Samejima et al., 1969, 1981, 1982, 1985, 1988; Deng et al., 1976; Cheng and Parrish, 1979; Yasui et al., 1979, 1980, 1982; Acton et al., 1982; Liu et al., 1982; Lanier et al., 1982; Ishioroshi et al., 1983; Montejano et al., 1983, 1984; Wu et al., 1985; Foegeding et al., 1986; Hermansson et al., 1986; Morita et al., 1987; Foegeding, 1990) and reviewed (Schmidt et al., 1981; Acton et al., 1983; Ziegler and Acton, 1984b; Asghar et al., 1985; Foegeding, 1988). Many investigators indicated that actomyosin, and in particular myosin, is responsible for development of high

gel strength (Fukazawa et al., 1961a, 1961b; Samejima et al., 1969; Nakayama and Sato, 1971a, 1971b; Yasui et al., 1982). Actin by itself does not exhibit any of the viscoelastic properties of myosin or actomyosin gels (Samejima et al., 1969; Deng et al., 1976; Yasui et al., 1980). However, F-actin and myosin exerts a synergistic effect on gelling properties. The maximum gel strength of the proteins from rabbit skeletal muscle when solubilized in 0.6M KCl at pH 6.0 is obtained when the molar ratio of myosin to actin is 2.7:1, which corresponds to the weight ratio of 15:1 (Ishioroshi et al., 1980; Yasui et al., 1980, 1982). At this ratio, 15-20% of the total protein exists as an actomyosin complex and the remainder is free myosin. This corresponds to a weight ratio of 4:1 as the optimum gelation ratio for myosin and actomyosin . The F-actin acts as a crosslinker with free myosin on heating (Yasui et al., 1982). Tropomyosin and troponin contribute little to the microstructure and gel texture of myosin or actomyosin gels (Nakayama and Sato, 1971b, 1971c; Samejima et al., 1982).

Due to the high gelling potential of myosin, efforts have been made to understand the gelling characteristics of myosin subfragments. The gelling potential of myosin is solely dependent on myosin heavy chain (Samejima et al., 1984). Although light chains contribute little to the gelling power of myosin, they possibly provide some stability to the gel if the pH is above 6.0 (Samejima et al., 1984). Samejima et al. (1981) reported that intact

myosin and myosin rods form firm gels. The globular head portion of myosin heavy chains (HMM-S1) alone form a very weak gel compared to that formed by intact myosin molecules. The HMM-S1 is closely associated with the oxidation of SH groups during aggregation (Samejima et al., 1981, 1984, 1988). The rod or tail portion of myosin and F-actomyosin have demonstrated the potential to form a network through the interaction between their tail portions at high salt concentrations (Samejima et al., 1981; Yasui et al., 1982; Ishioroshi et al., 1983).

Factors which affect heat-induced gelation properties of protein include heating rate, temperature, pH, ionic strength and protein concentration. Each of these factors is related to one another. The rigidity and stability of muscle gels are heating rate dependent within certain temperature ranges (Montejano et al., 1983, 1984; Foegeding et al., 1986). It was suggested that slower heating rates allowed more time for proteins to unfold and interactions to occur thus enabling a stronger gel matrix to form (Saliba et al., 1987).

The heat-induced transitional changes in viscosity and rigidity of myosin and actomyosin can be represented by different reaction stages. These occur in separate temperature ranges and involve distinctly different segments of the myosin molecule (Samejima et al., 1983; Morrissey et al., 1987). These changes can be studied through transition temperature (Tm), which can be obtained using different

techniques. These include shear modulus (SM), differential scanning calorimetry (DSC), thermal scanning rigidity modulus (TSRM), optical rotation (OR) and circular dichroism (CD). The Tm has been used to identify points at which protein conformational changes or shifts occur upon absorption of thermal energy. The differences in Tm of myosin depend upon the pH and salt concentration (Samejima et al., 1983; Wright and Wilding, 1984).

Differences in pH will alter the charges on the myosin molecule and thus affect the balance between electrostatic forces. Once this change has occurred, the protein-protein interaction will be altered and cause changes in Tm (Deng et al., 1976).

Salt concentration is another factor influencing gelation characteristics. Myosin can form two completely different gel structures in the pH range 5.5-6.0, depending upon the ionic strength of the solution (Hermansson et al., 1986). Myosin exists as filaments and forms fine stranded gel structures at lower salt concentrations (< 0.30M). At salt concentrations greater than 0.30M (high ionic strength), myosin is primarily monomeric, soluble at high ionic strength and forms a more coarse gel network when compared to myosin in low ionic strength solutions (Kaminer and Bell, 1966; Ishioroshi et al., 1979; Hermansson et al., 1986). However, Wang (1990) reported that there were no significant differences in dynamic testing of chicken SSP at 0.15M and 0.30M NaCl compared with SSP at 0.60M NaCl. In

addition, the effect of NaCl concentration on the viscoelastic properties of chicken SSP was less than the effect of pH changes (Wang, 1990). Wang (1990) concluded from rheological properties, that chicken SSP prepared at pH 6.5-7.5, 0.6M NaCl and heat-induced gelation at 65°C-70°C exhibited a continuous filamentous matrix, which corresponded with high water-holding capacity.

The muscle type and animal species also influence the rheological properties of heat-induced gelation. White muscle from chicken (Asghar et al., 1984; Morita et al., 1987) and bovine muscle (Fretheim et al., 1986) generally formed superior gels at pH 6.0 when compared to gels from red muscle. Montejano et al. (1984) reported that the final gel strength of minced turkey paste was similar to gel strength of fish surimi and almost twice as large as gels from beef and pork.

2.3.4. Texture Analysis of Meat Protein Gelation

The rheological methods for determining stress-strain conditions at structural breakdown (failure point) of heat-induced protein gels and meat products include: texture profile analysis (TPA) (Bourne, 1978); compression tests (Lee et al., 1987) and torsion failure tests (Montejano et al., 1983).

Texture profile analysis is used as a measurement of texture properties of food with an Instron testing machine. Seven textural parameters are generated from each piece of

food of known dimension which have been compressed twice. These parameters include: hardness, fracturability, cohesiveness, adhesiveness, springiness, gumminess and chewiness. The definition of each of these parameters are discussed by Bourne (1978).

Uniaxial (axial) compression of specimens of known size and shape is the most common test used that yields stress and strain at failure (Hamann, 1983). It utilizes a compression cell which is composed of parallel plates between which protein gels (cylinder in shape) are compressed. Apparent stress and apparent strain at failure can be calculated (Diehl and Hamann, 1979; Hamann, 1983).

The torsion test is a two step procedure. The first step is to shape protein gels or other processed meat products into a dumbbell shape with a minimum diameter of 1 cm at the midsection. The next step is twisting the dumbbell shaped specimen using an Instron universal testing machine or digital viscometer. Pure shear stress and strain can be calculated from the measurements taken from each sample (Montejano et al., 1983; Amato et al., 1989). Specimens are tested at room temperature. Several researchers (Montejano et al., 1983, 1984; Hamann, 1987; Hamann et al., 1987; Saliba et al., 1987; Amato et al., 1989; Foegeding, 1990) have found that the torsion test has worked well for processed meat products, including frankfurters as well as protein gels. The torsion test offers several advantages: (1) no appreciable change in

volume occurs, (2) it produces failure even in highly deformable materials, and (3) tension, compression, and shear stress of equal magnitude are created; therefore, the material will fail due to the stress for which it has the least strength (Montejano et al., 1983; Hamann et al., 1987).

The term shear stress at failure is highly correlated with TPA hardness or firmness (Voisey et al., 1975; Montejano et al., 1985; Lee et al., 1987). Hardness is defined in objective terms as the force required to attain a given deformation (Szczesniak, 1963). For moderately deformable muscle gels, shear stress at failure calculated from rupture forces like those of Voisey et al. (1975) agree with shear stress calculated from torsion testing (Montejano et al., 1984).

The shear strain at failure is related to elasticity (springiness) and cohesiveness (Montejano et al., 1985; Hamann et al., 1987; Hamann, 1988). The elasticity is defined as the rate at which a deformed material goes back to its unreformed condition after the deforming force is removed (Szczesniak, 1963). Hamann (1988) reported that for a frankfurter rupturing at shear strain below 1.5, either axial compression or torsion testing can be used and the results would correlate about equally well with sensory texture. However, specimens may change shape in axial compression tests and account for variability of results (Christianson et al., 1985).

Hamann et al. (1987) and Hamann (1988) reported that failure stress and strain can be considered as a primary indicator of processed meat product structure. The failure stress is strongly influenced by protein concentration or changes in process schedule (Wu et al., 1985; Lee et al., 1987; Saliba et al., 1987; Hamann, 1988; Amato et al., 1989; Foegeding, 1990). Strain in a model product is a good measure of cohesive structure of comminuted muscle (Hamann et al., 1987).

2.3.5. Water-Binding Properties of Meat Proteins

The ability of proteins to bind or entrap water is responsible for many of their desirable functional properties. The water-binding property of food is commonly referred to as "water-holding capacity" (WHC). The WHC can be further described by three terms: (1) water-binding potential, (2) expressible moisture, and (3) free drip (Jauregui et al., 1981). Water-binding potential (WBP) and expressible moisture (EM) both refer to the ability of a protein system to hold water with applied external force. Free drip is the amount of liquid lost by a protein system without application of external force other than gravity.

The factors that influence water binding by protein include amino acid composition (type of protein), protein conformation, pH, ionic species and concentration, presence of phosphates, and product final cooked internal temperature. It is generally accepted that only myosin,

actin, and to some extent tropomyosin, are responsible for the WHC of meat (Nakayama and Sato, 1971c). Gels with a fine network structure may have better water-binding properties than gels with a coarser network structure (Wang, 1990). Actomyosin has minimum WHC at isoelectric pH (pI, pH around 5), and has a higher WHC at pH values above or below the pI of meat. The increase in WHC on the addition of salt is regarded as an effect of the binding of anions (for example, chloride ions) to the structure-forming meat proteins, thus increasing the net negative charge and repulsive forces. This means that more water can be imbibed by the protein network (Hermansson and Åkesson, 1975; Asghar et al., 1985). Salt is also used to extract myofibrillar proteins which contribute to binding water, fat and/or non-meat components (Barbut, 1988). Both NaCl and KCl generally exhibit a stabilizing effect on meat batters; while $MgCl_2$, $ZnCl_2$ and $CaCl_2$ (divalent cations) have a reverse effect (Weinberg et al., 1984; Asghar et al., 1985; Barbut and Mittal, 1988; Gordon and Barbut, 1989, 1990b). The maximum added salt concentration in raw meat systems is generally about 0.68M (4%) NaCl to provide a maximum in water-binding effect in final products (Hermansson and Åkesson, 1975; Acton et al., 1982; Offer and Trinick, 1983).

Alkali polyphosphates have a synergistic effect with NaCl (0.15M to 0.43M) and are very effective in increasing the WHC and emulsion stability of meat products (Siegel et al., 1978; Asghar et al., 1985; Trout and Schmidt, 1986;

Barbut, 1988). The extent of the synergistic effect decreases linearly as the chain length of the phosphate increases. The possible mechanisms for the effect of phosphates on meat systems includes higher pH and ionic strength, interaction with specific proteins including dissociation of actomyosin by pyrophosphate, and chelation of cations (Asghar et al., 1985; Whitting, 1988).

Heating temperature also influences WHC. Thomsen and Zeuthen (1988) reported that final internal temperatures from 50° to 70°C increased the WHC of a pork sausage model system. Heating above the gelation temperature (70°C) often results in a decrease of WHC in protein gels (Hermansson, 1986).

2.4. THE EFFECTS OF LIQUID SMOKE CONSTITUENTS (ACIDS, CARBONYLS AND PHENOLS) ON PROTEIN FUNCTIONALITY

The smoking process will cause changes in meat proteins as well as palatability and aroma (Krylova et al., 1962). Randall and Bratzler (1970a, 1970b) found that the smoking process causes changes in protein properties of pork muscle. They observed a decrease in protein solubility, pH, free sulfhydryl groups and myofibrillar protein nitrogen of cooked and smoked meat products. Changes in protein electrophoretic patterns were also observed. Sink and Hsu (1977, 1979) reported that various smoke processing systems would affect the palatability characteristics of meat products, especially flavor, tenderness, juiciness, and

overall acceptability. The types of smoke processing systems used for frankfurters included wood generated aerosol, LS generated aerosol, LS dip and LS mix or incorporated. They found that LS incorporated into the meat emulsion had significantly lower juiciness, tenderness and shear force value when compared to other types of smoking processes (Sink, 1979; Sink and Hsu, 1979). These results indicated that incorporating LS with the meat emulsion distributes smoke compounds evenly, and may cause extensive chemical reactions between smoke components and the proteins. Daun (1981) reported that phenolic fractions reacted with sulfhydryl groups of the protein. Carbonyls and acids also reacted with amino groups. The possible reactions of LS components with meat proteins will be discussed below.

2.4.1. Effect of Acids in Smoke on Protein Functionality

As discussed previously (see 2.2.2.1.), LS has a pH range of 2 to 4, with 5-10% acidity. At this high acidity, the manufacture of meat products using various smoke processing methods (LS used as aerosol, dipping or mixing) would cause significant change in pH and shear force of finished products (Sink and Hsu, 1977). Frankfurters manufactured using LS application by dipping or mixing into the emulsion resulted in significantly lower pH values when compared to the controls (no smoke). Frankfurters processed by dipping also had significantly higher shear force values

when compared with the control. These observations indicated that the acid in LS may cause a decrease in pH of processed meat products and changes in protein conformation. At a pH near the pI of the protein, the protein-protein interaction is stronger than the protein-solvent interaction, thus resulting in protein coagulation, decreases in protein solubility and loss of functional properties (Morrissey et al., 1987). This agrees with observations made by Wang and Smith (1992) that solubility of chicken SSP in 0.6M NaCl were 98, 13 and 2% at pH 6.5, 5.5 and 4.5, respectively.

2.4.2. Possible Reactions Between Protein and Carbonyls from Smoke

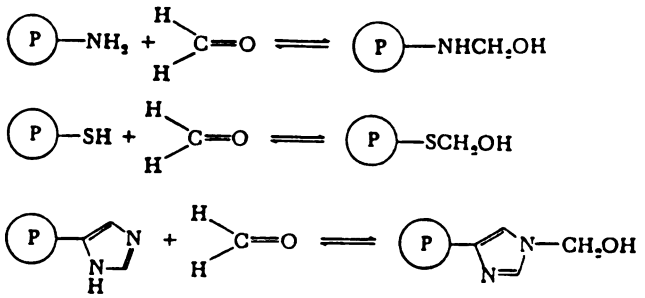
Reaction of aldehydes with free amino groups are well known. The carbonyl-amino types of reaction include: (1) reactions between carbonyls and the free amino acids; (2) the binding of the free amino acids of the protein with carbonyls groups from reducing sugars and carbonyls from fat oxidation (Carpenter et al., 1962); and (3) reactions between amino acids and carbonyl groups in smoke components and/or flavor components (Krylova et al., 1962; Dvořàk and Vognarovà, 1965; Ruiter, 1970, 1979; Chen and Issenberg, 1972; Hollenbeck, 1977; Damodaran and Kinsella, 1980).

The interactions of smoke components with ϵ-amino groups in proteins have been studied (Dvoŕàk and Vognarovà, 1965; Chen and Issenberg, 1972). Dvoŕàk and Vognarovà

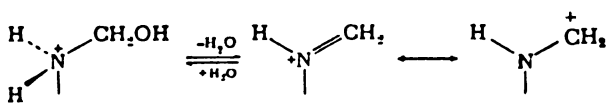
(1965) reported that loss of available lysine in meat treated with wood smoke was attributed to reactions of formaldehyde with the ϵ-amino group of lysine. Ruiter (1970) suggested that glycolaldehyde and pyruvaldehyde can react with meat protein. Chen and Issenberg (1972) studied the interaction of different smoke fractions with ϵ-amino groups in proteins. Beef homogenate treated with acid, neutral or phenolic fractions of smoke condensate, lost 14, 45 and 38% of the available lysine, respectively. They also observed that sinapaldehyde and coniferaldehyde cause the reduction of available ϵ-amino groups. However, no significant interaction was detected when bovine serum albumin (BSA) was treated with phenol, cyclotene, eugenol, or syringol (components from the phenolic fraction).

The interaction of flavor carbonyls with BSA and soy protein has also been reported by Damodaran and Kinsella (1980, 1981a, 1981b). They found that the affinity of ketones for BSA (0.6% solution) increased with chain length and the binding of α-nonanone induced conformational changes in BSA. However, at higher protein concentrations and with different types of protein, the binding affinities may be different.

The reaction between carbonyls and amino acids has been illustrated by Means and Feeney (1971). The most reactive component in the carbonyl fraction, formaldehyde, will react with amino groups or thiol groups in proteins as below:



The results from the reaction of formaldehyde with tyrosine, tryptophan, histidine, asparagine and cysteine are shown in Fig. 3, compounds I through V, respectively (French and Edsall, 1945; Means and Feeney, 1971). The formation of these compounds are presumed to occur by the formation of electrophilic immonium cations (see below), which react with the adjacent amino acid side chains.



Similar reactions also produce protein linking the ε-amino groups of lysine residues via methylene bridges to neighboring side chains (Fraenkel-Conrat et al., 1947; Fraenkel-Conrat and Mecham, 1949; Means and Feeney, 1971). For example, the formation of the methylene bridged lysine-tyrosine compound VI (Fig. 3).

One can postulate that once the reactions between carbonyls and amino acids occur, the molecular weight of the protein would increase and crosslinking between amino acids will occur. This will cause conformational changes of the

proteins. However, the exact mechanisms of the reactions of carbonyls with protein and the subsequent conformational changes are still not known.

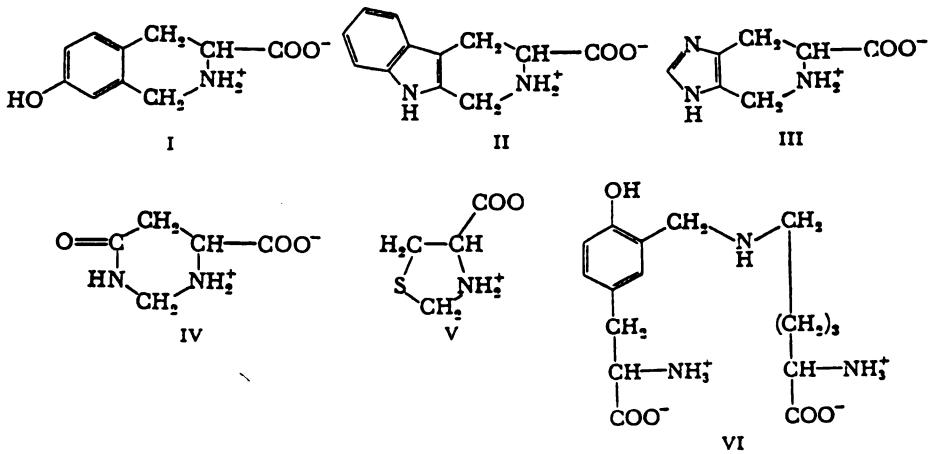


Figure 3. Compounds formed by the reaction of formaldehyde with specific amino acids (I=tyrosine, II=tryptophan, III=histidine, IV=asparagine, V=cysteine and VI=lysine-tyrosine; adapted from Means and Feeney, 1971).

2.4.3. Possible Reactions Between Protein and Phenols from Smoke

The existence of strong interaction between proteins and phenols has long been recognized. The major phenolic constituents of plants can be divided broadly into two main groups: phenolic acids including cumarins, and flavonoids, including anthocyanidins (Salunkhe et al., 1990b). According to Loomis (1974), four types of linkages may be involved in the formation of the protein-phenol complex. They are hydrogen bonding, hydrophobic interaction, electrostatic attraction, and covalent coupling associated

with oxidation.

The hydrogen bonds formed between phenolic hydroxyl groups and the peptide carboxyl groups is relatively strong. Binding decreases at high pH due to the ionization of hydroxyl groups (Loomis and Battaile, 1966). Hydrophobic interaction may occur between the aromatic ring structure (hydrophobic sites) of the phenolics and the hydrophobic regions of the protein (Loomis, 1974; Oh et al., 1980). The electrostatic attraction or salt linkage may occur between basic amino acid residues of proteins and phenolic hydroxyl groups at high pH due to the high pK value of phenolic hydroxyl (Loomis, 1974). Permanent covalent linkage may occur between phenols and proteins when the oxidation of phenols occurs. The sulfhydryl groups in protein are particularly reactive; free amino and imino groups also react with hydroxyl groups of phenols (Loomis and Battaile, 1966; Pierpoint, 1969a, 1969b; Van Sumere et al., 1975). Rapid crosslinking of protein often results (Loomis and Battaile, 1966).

The phenolic fraction of LS, which originates from pyrolysis of plant lignin, probably will have similar interactions with meat proteins as plant phenolic compounds. They interact with enzymes (Goldstein and Swain, 1965; Loomis and Battaile, 1966; Loomis, 1969, 1974; Haslem, 1974), milk protein (Brown and Wright, 1963), gelatin from calfskin (Oh et al., 1980), and other proteins and/or amino acid solutions (Hagerman and Butler, 1981; Watanabe et al.,

1981a, 1981b; Ozawa et al., 1987). The principle linkages involved in the formation of tannin-protein complexes include hydrogen bonds and hydrophobic interactions (Salunkhe, 1990a). Swain (1965) stated that a tannin should have a high molecular weight (between 500 and 3000 MW) and a sufficiently large number of phenolic hydroxyl or other suitable groups to enable it to form effective crosslinks between proteins and/or other macromolecules. Swain (1965) further stated that low molecular weight phenolic compounds may be too small to form effective crosslinks. The possible interaction of the phenolic fraction in LS which contains low molecular weight compounds with meat proteins may exhibit a different pattern of crosslinking.

Pusztai (1966) indicated that BSA and cytochrome C had a solubility preference in a phenolic rich solution instead of water in a two phase system. Several reports suggest that phenols in smoke bind with meat proteins primary through the interaction of phenols with the thiol groups of the proteins (Burton and Stoves, 1950; Krylova et al., 1962; Kakō, 1968; Randall and Bratzler, 1970a; Daun, 1981). Krylova et al. (1962) studied the interaction of smoke components with amino and SH-groups of proteins using free amino acids and meat protein. Their results indicated that a sharp decrease of SH-groups occurred in cysteine and glutathione solutions after smoke treatment. In meat, 40% of the SH-groups are lost after smoking. The phenol fraction caused about a 30% decrease in SH-groups of

cysteine, glutathione and fresh beef solutions. Krylova et al. (1962) studied in further detail the reaction of SH-groups with the phenolic fraction. The phenol fractions with a higher boiling point, containing methyl ethers of pyrogallol and its derivatives, react better with SH-groups than the low boiling fraction containing mainly phenol, guaiacol, m-cresol, methyl-guaiacol. Randall and Bratzler (1970a) observed that a heated, smoking process causes a significant reduction of free SH-groups of pork *longissimus dorsi* muscle. The myofibrillar proteins were especially affected.

The use of LS in food has become popular. Many of the components in LS have been identified and specific reactions of these components have been reviewed. However, few studies have measured the effects of LS on meat protein functionality. The mechanisms of interaction between polyphenols and carbonyls in LS and meat proteins are poorly understood and warrant further investigation.

MATERIALS AND METHODS

3.1 EXTRACTION OF CHICKEN SALT-SOLUBLE PROTEINS

Chicken salt-soluble proteins (SSP) were isolated by the procedure described by Wang (1990). Boneless, skinless chicken breasts were purchased from a local retail store. Visible fat and connective tissue were removed prior to grinding. The meat was ground twice through a Hobart Kitchen Aid food grinder (model KF-A, Troy, OH) using a 4 mm plate. All solutions used for extracting SSP were adjusted to pH 6.5 except when otherwise indicated. The extraction process was completed using two low salt buffered extractions (0.1M NaCl, 0.05M Na-phosphate buffer), followed by one high salt buffer (0.6M NaCl, 0.05M Na-phosphate buffer; buffered system) or high salt solution (0.6M NaCl; non-buffered system) extraction. All extraction procedures were completed at temperatures between 2° to 4°C. For the low salt extraction, the ground meat was blended with four volumes of the low salt buffer in a Waring Blender (model 1120, Winsted, CT) for 90 sec, and stirred with a motorized propeller at moderate speed (avoiding foaming) for 1 hr. The solution was centrifuged for 15 min at 5,860 x g. The supernatant was decanted and discarded. The pellet was resuspended in four volumes of the low salt buffer, stirred,

centrifuged and the supernatant discarded. The pellet obtained after low-salt extraction was dissolved in a 2.4M NaCl buffer or solution until an approximate final 0.6M NaCl concentration was reached. About three volumes of the high salt buffer or solution were added and stirred for 1 hr. After stirring, the suspension was centrifuged at 20,000 x g for 30 min to remove connective tissue (stroma protein) and denatured myofibrillar protein. The pellet was discarded. Five volumes of cold (2°-4°C) deionized distilled water were added to the supernatant to precipitate the SSP. The solution was centrifuged at 20,000 x g for 30 min. Pellets were combined and centrifuged to concentrate them. The final pellet was solubilized with a one-third volume of 2.4M NaCl buffer or solution until a final salt concentration of 0.6M was reached. The pH of the SSP solution was adjusted to 6.5 with 0.1M HCl or 0.1M NaOH. The SSP solution was held in a cooler (2°-4°C) overnight, and protein concentration was determined using micro-Kjeldahl (AOAC, 1984). Salt-soluble proteins were diluted to 4% (40mg/ml) with a 0.6M NaCl buffer or solution and designated as total protein fractions. The treatments included three brands of commercial LS (F, G and H) at three concentrations (0.125%, 0.25% and 0.50% w/w or 31.2, 72.1 and 125 mg LS/g protein, respectively). Liquid smoke fractions (phenols and carbonyls from F or G) were added to the 4% SSP based on phenol and carbonyl content at the 0.5% w/w LS level, or 0.05% w/w smoke fraction addition were also studied. All LS

fractions contained 10% Tween-20 (polyoxyethylenesorbitan or monolaurate) as an emulsifier. Each commercial LS was designed for addition into food products. The treatments in the buffered system were adjusted to pH 6.5 after the addition of LS or smoke fractions. No pH adjustment was made for treatments in the non-buffered system. All samples were placed in a cooler (2°-4°C) overnight to allow reaction of SSP and LS before further testing.

3.2. CHARACTERIZATION OF SALT-SOLUBLE PROTEINS AFTER LIQUID SMOKE ADDITION

3.2.1. Solubility

Solubility was determined as described by Morr et al. (1985) with modifications. About 20 g of various LS treated SSP (40mg/ml) were diluted to 10mg/ml using a 0.6M NaCl buffer or solution. The diluted samples were stirred using a magnetic stir bar for 20 min in a cold room and allowed to stand overnight. The following day, samples were stirred (2°C) once prior to centrifuging at 20,000 x g for 30 min at 2°C. The protein concentration of the supernatant was determined by micro-Kjeldahl and designated as soluble protein fractions. The protein concentration of the control (SSP without LS addition, 10mg/ml) was also measured as total protein before centrifugation. Percent solubility was determined by dividing the supernatant protein concentration by the total protein concentration and multiplying by 100. Three values of solubility was determined for each observation.

3.2.2. Electrophoresis

Total protein (40mg/ml) and soluble protein (10mg/ml) fractions of LS treated SSP treatments were characterized by Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), described by Laemmli (1970) with modifications. Samples were diluted to 2mg/ml using a sample buffer (pH 6.8 0.0625M Tris buffer, 2% SDS, 10% glycerol, 5% β-mercaptoethanol and 0.2% bromophenol blue) and mixed well using a stir bar before heating in boiling water for 10 min.

A tris-glycine electrode buffer system (0.025M Tris, 0.192 glycine and 0.1% SDS, pH 8.3), described by Laemmli (1970), was used in a SDS-PAGE SE 600 series vertical slab unit (Hoefer Scientific Instrument, San Francisco, CA). The acrylamide stock solution was prepared by dissolving 30% acrylamide and 0.8% N'-N methylenebisacrylamide. Twelve percent acrylamide in a Tris-SDS solution (0.375M Tris buffer, 0.1% SDS, pH 8.8) and 4% acrylamide in Tris-SDS solution (0.125M Tris buffer, 0.1% SDS, pH 6.8) were prepared for resolving gels and stacking gels, respectively. Twenty micrograms of molecular weight standard (MW-SDS-200 kit, SDS-6H, Sigma Chemical Co., St. Louis, MO) and 42μg of protein solution were applied into sample wells using a Hamilton Syringe.

Constant currents of 25mA and 45mA were applied using a Bio-Rad power supply (Model 1000/500, Richmond, CA) for protein migration into the stacking gel and resolving gel, respectively. The gels were stained with 0.25% coomassie

brilliant blue in acetic acid-methanol-water (9:45:45, v/v/v) overnight. Destaining was first accomplished with acetic acid-methanol-water in a ratio of 1:5:4 for one hour, and followed by several changes of destaining solution (6:4:7 acetic acid-methanol-water) until the gel background was clear. The gels were preserved in 7.5% acetic acid solution. The molecular weight of the respective protein bands was determined by comparing relative mobility to a standard curve, obtained from plotting mobility distance vs log molecular weight of the molecular weight standard.

3.3. GEL PREPARATION

Four percent (40mg/ml) SSP gels were prepared as described by Beuschel (1990). About 8 g of each 4% SSP solution were carefully pipetted using a 60 cc plastic syringe (Becton Dickinson & Co., Rutherford, NJ) into glass tubes (10 x 130mm) stoppered at one end and covered with plastic caps. The air bubbles were removed by centrifugation (250 x g for 5 min) before heating in a water bath (~73°C) to an internal temperature of 70°C. The gels were held at an internal temperature of 70°C for 15 min. Sample tubes were put into ice water immediately after the desired temperature and time were reached. The temperature was monitored in a blank tube (containing 4% SSP) using a thermocouple. After the protein gels were cooled, yield and expressible moisture was measured. The remaining gels were stored at 2° to 4°C overnight until further analyses.

3.4. GEL EVALUATION

3.4.1. Yield

Percent yield of protein gels was determined by dividing the weight of drained gels by the weight of uncooked SSP and multiplying by 100. Two protein gels were evaluated in duplicate.

3.4.2. Expressible Moisture

A low speed centrifugation method described by Jauregui et al. (1981) was modified to measure the expressible moisture (EM). Three pieces of Whatman #2 filter paper, 9 cm in diameter, were folded into a cone shape and placed in a 50 ml polycarbonate centrifuge tube. One piece of 4 mm^2 polyethylene square mesh (230 microns, Spectrum Medical Industries, Inc., Los Angeles, CA) was placed inside the folded filter paper to prevent sticking. A gel (1.5±0.3 g) was placed inside the filter paper cone and centrifuged at 755 x g for 5 min at 2°C. The weight gain of the filter papers was recorded. Percentage EM of the SSP gels was calculated by dividing weight gain of the filter paper by original weight of the gel sample and multiplying by 100. Four values of EM were obtained for each observation.

3.4.3. Apparent Stress and Strain at Failure

Apparent stress and apparent strain at failure of the SSP gels were evaluated using an Instron Universal Testing Machine (Model 4202, Canton, MA). A crosshead speed of

50mm/min was used with a 50N compression cell. A gel was cut into four cylinder shaped pieces (10mm x 10mm). The gel cores were uniaxial compressed to 80% of original height by two flat plates. Measurements of protein gel texture were completed at 2°-4°C. The equations for calculated strain (ϵ), apparent strain (ϵ_{app}) and apparent stress (σ_{app}, KPa) were described by Hamann (1983) in equation 1, 2 and 3, respectively. Strain was related to the distance the crosshead travelled at structural failure. Stress was the force that the crosshead achieved before sample fractured. If the gel did not fail during an 80% compression, apparent stress and strain were recorded at that point. Twelve to sixteen gel cores were averaged per determination.

Strain was calculated as:

$$\epsilon = \Delta L / L_o \qquad (1)$$

where ΔL = Original length minus length of core at failure (mm)
L_o = Original length (mm)

Apparent strain was calculated as:

$$\epsilon_{app} = -\ln (1-\epsilon) \qquad (2)$$

Apparent stress was calculated as:

$$\sigma_{app} = F / \pi R^2 (1+\nu\epsilon)^2 (1000) \qquad (3)$$

Where F = Force at failure from chart (N)
π = 3.14159
R = Radius of core (m)
ν = Poisson's ratio (0.48)
ϵ = Strain

3.4.4. Scanning Electron Microscopy

Microstructure of protein gels from the control, three brands of LS (0.5%, w/w), and two types of LS (F and G) fractions (carbonyls and phenols) were evaluated by scanning electron microscopy (SEM). The chemical fixation procedure used was the modified osmium tetroxide-tannic acid-uranyl acetate (OTU) method described by Woodward and Cotterill (1985). The gels were cut into about $3mm^2$, placed into 4 ml vials and fixed with 2% glutaraldehyde in a 0.1M phosphate buffer (pH 6.5) overnight. After chemical fixation, the specimens were rinsed 3 times for 15 min in deionized water (H_2O). The specimens were post-fixed in a 1% osmium tetroxide (OsO_4) in H_2O for 2 hr and rinsed with H_2O. They were placed in a 1% tannic acid buffer (pH 6.0) for 1 hr and washed with H_2O. The final fixation was performed in 0.5% uranyl acetate for 1 hr. The specimens were rinsed with H_2O, stored in H_2O overnight and rinsed with three changes of H_2O (15 min each) the following day before dehydration. All fixation steps were completed at room temperature. Dehydration of specimens was performed in a graded ethanol series of 25, 50, 75, 95 and 100% for 15 min per step and stored in 100% ethanol overnight. Three additional changes of 100% ethanol (15 min each) were done before liquid carbon dioxide critical point drying (Balazer Fl 9496 Critical Point Dryer, Balzer's Union, Furstentum, Liechtenstein, West Germany). Dried specimens were mounted on aluminum stubs (Electron Microscopy Sciences, Ft. Washington, PA) with

conductive colloidal graphite (Ladd 60780, Burlington, VT), and coated with a thin layer of gold in a Emscope Sputter Coater (Model SC 500, Kent, England). All coated specimens were then examined at 2,000 X and 6,000 X magnification with a JEOL Scanning Microscope (Model JSM-35CF, Osaka, Japan) at an accelerating voltage of 10 KV with a working distance of 15 mm and condenser lens setting of 600.

3.5. CHARACTERIZATION OF SALT-SOLUBLE PROTEINS AND SALT-SOLUBLE PROTEIN GELS AFTER TREATING WITH LIQUID SMOKE FRACTIONS

3.5.1. Fractionation of Liquid Smoke

The neutral (carbonyl), phenol and acid fractions were prepared from LS flavorings F and G by fractionation based on acidity (Braus et al., 1952; Lustre and Issenberg, 1969). A 40 ml volume of LS was diluted to a volume of 400 ml and brought to pH 12 using 40% NaOH. The solution was extracted consecutively with two 800 ml and one 400 ml volumes of anhydrous diethyl ether. The ether phase contained the neutral fraction. The alkaline aqueous phase was neutralized using dry ice (solid CO_2) to pH 6.8 for phenol extraction. The extraction process was completed using two 800 ml and one 400 ml volumes of diethyl ether. The ether layer contained the phenolic fraction and free acids remained in the aqueous phase. The carbonyls and phenols were dried with anhydrous sodium sulfate and concentrated in a rotavapor rotary vacuum evaporator (Model B-465, Brinkmann Instruments, Inc., Westbury, NY) at 20°C to a volume of

approximately 5 ml. Fifteen milliliter of 100% ethanol was used to rinse the fraction from the round bottom flask into a 20 ml vial. The fractions (in ethanol) were further concentrated to about 5 ml by careful evaporation of the solvent in a stream of nitrogen. They were stored in the dark at 4°C, and were used within one week after extraction.

The terms "neutral fraction," "phenolic fraction" and "acid fraction" describe the general nature of these fractions. The procedures described are a crude separation. Many components are expected to be present in each fraction (Lustre and Issenberg, 1969).

3.5.2. Measurement of The Concentration of Liquid Smoke Fractions

3.5.2.1. Acid determination

Total acidity (as acetic acid) in LS was measured by titrating 5 ml of diluted LS (LS:H_2O = 1:2) to an endpoint at pH 7.0 with 0.1N NaOH. The percentage of total acid was calculated as follows:

$$\text{Percentage Acid (as acetic acid)} = LNMD(100)/S \qquad (4)$$

Where L = NaOH titrated (liter)
N = Normality of NaOH (0.1N)
M = Molecular weight of acetic acid (60)
D = Dilution factor (3)
S = Liquid smoke (ml)

3.5.2.2. Carbonyl determination

Carbonyls in LS and LS fractions were measured by a colorimetric (2,4-dinitrophenylhydrazine) method (Iddles and Jackson, 1934; Lappin and Clark, 1951). One liter of

carbonyl-free methanol was prepared by adding 10 g of 2,4-dinitrophenylhydrazine (DNP, Sigma Chemical Co., St. Louis, MO) and a few drops of concentrated HCl to 1 liter of methanol. The mixture was refluxed 2 to 3 hrs and then distilled. The methanol remains suitable for several months if kept tightly stoppered. All methanol used in measurements was carbonyl-free, and all solutions were stored at 4°C. A saturated DNP solution was prepared using methanol and used within 1 week. A 10% KOH solution was prepared by dissolving 10 g of KOH in 20 ml H_2O and making to 100 ml with methanol. Methyl ethyl ketone (2-Butanone, Mallinckrodt Inc., Paris, KY) in appropriate dilutions was used to prepare a standard curve. The LS or LS fractions were diluted to a suitable concentration using water (usually to less than 10^{-3} molar carbonyls). One milliliter of the above solution was diluted to a 10 ml solution using methanol and used for the carbonyl measurement.

Color development was obtained by combining 1 ml of diluted sample or standard solution or carbonyl-free methanol (as blank), 1 ml of the DNP reagent, and 50 µl concentrated HCl in a 25 ml volumetric flask. The flasks were stoppered and heated in a water bath at 50°C for 30 min. After cooling (ca. 5 min) at room temperature, 5 ml of KOH solution was added and the volume made up to 25 ml with methanol. A wine red solution color developed.

The optical density of each solution was determined using a Lambda 4B UV/VIS Spectrophotometer (Model C688-0003,

Perkin-Elmer Corp., Norwark, CT) with a Lamda 4B operating software spectral processing (C688-011) at 440 nm. Each analysis was performed in duplicate.

3.5.2.3. Phenol determination

The indophenol method of Gibbs (1927) and Tucker (1942) was used to estimate total phenols in LS and LS fractions with modifications. Guaiacol or 2,6-dimethoxyphenol (99%, F.W. 154.17, Aldrich, Milwaukee, WI) was used as a standard for total phenols in a concentration range of 0 mg to 10 mg per 100 ml H_2O. The color reagent stock solution was prepared by dissolving 0.25 g of 2,6-dichloroquinone-4-chlorimide (Gibb's reagent, Sigma Chemical Co., St. Louis, MO) in 30 ml absolute ethanol and refrigerated. A boric acid-potassium buffer (pH 8.3) was prepared by combining 0.4M boric acid, 0.4M KCl, 0.2M NaOH and H_2O in a ratio of 25:25:8:142.

To 5 ml of diluted LS, LS fractions (diluted to an appropriate concentration), H_2O (as blank) or 2,6-dimethoxyphenol solution, 5 ml of the pH 8.3 buffer was added. One milliliter of 0.6-0.7% NaOH was added to adjust the pH of the solution to 9.8. Color was developed by adding 1 ml of diluted color reagent (1 ml of color reagent stock solution diluted to 15 ml with H_2O) and incubating at room temperature for 25 min. The optical density of each solution was determined at a wavelength of 580 nm. Each sample was analyzed in quadruplicate.

3.6. FRANKFURTER PREPARATION

Frankfurters were prepared using mechanically deboned chicken (MDC) meat (14.6% protein, 14.2% fat, 66.5% moisture; Nottawa Gardens Corps, Athens, MI), water, salt with or without LS. The formulation was designed to contain 74.0% moisture, 10.5% protein, 10.1% fat, 2.0% salt with/without 0.5% LS. Two preparations of commercial LS flavoring were used.

Finely ground MDC was thawed 2 days in a 2°C cooler. The meat, salt, ice with or without LS were chopped in a Hobart bowl chopper (Model 84181 D, Troy, OH) for 5 min at 2°C. The final temperature of batter was approximately 1°C. The batter was stuffed into 24 mm diameter cellulose casings (Viskase, Chicago, IL) using a continuous vacuum stuffer (Model Robot 500 Type 128, Robert Reiser & Co., Inc., Canton, MA). The frankfurters were left unlinked, weighed, hung vertically and held at 2°C until cooking. Frankfurters were processed in a forced-air, microprocessor-controlled smokehouse according to the schedule in Table 2 to a maximum internal temperature of 72°C. The frankfurters were showered for 8 min to a final internal temperature (ca. 21°C) after cooking. The frankfurters were held for 15 min at ambient temperature and weighed before the casings were removed. The products were sealed in polyethylene bags and stored at 2°C until subsequent analysis. Samples used for the torsion test were held at room temperature overnight and analyzed the following day. The experiment was replicated 3

times.

Table 2. Cooking cycle for frankfurters

Cycle	Time	Wet Bulb °C	Dry Bulb °C
1	25 min	71	44
2	21 min	88	60
3	15 min	71	63
4	3 min	38	24
5	8 min	Shower	

3.7. FRANKFURTER EVALUATION

3.7.1. Cooked Yield

Cooked yield was determined by dividing the weight of the cooked frankfurter by the weight of the uncooked batter and multiplying by 100. The percent cooked yield was the average of 4 to 5 determinations.

3.7.2. Severe Reheat Yield

Severe reheat yield was measured using the modified method described by Smith and Brekke (1985a). A 24±0.5 g frankfurter portion was placed in 100 ml boiling distilled water, heated at 95°C for 10 min, removed and cooled at room temperature for 5 min before reweighing. The percent severe reheat yield was calculated by dividing the final weight by the initial weight and multiplying by 100. Each analysis was performed in triplicate.

3.7.3. Torsion Test

The torsion failure test was performed at room temperature using the method described by Montejano et al. (1983) and Wu et al. (1985). Frankfurters with approximately 19 mm diameters were cut into 28.7 mm lengths with a guillotine jig. Whatman filter papers were used to absorb small amounts of moisture and fat at the two ends of the sample for ease of gluing. Plastic disks, designed to fit the torsion apparatus, were glued onto the two ends of the samples using cyanoacrylate glue (Borden Inc., HPPG, Columbus, OH). The cylindrical frankfurter was shaped into torsion specimen geometry (dumbbell shape) by carefully rotating the specimen into a rotating cutting wheel (Specimen Milling Machine, Model 91, Gel Consultants Inc., Raleigh, NC) until the diameter was 10 mm at the midsection. Twisting was accomplished by placing in a torsion assembly (Gel Consultants Inc., Raleigh, NC) attached to a Brookfield digital viscometer model 5XHBTD DV-1 (Brookfield Engineering Laboratories, Stoughton, MA). The upper plate of the torsion assembly rotated at 2.5 rpm, while the bottom plate remained stationary. The specimen was twisted until failure. The maximum viscometer reading was manually recorded. The torque reading (visco. reading) and distance (mm) was obtained from a chart recorder (Model 285/E1, Linear Instrument Corp., Irvine, CA). Eight samples from each batter preparation were tested.

Fracture shear stress and shear strain were calculated

as described by Hamann (1983). Fracture shear stress (τ_{max}, Pa) was calculated based on assumption that the ratio of shear stress and strain remain constant as follows:

$$\tau_{max} = [\ KM_t r_{min}\ ] / J = 1581\ [\text{Visc. reading}] \quad (5)$$

Where K = Shape factor constant (1.08)
M_t = Torque
r_{min} = Radius at smallest crossection
J = Polar moment of inertia of smallest crossection
$= (\pi r_{min}^4) / 2$

Uncorrected shear strain (Y_{max}) was calculated as:

$$Y_{max} = [\ 2\ K\Psi_Q\ ] / [\ \pi r_{min}^3 Q\ ] \quad (6)$$

$$= 0.150\ S^{-1}\ [(\text{chart distance} / \text{chart vel. S})] - 0.00847\ [\ \text{Visc reading}\ ] \quad (7)$$

where K = Shape factor constant
Ψ_Q = Angular rotation in the Q section
r_{min} = Radius at smallest crossection
Q = Constant for the machined Q section
Chart distance: The distance of the travelled from start point to failure point
Chart vel: Chart speed

Fracture shear strain (Y_{true}) was calculated as:

$$Y_{true} = \ln [\ 1 + (Y_{max}^2/2) + Y(\ 1 + Y_{max}^2/4)^{\frac{1}{2}}\ ] \quad (8)$$

3.7.4 Skin Strength Test

Skin strength or hardness was measured using a Texture Testing System (Model TMS-90, Food Technology Corp., Rockville, MD), equipped with penetration test cell (Model PT-1). Six frankfurters (about 6 cm long) per analysis were measured in triplicate at room temperature. The maximum

force (N) needed to penetrate the frankfurter skin was recorded as skin hardness.

3.7.5. Proximate Analysis

Protein content was determined by micro-Kjeldahl according to AOAC (24.038-24.039, 1984). Fat and moisture content of MDC and frankfurters were measured by the methods described in AOAC (24.005a, 24.003, 1980). Determinations were performed in triplicate.

3.8 STATISTIC DESIGN AND ANALYSIS

The results of LS and LS fraction treatments in three replications were analyzed using a randomized complete block design by using MSTATC software (1989). Tukey's honestly significant difference test ($P < 0.05$) (MSTATC, 1989) was used to evaluate the significant differences between treatment means.

RESULTS AND DISCUSSION

4.1. COMPOSITION OF LIQUID SMOKE (LS)

The smoke constituents of three brands of commercial LS (named as F, G and H) are listed in Table 3. The smoke constituents can be crudely separated into three classes: acids, carbonyls and phenols. Liquid smoke F had higher ($p < 0.05$) acidity (6.9%) and carbonyl (72.5 mg/ml) content when compared to the other two LS. Liquid smoke G exhibited different concentrations when compared with F. It was three times higher ($p < 0.05$) in phenols (47.5 mg/ml) than F and H, with half ($p < 0.05$) the acid (3.1%) and carbonyl (38.2 mg/ml) content of F. Smoke components of H ranged between F and G. Liquid smoke F had significantly lower ($p < 0.05$) acid (5.40%), carbonyl (49.1 mg/ml) and phenol (9.1 mg/ml) content than F. However, it was higher ($p < 0.05$) in acids and carbonyls than G. The significant smoke component patterns in each LS may exhibit different effects on protein functionality after direct addition of LS to salt-soluble proteins (SSP).

Table 3. Acid, carbonyl and phenol content of three types of commercial liquid smoke (LS)[1].

Liquid Smoke	Acids[2,3,6] (%, v/v)	Carbonyls[4] (mg/ml)	Phenols[5] (mg/ml)
F	6.9±0.01[a]	72.5±2.2[a]	11.0±0.03[b]
G	3.1±0.02[c]	38.2±1.6[c]	47.5±0.11[a]
H	5.4±0.01[b]	49.1±1.1[b]	9.1±0.01[c]

1: Three brands of commercial LS identified as F, G and H.
2: Means (n=3) within columns followed by the same letter do not differ significantly ($p > 0.05$).
3: Acids measured as acetic acid.
4: Carbonyls measured as 2-butanone.
5: Phenols measured as 2-methoxyphenol.
6: ± = Standard deviation.

4.2. EFFECTS OF LIQUID SMOKE ON CHARACTERISTICS OF SALT-SOLUBLE PROTEINS (SSP)

4.2.1. Solubility

Solubility is used as an indicator of protein denaturation. The changes in solubility of 4% SSP treated with three brands of LS at three concentrations in a buffered (0.6M NaCl, 0.05M Na-phosphate buffer, pH 6.5) system is reported in Table 4. The solubility of LS F at a 0.5% concentration was significantly ($p < 0.05$) less compared to the control (SSP without LS addition). All other treatments were not significantly different ($p > 0.05$). High standard deviations were observed in all treatments. This was due to variation between batches of SSP extracted from replication to replication. To reduce the standard deviations, one treatment (LS at the 0.5% level) was tested in three replications of SSP prepared from one batch of chicken muscle.

The data presented in Table 5 indicate that SSP prepared from the same batch effectively reduced the variation. In the buffered system, LS F treated SSP had lower ($p < 0.05$) solubility when compared to the control and the other two LS. In the non-buffered (0.6M NaCl) system, all LS treatments had different ($p < 0.05$) solubilities (Table 5). The LS treated SSP had lower ($p < 0.05$) solubilities, 14.5% and 69.2% for F and H, respectively. However, LS G treatments were higher ($p < 0.05$) in

Table 4. Solubility of 4% chicken breast salt-soluble proteins (SSP) in a buffered system (0.6M NaCl and 0.05M Na-phosphate buffer, pH 6.5) treated with liquid smoke (LS).

Treatments[1]	Solubility[2,3] (%)
SSP	95.8± 2.3[a]
F (0.125%)	92.6± 4.1[a]
F (0.250%)	87.8±11.9[ab]
F (0.500%)	74.6±11.3[b]
G (0.125%)	97.1± 2.0[a]
G (0.250%)	96.7± 3.8[a]
G (0.500%)	96.3± 5.0[a]
H (0.125%)	93.6± 3.5[a]
H (0.250%)	93.3± 2.5[a]
H (0.500%)	92.0± 5.6[a]

1: SSP represents the control without LS addition. F, G and H represents three brands of commercial LS. The number in parenthesis represents the % added LS w/w based on the solution concentration of 4% SSP (or 31.25, 72.12 and 125 mg LS/g protein, respectively).
2: Means (n=3) within columns followed by the same letter do not differ significantly ($p > 0.05$).
3: ± = Standard deviation.

Table 5. Solubility and/or pH of 4% chicken breast salt-soluble proteins (SSP) in a buffered (0.6M NaCl and 0.05M Na-phosphate buffer, pH 6.5) or non-buffered (0.6M NaCl) system treated with liquid smoke (LS).

Treatments[1]	Solubility[2,4] (%)		pH[2,3]
	Buffered	Non-Buffered	
SSP	96.5±0.5[a]	84.1±1.9[a]	6.50[a]
F (0.50%)	90.9±1.3[b]	14.5±0.8[b]	5.87[c]
G (0.50%)	96.7±1.0[a]	93.1±0.8[c]	6.12[b]
H (0.50%)	94.3±0.9[a]	69.2±2.9[d]	5.93[c]

1: SSP represents the control without LS addition. F, G and H represents three brands of commercial LS. The number in parenthesis represents the % added LS w/w based on the solution concentration of 4% SSP (or 125 mg LS/g protein).
2: Means (n=3) within columns followed by the same letter do not differ significantly ($p > 0.05$).
3: pH values are reported for the non-buffered system.
4: ± = Standard deviation.

solubility when compared to the control. In the non-buffered system, the pH values of the three brands of LS treated SSP were lower ($p < 0.05$) than the control (Table 5). However, both F and H treatments had lower ($p < 0.05$) pH values than G.

The LS F treatment was lower ($p < 0.05$) in SSP solubility than the control in both systems. This may be explained by higher ($p < 0.05$) acids and carbonyls in F compared with G and H. The high acid content of LS F (6.9%) and H (5.4%), decreased ($p < 0.05$) the pH of SSP model system and may be responsible for changes in solubility of the non-buffered system. Stronger protein-protein interaction occurred when the pH of LS treated SSP decreased. This may result in decreasing of SSP solubility and loss of other functional properties (Morrissey et al., 1987). The carbonyls may react with SSP, increasing the molecular weight of the proteins, thus decreasing the solubility in both systems. The reaction may be through carbonyl-amino type reactions as illustrated by Means and Feeney (1971) and discussed by other workers (Krylova et al., 1962; Chen and Issenberg, 1972). However, in the non-buffered system, SSP solubility decreased dramatically with LS F. This response may either involve the acid fraction or a combination of both the acid and carbonyl fractions. Liquid smoke G was high in phenols and low in acidity, and had higher ($p < 0.05$) solubility than other treatments in the non-buffered system. The actual mechanism for the

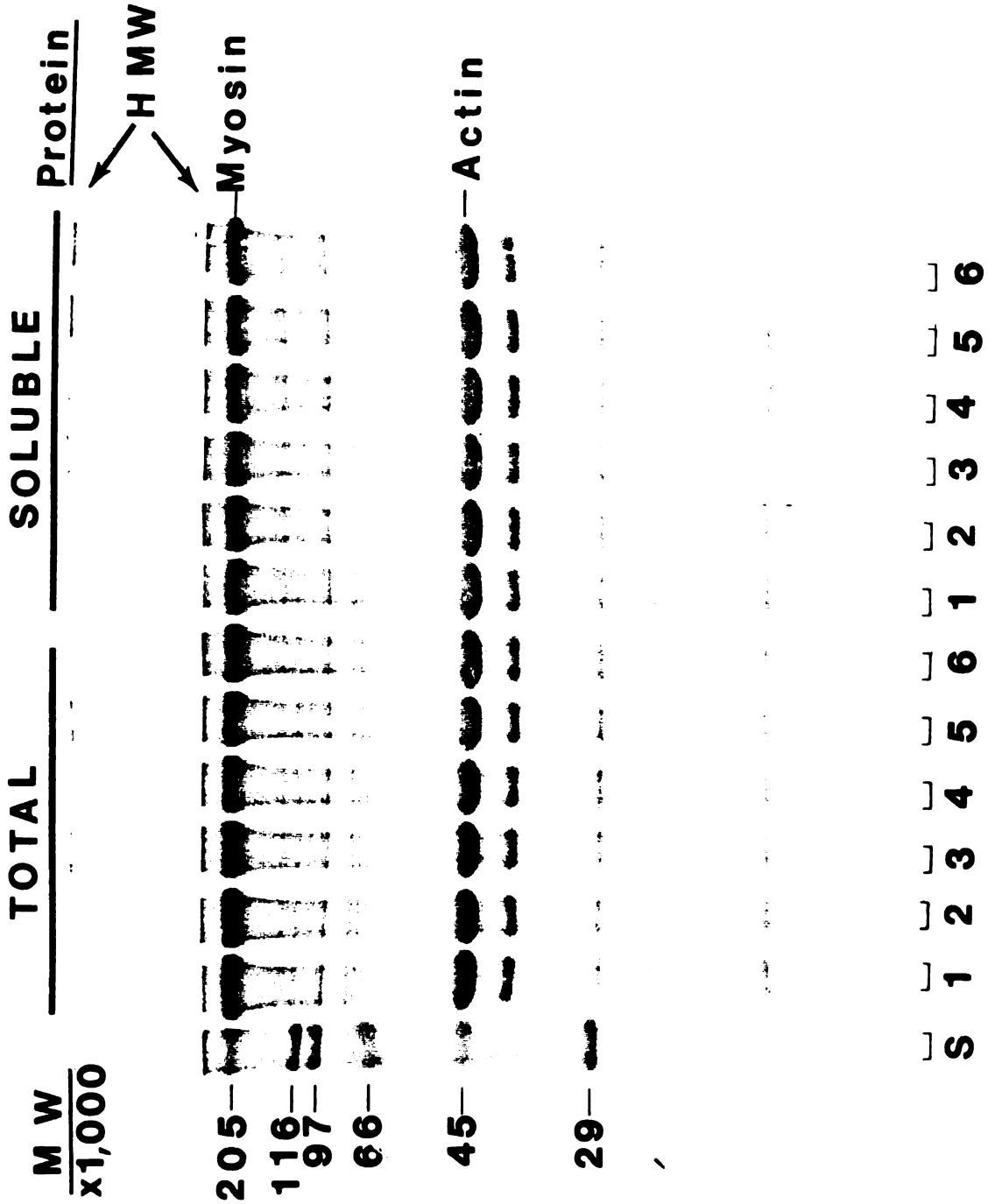
increase in solubility is not known. However, one may propose that there may be interaction between smoke components (especially phenols) and proteins as reviewed in sec 2.4.3.

4.2.2. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The SDS-PAGE profiles of the total protein fractions of the control (SSP only) and the LS treated SSP in buffered systems are presented in Fig. 4. Typical protein components of chicken breast SSP were exhibited as reported by Xiong and Brekke (1989). The term "TOTAL" and "SOLUBLE" in Fig. 4 represent SSP treatments before and after centrifugation (20,000 X g, 30 min), respectively. The major protein bands indicated in Fig. 4 were myosin heavy chain (MHC, MW about 195 KDa) and actin (MW about 43.6 KDa), which have a reported MW of 200 KDa and 45 KDa, respectively (Porzio and Pearson, 1977). The other minor bands may include unidentified (MW about 88.6, 85.3 and 79.2 KDa), troponin/tropomyosin (MW about 35 KDa), myosin light chain (MW about 24 and 17.6 KDa) and troponin-C (MW about 18 KDa) (Porzio and Pearson, 1977, Xiong and Brekke, 1989). Visual observations were used to make comparisons between protein band patterns.

The composition and intensity of protein bands in the control and the three brands of LS treated SSP presented in Fig. 4 were similar. An interesting observation was the

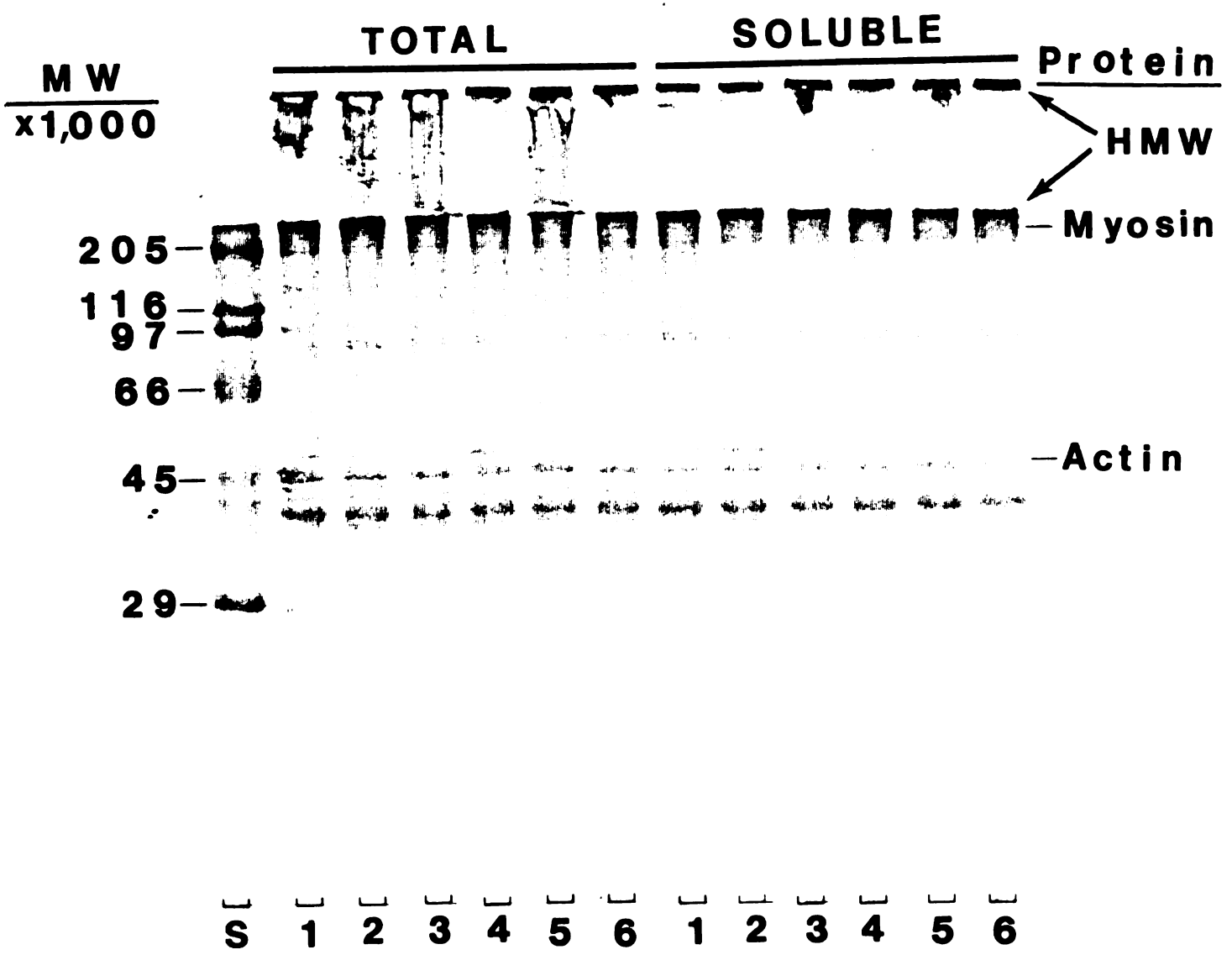
Figure 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of total and soluble protein fractions of 4% salt-soluble proteins (SSP) treated with three brands of liquid smoke (LS) in a buffered system (0.6M NaCl, 0.05M Na-phosphate buffer, pH 6.5). Forty-two ug protein were applied to each sample well of total proteins. For soluble proteins, the load amounts were variable, depending upon solubility (maximum load was 42 ug). Lane S was the molecular weight standard. Lane 1 contained total and soluble proteins of the control (SSP without LS treatment). Lanes 2-6 represent the total and soluble proteins from SSP treated with various brands and concentrations of LS (F, 0.25%; F, 0.50%; G, 0.25%; G, 0.50% and H, 0.50%, respectively).


Protein
SOLUBLE
TOTAL
M W
x1,000
H MW
Myosin
Actin
205
116
97
66
45
29
S 1 2 3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6

high molecular weight proteins (HMW) or protein aggregates which did not enter the SDS gel matrix but stayed at the top of the stacking (4% acrylamide) or resolving (12% acrylamide) gels in LS treated SSP (Fig. 4, Lane 2-6). Also, the unidentified protein bands in the soluble protein fraction of the LS treatments appear to be slightly decreased (Fig. 4, Lanes 2, 3, 5 and 6, SOLUBLE). This observation indicated that LS treatments appeared to cause slight changes in SSP bands.

To further investigate the type of bonding involved in the increased molecular weight or aggregates, the control and LS treatments were prepared in the SDS-sample buffer without β-mercaptoethanol (βMCE) (Fig. 5). The major function of βMCE is to reduce the disulfide bonds between protein subunits to sulfhydryl groups (Cooper, 1977). In Fig. 5, a higher proportion of protein material did not enter the resolving gel when βMCE was omitted from the sample buffer. This agrees with the results of Singh and Creamer (1991). They suggested that disulfide-linked protein components exist which cannot enter the resolving gel without treatment by a reducing agent (βMCE). This also agrees with the hypothesis suggested by Schmidt et al. (1981) that disulfide bonds are involved in crosslinking of protein gels (sec 2.3.2.). By visual observation, less aggregate proteins or high molecular weight proteins were observed at top of the stacking gel (Fig. 5, TOTAL) of LS G (Lane 4) and H (Lane 6) treatments than others (Lanes 1, 2,

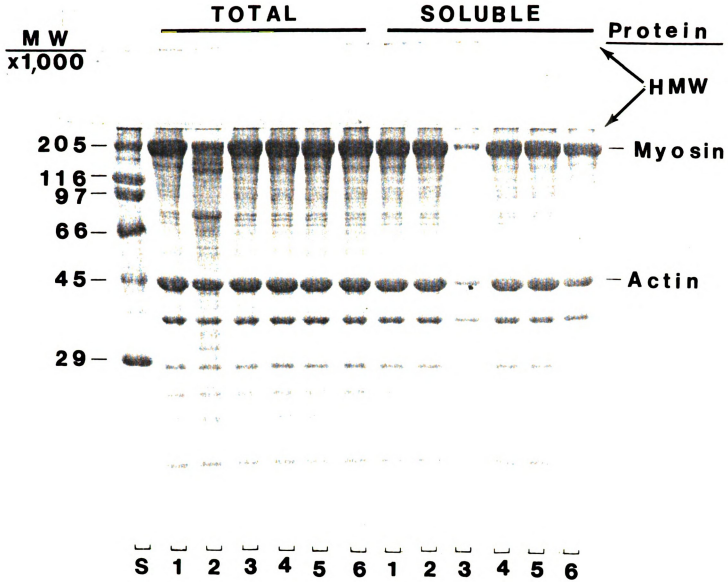
Figure 5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis without β-mercaptoethanol (βMCE) reaction of total and soluble protein fractions of 4% salt-soluble proteins (SSP) treated with three brands of liquid smoke (LS) in a buffered system (0.6M NaCl, 0.05M Na-phosphate buffer, pH 6.5). Forty-two ug protein were applied to each sample well of total proteins. For soluble proteins, the load amounts were variable, depending upon solubility (maximum load was 42 ug). Lane S was the molecular weight standard. Lane 1 contained total and soluble proteins of the control (SSP without LS treatment). Lanes 2-6 represent the total and soluble proteins from SSP treated with various brands and concentrations of LS (F, 0.25%; F, 0.50%; G, 0.25%; G, 0.50% and H, 0.50%, respectively).


TOTAL
SOLUBLE
Protein
MW
x1,000
HMW
Myosin
205
116
97
66
Actin
45
29
S
1
2
3
4
5
6
1
2
3
4
5
6

3 and 5). Soluble protein fractions (Fig. 5) of LS F (Lane 3) and G (Lane 5) had more high molecular weight proteins than other lanes. The high molecular weight proteins or protein aggregates in the soluble fraction may not have been large enough to sediment during centrifugation (20,000 x g, 30 min). This means that soluble high molecular weight proteins were present in all treatments. Most of the myosin heavy chain and actin bands in Fig. 5 disappeared when compared to similar protein patterns in Fig. 4. The SSP were insolubilized and could not be dispersed into individual components in the absence of βMCE. The LS F and G treatments caused similar changes in SSP characteristics by increasing molecular weight of proteins or protein aggregates. Therefore LS treatments may cause a different degree or type of crosslinking in SSP. Treatments without βMCE may have sulfhydryl oxidize to disulfide groups during confounding the above observations. In the future, it is recommended that the proteins be carboxymethylated before running on the SDS gels.

The SDS-PAGE profile of the LS treatments in the non-buffered system (Fig. 6) was similar to those in the buffered system (Fig. 4). The soluble protein fractions of the control (Lane 1), LS F (Lane 2) or G (Lanes 4 and 5) treated SSP had similar patterns on SDS-PAGE when compared to those of the total protein fraction. This indicates most of the proteins were soluble. However, LS F treated samples lost most of the protein components. Very light bands were

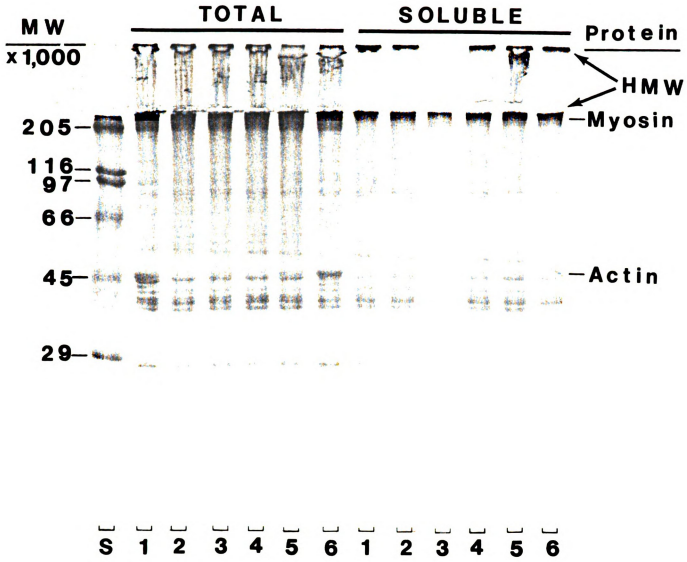
Figure 6. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of total and soluble protein fractions of 4% salt-soluble proteins (SSP) treated with three brands of liquid smoke (LS) in a non-buffered system (0.6M NaCl). Forty-two ug protein were applied to each sample well of total proteins. For soluble proteins, the load amounts were variable, depending upon solubility (maximum load was 42 ug). Lane S was the molecular weight standard. Lane 1 contained total and soluble proteins of the control (SSP without LS treatment). Lanes 2-6 represent the total and soluble proteins from SSP treated with various brands and concentrations of LS (F, 0.25%; F, 0.50%; G, 0.25%; G, 0.50% and H, 0.50%, respectively).


TOTAL
SOLUBLE
MW
x1,000
Protein
HMW
205
116
97
66
45
29
Myosin
Actin
S
1
2
3
4
5
6
1
2
3
4
5
6

observed for myosin, actin and troponin/tropomyosin (Lane 3, SOLUBLE). The LS H treated protein visually decreased slightly in band density of the soluble protein fraction when compared with the control (Lanes 6 vs 1, SOLUBLE). These observations (Lanes 3 and 6, SOLUBLE) agree with the solubility results presented in Table 5 (sec. 4.2.1.). Electrophoretic patterns of LS treated samples in the non-buffered system dispersed in the SDS-sample buffer without βMCE are shown in Fig. 7. Similar patterns were observed when compared to those in buffered system (Fig. 7 vs Fig. 5). The exception was the soluble fraction of LS G (Fig. 7, Lane 5) treatment exhibited a different electrophoretic pattern in the stacking gel when compared to other LS treated SSP. The LS G treatments may have had more high molecular weight proteins which were not large enough to be precipitated during centrifugation and remained in the soluble fraction. One may assume that higher molecular weight protein aggregates remained in the soluble fraction and consequently, explains why the LS G treated SSP had a significantly higher ($p < 0.05$) solubility than other treatments (Table 5). However, this could be verified by carboxymethylation of protein discussed in the previous paragraph.

The high molecular weight proteins produced in LS treated SSP indicated crosslinking reactions may have occurred. Krylova et al.(1962) stated that smoke components, especially phenol and carbonyl compounds, had an

Figure 7. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis without β-mercaptoethanol (βMCE) reaction of total and soluble protein fraction of 4% salt-soluble proteins (SSP) treated with three brands of liquid smoke (LS) in a non-buffered system (0.6M NaCl). Forty-two ug protein were applied to each sample well of total proteins. For soluble proteins, the load amounts were variable, depending upon solubility (maximum load was 42 ug). Lane S was the molecular weight standard. Lane 1 contained total and soluble proteins of the control (SSP without LS treatment). Lanes 2-6 represent the total and soluble proteins from SSP treated with various brands and concentrations of LS (F, 0.25%; F, 0.50%; G, 0.25%; G, 0.50% and H, 0.50%, respectively).


MW
x1,000
TOTAL
SOLUBLE
Protein
HMW
Myosin
Actin
205
116
97
66
45
29
S
1
2
3
4
5
6
1
2
3
4
5
6

extremely high reactive activity with meat protein functional groups. They further postulated the reactions between phenols and proteins were through disulfide and sulfhydryl groups. These reactions often result in crosslinking of proteins (Loomis and Battaile, 1966). The carbonyl compounds in smoke may react with the amino groups of protein (Krylova et al., 1962). The decreased solubility and increased in amounts of high molecular weight proteins observed in LS F treatments may indicate that smoke components in LS caused conformational changes of the proteins. However, further research is necessary to study the effects of phenols or carbonyls in smoke components on crosslinking between the proteins and subsequent conformational changes.

4.3. EFFECTS OF LIQUID SMOKE ON SALT-SOLUBLE PROTEIN GELS

4.3.1. Expressible Moisture and Yield

Expressible moisture (EM) and yield describe the ability of proteins to entrap water. Expressible moisture methodology uses external force. The EM was measured in buffered SSP gels treated with three brands of LS at three concentrations (0.125%, 025% and 0.50%, w/w). The data in Table 6 indicates no significant differences ($P > 0.05$) for all treatments. This is due to the high standard deviations for reasons discussed in sec 4.2.1. Liquid smoke G (0.50%) had lowest EM (30.71%) when compared with control (38.82%) and other LS treatments.

Table 6. Expressible moisture (EM) of 4% chicken breast salt soluble protein (SSP) gels in a buffered system (0.6M NaCl and 0.05M Na-phosphate buffer, pH 6.5) treated with liquid smoke (LS).

Treatments[1]	Expressible moisture[2,3] (%)
SSP	38.8±3.4[a]
F (0.125%)	40.1±2.7[a]
F (0.250%)	37.9±3.8[a]
F (0.500%)	32.7±2.4[a]
G (0.125%)	34.5±1.6[a]
G (0.250%)	34.5±4.3[a]
G (0.500%)	30.7±3.0[a]
H (0.125%)	38.4±5.2[a]
H (0.250%)	39.7±3.7[a]
H (0.500%)	39.1±4.3[a]

1: SSP represents the control without LS addition. F, G and H represents three brands of commercial LS. The number in parenthesis represents the % added LS w/w based on the solution concentration of 4% SSP (or 31.25, 72.12 and 125 mg LS/g protein, respectively).
2: Means (n=3) within columns followed by the same letter do not differ significantly ($p > 0.05$).
3: ± = Standard deviation.

Expressible moisture and yield were calculated for LS treated SSP gels in buffered or non-buffered systems (Table 7. The EM for the buffered treatments did not differ ($P > 0.05$) when compared to the control. Liquid smoke F and G treated SSP gels had lower ($p < 0.05$) yields than the control. In addition, F treatments were lower ($p < 0.05$) in yield than G treatments. When SSP were treated with LS in a non-buffered system, the EM and yield were different ($p < 0.05$) when compared to the control. Liquid smoke G gels had lower ($p < 0.05$) EM, while LS F and H had higher ($p < 0.05$) EM than the control. The yield of the control was higher ($p < 0.05$) than all LS treatments and LS F had lowest ($p < 0.05$) yield.

The results in Table 7 coincide with solubility results presented in Table 5 earlier. The LS F treated SSP had lower ($p < 0.05$) solubility and gel yield in both systems. The lower pH and higher EM values were also present in the non-buffered system. A similar pattern was also observed with LS H treated SSP in the non-buffered system. These results indicate that decreases in protein solubility due to LS treatment may cause decreases in moisture retention. These observations are similar to those obtained by Xiong and Brekke (1989), which indicate an increase in chicken SSP solubility during storage may contribute to increased water holding capacity (WHC). However, a different trend was observed in LS G treatments. They were higher ($p < 0.05$) in solubility, but lower in both ($p < 0.05$) yield and EM. This

Table 7. Expressible moisture (EM) and yield of 4% chicken breast salt soluble protein (SSP) gels in buffered (0.6M NaCl and 0.05M Na-phosphate buffer, pH 6.5) and non-buffered (0.6M NaCl) systems treated with liquid smoke (LS).

Treatments[1]	Expressible[2,3] Moisture (%)	Yield[2] (%)
	Buffered	
SSP	30.3±0.8[a]	97.0±0.5[a]
F (0.50%)	32.6±2.5[a]	91.1±1.3[c]
G (0.50%)	28.9±1.3[a]	93.9±0.9[b]
H (0.50%)	33.1±0.4[a]	96.1±0.3[a]
	Non-Buffered	
SSP	38.6±3.0[a]	96.5±1.1[a]
F (0.50%)	51.0±0.0[b]	55.8±2.1[b]
G (0.50%)	27.0±1.5[c]	84.1±0.5[c]
H (0.50%)	49.4±2.9[b]	80.7±1.8[c]

1: SSP represents the control without LS addition. F, G and H represents three brands of commercial LS. The number in parenthesis represents the % added LS w/w based on the solution concentration of 4% SSP (or 125 mg LS/g protein).
2: Means (n=3) within columns followed by the same letter do not differ significantly ($p > 0.05$).
3: ± = Standard deviation.

can be explained by the formation of a denser protein network because of a concentration effect of SSP during cooking. As was done using electrophoretic patterns (sec. 4.2.2.), one can postulate that when the high molecular weight proteins or protein aggregates are formed during LS treatments, stronger protein-protein interaction occurred, thus increasing the free drip of LS treated SSP gels. Liquid smoke constituents may be in part responsible as intermediates of enhanced protein-protein interaction.

4.3.2. Apparent Stress and Apparent Strain at Failure

Apparent stress and apparent strain at failure were related to hardness and elasticity of food proteins, respectively (Hamann, 1983). The data in Table 8 shows apparent stress and apparent strain at failure for 4% SSP gels prepared using three brands of LS (F, G and H) at three concentrations (0.125%, 0.25%, 0.50%; w/w). Liquid smoke treatments did not significantly affect the apparent stress and apparent strain of gels ($p > 0.05$). However, a general trend observed was that SSP alone formed weaker (22.7 KPa) gels than LS treated gels (28.1-38.8 KPa). Liquid smoke G (0.50%) treatments had highest gel strength (38.2 KPa) with the apparent stress almost doubled. High standard deviations in apparent stress may be responsible in part for explanation why no significant differences exist between treatments (described in sec. 4.2.1.). Elastic (apparent strain about 1.60) gels were formed in all treatments.

Table 8. Apparent stress (KPa) and strain at failure for liquid smoke (LS) treated 4% salt-soluble protein (SSP) gels in a buffered system (0.6M NaCl and 0.05M Na-phosphate buffer, pH 6.5).

Treatments[1]	Apparent Stress[2,3] (KPa)	Apparent Strain[2]
SSP	22.7± 1.4[a]	1.60±0.00[a]
F (0.125%)	38.8± 7.6[a]	1.58±0.02[a]
F (0.250%)	35.1± 4.3[a]	1.58±0.02[a]
F (0.500%)	31.6± 3.6[a]	1.52±0.09[a]
G (0.125%)	31.6± 8.0[a]	1.59±0.02[a]
G (0.250%)	29.1± 6.0[a]	1.59±0.02[a]
G (0.500%)	38.2±12.0[a]	1.59±0.02[a]
H (0.125%)	28.1±10.8[a]	1.59±0.02[a]
H (0.250%)	32.8± 9.3[a]	1.59±0.02[a]
H (0.500%)	32.1± 5.8[a]	1.56±0.07[a]

1: SSP represents the control without LS addition. F, G and H represents three brands of commercial LS. The number in parenthesis represents the % added LS w/w based on the solution concentration of 4% SSP (or 31.25, 72.12 and 125 mg LS/g protein, respectively).
2: Means (n=3) within columns followed by the same letter do not differ significantly ($p > 0.05$).
3: ± = Standard deviation.

Apparent stress and apparent strain at failure for LS (F, G and H) treated SSP gels in buffered and non-buffered systems are presented in Table 9. In the buffered system, apparent stress for treatments F and G differ ($p < 0.05$) from the control, while LS H treatments were similar. The firmest gels were LS G treatments, followed by LS F treatments. Less elastic gels ($p < 0.05$) were formed after LS F and H treatment. Apparent strain of LS G was similar to the control. These results indicate that LS G treatments had positive effects on protein gels. Liquid smoke G increased the hardness without decreasing the elasticity of the gel. Apparent stress of LS H treated SSP gels were similar to the control in the non-buffered system (Table 9). Liquid smoke F treatments produced weak ($p < 0.05$) gels (12.1 KPa) compared to the controls (24.5 KPa) and LS G treatments (86.1 KPa). Very firm gels were produced using LS G. The apparent strain indicated similar trends as in the buffered system. However, the elasticity of LS F and H treatments was reduced about 35% when compared to the control or LS G treatments.

As demonstrated above, LS F and G caused dramatic changes in the apparent stress of SSP gels. This agrees with Hamann (1987), who showed that rupture shear stress was strongly influenced by protein concentration (sec. 2.3.4). As one compares smoke treatments on solubility, electrophoretic patterns, yield and EM to apparent stress, one can conclude that as carbonyl and/or acid content

Table 9. Apparent stress (KPa) and strain at failure for liquid smoke (LS) treated 4% salt soluble protein (SSP) gels in buffered (0.6M NaCl and 0.05M Na phosphate buffer, pH 6.5) and non-buffered (0.6M NaCl) systems.

Treatments[1]	Apparent Stress[2,3] (KPa)	Apparent Strain[2]
	Buffered	
SSP	29.2±3.0[a]	1.57±0.00[a]
F (0.50%)	36.9±2.2[b]	1.35±0.04[b]
G (0.50%)	44.5±0.4[c]	1.55±0.02[a]
H (0.50%)	29.8±0.8[a]	1.33±0.04[b]
	Non-Buffered	
SSP	24.5±1.3[a]	1.50±0.00[a]
F (0.50%)	12.1±2.4[b]	0.87±0.09[b]
G (0.50%)	86.1±6.5[c]	1.49±0.04[a]
H (0.50%)	22.7±1.5[a]	1.00±0.05[b]

1: SSP represents the control without LS addition. F, G and H represents three brands of commercial LS. The number in parenthesis represents the % added LS w/w based on the solution concentration of 4% SSP (or 125 mg LS/g protein).
2: Means (n=3) within columns followed by the same letter do not differ significantly ($p > 0.05$).
3: ± = Standard deviation.

increased, solubility and moisture retention decreased. The decreased in moisture retention of gel may increased the protein concentration of gel and may take part of responsible in increasing gel hardness. This trend seems apparent in LS F treatments in buffered systems. In non-buffered systems, LS F treated gels yield about 55% (Table 7). This may indicate that the protein gels are highly aggregated and thus, most of their functional properties are lost (low in apparent stress and strain as demonstrated in Table 9). These observations are similar to Hamann (1987) and Wang (1990). High carbonyl and acid content may explain this observation in part. Liquid smoke G (high in phenols) had positive effects on SSP gels when compared to LS F treatments. They increased dramatically in hardness without decreasing the elasticity of the gels and the solubility of the SSP. From the discussion in sec. 4.2.2., LS G treatments moderately increase the molecular weight of proteins and yet remain soluble. This may partially explain why firm gels were produced when SSP were treated with LS G. The pH may be responsible for part of the higher gel hardness observed with LS G treatments in non-buffered systems when compared to the hardness of gels in the buffered systems. The effect of pH in decreasing yield and increasing protein concentration of gel is part of explanation in higher gel hardness. However, both LS F and H treatments had lower pH values than LS G treatments. Therefore, stronger acid effects on SSP were produced. The

effects of pH on SSP is well demonstrated (Wang, 1990). Further investigations on gel properties affected by LS high in phenols at various pH ranges in buffered systems are needed.

4.3.3. Scanning Electron Microscopy

The microstructure of LS F, G and H (0.5%, w/w) treated 4% SSP gels were examined using magnifications of 2,000 X and 6,000 X. In the buffered system, SSP alone (control, Fig. 8A) and LS G treated gels (Fig. 8C) had denser or thicker protein network structure with small (0.7 um) and evenly distributed void areas when compared to LS F and H (Fig. 8B and 8D, respectively). Examination at higher magnification (6,000 X; Fig. 9A and 9C, respectively) reveal a dense network made up from similar sized globular particles which clump together to form globular aggregates in structure. This microstructure is similar to that of bovine myosin extracted with high salt (0.6M KCl) concentrations illustrated by Hermansson et al. (1986) where globular aggregates predominated. The dense structures of the control and LS G treatments are patterned with high elasticity and moisture retention as discussed previously. The dense proteins surrounding the evenly distributed small void areas may be suitable for trapping water which explains EM and yield observations. Differences in gel hardness of control and LS G treatments were not consistent with the gel microstructure. This may be due to either artifacts

Figure 8. Scanning electron micrographs of salt-soluble proteins (SSP) gels prepared by direct addition (0.50%, w/w) of three brands of liquid smoke (F, G and H) to 4% SSP in a buffered system (0.6M NaCl, 0.05M Na-phosphate buffer, pH 6.5). The gels were heated at 70°C for 15 min. (A) Control (SSP without LS treatment); (B) F; (C) G; (D) H.

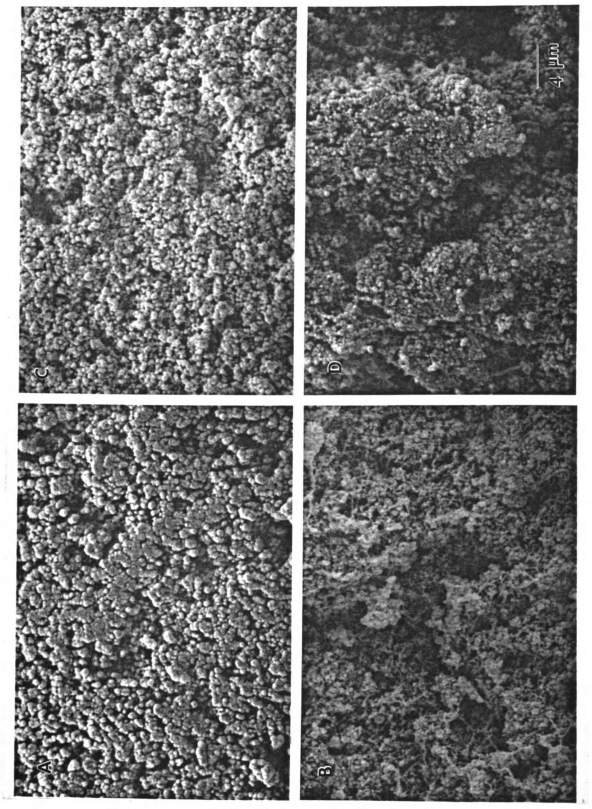
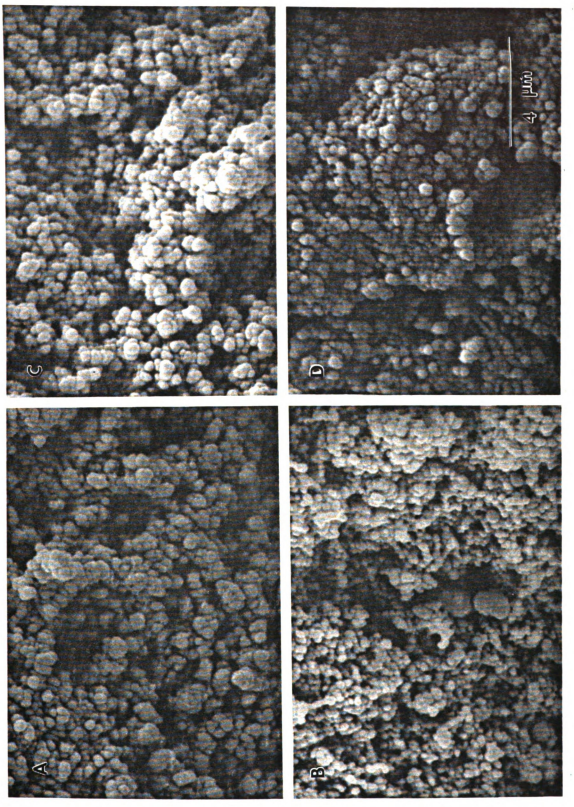

A
B
C
D
4 μm

Figure 9. Scanning electron micrographs of salt-soluble proteins (SSP) gels prepared by direct addition (0.50%, w/w) of three brands of liquid smoke (F, G and H) to 4% SSP in a buffered system (0.6M NaCl, 0.05M Na-phosphate buffer, pH 6.5). The gels were heated at 70°C for 15 min. (A) Control (SSP without LS treatment); (B) F; (C) G; (D) H.


A
B
C
D
1 μm

resulting from the fixation methodology or the fact that only surface observations are made using SEM. Fine stranded, interconnecting networks with depth of field, irregular grape-like clusters and void areas were observed in gels treated with LS F (Fig. 8B). Further detail can observed in Fig. 9B. Small globular particles with variable diameters and irregular void areas make up the gel structure. Low elasticity and moisture retention values are consistent with this loose gel structure and agrees with observations of Wang (1990). Liquid smoke H (Fig. 8D) produced a microstructure with large irregular grape-like clusters (approximately 8-11 um) and void areas (about 4 um). The large clusters consisted of variable size globular particles (Fig. 9D). This type of uneven structure probably is responsible for decreased elasticity measurements of gels containing LS H.

In the non-buffered system (Fig. 10 and 11), the gels exhibited a different microstructure. More void areas were observed when compared to gels prepared in the buffered system. The microstructure of all treatments, except LS F, consists of small globular aggregates and strands (Fig. 10). Gels treated with LS F exhibited grape-like clusters with large and irregular void areas (4-9.3 um) (Fig. 10B). Further detail can observed in Fig. 11B. The grape-like clusters were loose compared to the others, as a result of weak interaction or no network formation, causing about one third of the structure to be large open pockets. These

Figure 10. Scanning electron micrographs of salt-soluble proteins (SSP) gels prepared by direct addition (0.50%, w/w) of three brands of liquid smoke (F, G and H) to 4% salt-soluble proteins in a non-buffered system (0.6M NaCl). The gels were heated at 70°C for 15 min. (A) Control (SSP without LS treatment); (B) F; (C) G; (D) H.

A
B
C
D
4 µm

Figure 11. Scanning electron micrographs of salt-soluble proteins (SSP) gels prepared by direct addition (0.50%, w/w) of three brands of liquid smoke (F, G and H) to 4% salt-soluble proteins in a non-buffered system (0.6M NaCl). The gels were heated at 70°C for 15 min. (A) Control (SSP without LS treatment); (B) F; (C) G; (D) H.

A
B
C
D
4 µm

coarse and highly aggregated microstructures were consistent with poor moisture retention and gel strength observed (Tables 7 and 9), and also agreed with results observed by Wang (1990). Control and LS H gels (Fig. 10A and 10D; or Fig. 11A and 11D, respectively) generally produced a similar microstructure, which was composed of globular aggregates. However, fine strands and larger void areas were observed in the LS H treatments. This agrees with the lower elasticity and moisture retention values when compared to the control. The SSP gels prepared using LS G exhibited a dense structure with small globular aggregates and strands (Fig. 10C). The micrograph (Fig. 11C) of higher magnification indicated that all globular aggregates making up the gel structure were well ordered and produced thick filamentous networks. This kind of dense structure may be responsible for the higher gel strength observed (Table 9).

The phase separated, highly aggregated or coarser microstructure may result from locally strong interactions between protein molecules (Hermansson, 1983, 1986; Wang, 1990). These interactions may include charge of the protein which is affected by pH and may have influenced the characteristics of the gel network (Barbu and Joly, 1953). The LS F treatments caused SSP to be less soluble resulting in undesirable gel structure. However, LS G treatments produced high molecular weight soluble proteins, which produced a dense microstructure resulting in high gel strength. These observations provide evidence that LS

components can cause positive and/or negative changes on microstructure of SSP gels. The changes in microstructure agree with measurements of functional properties of the SSP gels and support observations made by Hermansson (1983). However, one should be mention that is chemical artifacts may also involved in microstructure of gel when using SEM technique.

4.4. EFFECTS OF REACTION TIME BETWEEN SALT-SOLUBLE PROTEINS AND LIQUID SMOKE F ON PROTEIN SOLUBILITY AND GELLING PROPERTIES

Liquid smoke F dramatically changed SSP solubility and gelling abilities in a non-buffered system. Therefore, LS F was used to determine if SSP functionality was influenced by time of exposure to the LS treatment. The reaction times were 0, 6 and 24 hr. The 0 time represented treatments analyzed within 1 hr of LS addition. The pH, solubility, EM and yield of the control and LS F treatments at various times are presented in Table 10. An increase in apparent stress of the control from 0 to 6 hr was observed. This agrees with results of Xiong and Brekke (1989) who observed dramatic increases in viscosity, gel strength and WHC for chicken myofibrils suspended in 0.6M NaCl, pH 6.0 during an initial 10 hr storage at 4°C. This may be due to the formation of an orderly network structure upon standing. Gordon and Barbut (1990a) further stated that structure formation in meat systems could occur before heating. This confirms postulations made by Hermansson et al. (1986) that

Table 10. pH, solubility, expressible moisture (EM), yield, apparent stress and apparent strain 4% salt-soluble proteins (SSP) reacted with and without liquid smoke (LS) F (0.5% w/w or 125 mg LS/g protein) in a non-buffered system (0.6M NaCl) for 0, 6 and 24 hrs.

Treatments[1]	pH[2]	Solubility[2,3] (%)	EM[2] (%)	Yield[2] (%)	Apparent[2] Stress (KPa)	Apparent[2] Strain
SSP (0)	6.50[a]	92.1±0.4[a]	35.4±1.7[c]	99.0±0.2[a]	22.3±1.3[b]	1.46±0.02[a]
SSP (6)	6.50[a]	91.7±2.7[a]	33.4±0.5[c]	98.7±0.9[a]	25.5±2.4[ab]	1.49±0.08[a]
SSP (24)	6.50[a]	87.3±1.5[a]	33.8±2.3[c]	98.8±0.3[a]	27.3±1.3[a]	1.52±0.01[a]
LS (0)	5.82[b]	70.7±7.4[b]	49.3±1.5[b]	58.9±1.7[b]	7.7±0.1[c]	0.81±0.04[b]
LS (6)	5.82[b]	26.6±2.9[c]	51.3±2.0[ab]	52.6±1.5[d]	7.4±0.3[c]	0.88±0.06[b]
LS (24)	5.82[b]	17.7±0.7[d]	52.8±1.1[a]	55.9±1.0[c]	7.9±0.7[c]	0.84±0.05[b]

1: SSP represent the control without LS addition. LS represents brand F. The number in parenthesis represents the time (hours) held after LS addition to the SSP before heating to 70°C for 15 min. The 0 time represents treatments analyzed within 1 hr.
2: Means (n=3) within columns followed by the same letter do not differ significantly ($p > 0.05$).
3: ± = Standard deviation.

the conditions required for the formation of strand-like myosin gels are already present before the heat treatment. The LS F treatments were different in solubility, EM and yield from the control ($p < 0.05$). The pH of the treatment changed immediately after addition of LS F and remained constant during the three time periods. These results indicate that solubility of SSP changed soon after addition of LS F. After 6 hr of reaction, a significant portion of the proteins were denatured as measured by solubility. Apparent stress and apparent strain of the LS treatments were different ($p < 0.05$) from the control over the three time periods (Table 10). Less elastic (0.81 KPa) and weaker (7.7 KPa) gels were formed soon after the addition of LS F. These results agree with Krylova et al. (1962) who concluded that amino nitrogen and SH-groups of protein solutions decreased soon after addition of carbonyl and phenol fractions, respectively.

These results indicate that changes in functional properties of the SSP due to LS addition have a short reaction time (6 hr or less). The SSP used as controls improved in hardness over time. Choosing 24 hr for a standard reaction time in these experiments was appropriate.

4.5. EFFECTS OF LIQUID SMOKE CONSTITUENTS (PHENOLS AND CARBONYLS) ON CHARACTERISTICS OF SALT-SOLUBLE PROTEINS AND SALT-SOLUBLE PROTEIN GELS

Addition of LS F and G changed SSP functionality. Therefore, these brands were fractionated into phenol and

carbonyl fractions and further studied measuring functional properties of SSP in buffered systems to determine which fraction is responsible for functionality changes. The study was separated into two individual sections. First, the smoke fractions were studied at concentrations based upon the 0.5% w/w original LS volume. The second part is at a 0.05% added LS fraction w/w concentration based on the SSP concentration (or 12.5 mg LS fraction/g protein).

4.5.1. Liquid Smoke Fractions added to the Salt-Soluble Proteins Based on Phenol and Carbonyl Content of the 0.5% w/w Liquid Smoke Treatment Level

Phenol and carbonyl fractions were added to 4% SSP based on the constituent concentration at the original 0.5% LS treatment level. Therefore, phenol and carbonyl contents in the smoke fraction had a similar pattern as the original LS constituents (Table 3). The carbonyl content in LS F was higher than G, and the phenol content in LS F was lower than G.

All smoke fractions contained 10% Tween-20 (polysorbate 20) as an emulsifier. The phenol fraction was less soluble in the buffer after the acid fraction and/or emulsifier were removed during fractionation. Without an emulsifier, an uneven distribution of smoke fractions occurred in the SSP. Data generated without Tween-20 are given in appendix A. Addition of 10% Tween-20 to all smoke fractions reduced experimental variation. Table 11 presents results of SSP functionality after phenol and carbonyl fraction addition.

Table 11. Solubility, expressible moisture (EM), yield, apparent stress and apparent strain for 4% salt-soluble proteins (SSP) gels in 0.6M NaCl and 0.05M Na-phosphate buffer, pH 6.5 treated with liquid smoke (LS) fractions based on phenol and carbonyl contents of the 0.5% w/w LS level.

Treatments[1,2]	Solubility[3,5] (%)	Expressible[3] Moisture(%)	Yield[3,4] (%)	Apparent[3,4] Stress (KPa)	Apparent[3,4] Strain
SSP	98.3±1.4[a]	31.6±1.7[a]	99.5±0.4[a]	38.6±3.2[a]	1.56±0.01[a]
F (phenols)	94.2±0.1[b]	30.5±1.3[a]	97.5±0.2[b]	40.2±6.0[a]	1.56±0.00[a]
F (carbonyls)	88.7±1.3[c]	24.5±2.7[b]	88.7±1.1[cd]	60.5±4.9[b]	1.56±0.00[a]
G (phenols)	97.5±1.4[a]	32.6±1.6[a]	95.4±0.8[bc]	40.9±7.4[a]	1.56±0.00[a]
G (carbonyls)	97.0±0.5[ab]	32.6±2.6[a]	93.4±1.4[c]	44.0±2.6[a]	1.56±0.02[a]

1: SSP represent control without the addition of LS fractions. Liquid smoke F and G represent two brands of commercial LS. Phenols and carbonyls in parenthesis represent the type of the LS fraction added to SSP.
2: Phenol and carbonyl fractions (contained 10% Tween 20) were added to 4% SSP based on the constituent in the original 0.5% LS treatment level.
3: Means (n=3) within columns followed by the same letter do not differ significantly ($p > 0.05$).
4: The data were adapted from a second run due to the problem with the control during the first run.
5: ± = Standard deviation.

The carbonyl and phenol fractions from LS F had lower ($P < 0.05$) solubility than the control or LS G fractions. The LS F carbonyl treatments were lower ($p < 0.05$) in solubility, EM, yield and higher ($p < 0.05$) in apparent stress (firmest) than the phenol treatments. All fractions had lower ($p < 0.05$) yields than the control (99.5%). However, phenolic fractions had higher ($p < 0.05$) yields than carbonyl fractions.

The SDS-PAGE of treatments are shown in Fig. 12. The total and soluble protein fractions had similar patterns, indicating that most of the proteins were soluble. An interesting observation was noted on Lanes 3 and 5, more high molecular weight proteins (HMW) or protein aggregates stayed on the top of the stacking gel than other lanes. Lanes 3 and 5 contained SSP treated with carbonyl fractions of LS F and G, respectively. Also, a thinner band (MW about 205 KDa, myosin) was observed in Lane 3 compared with the others. This indicates that high molecular weight proteins produced were unable to migrate into the resolving gel, and explained why a denser band was observed on top of the stacking gel.

The SSP treatments exhibited a different electrophoretic pattern when samples were electrophoresed without βMCE (Fig. 13). Phenol fractions (Lanes 2 and 4) contained less banding changes than carbonyl fractions (Lanes 3 and 5) compared to SSP without LS. An interesting observation was the disappearance of the band at a MW of

Figure 12. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of total and soluble protein fractions of 4% salt-soluble proteins (SSP) treated with liquid smoke (LS) fractions (phenols and carbonyls) in a buffered system (0.6M NaCl, 0.05M Na-phosphate buffer, pH 6.5). The concentration of smoke fractions was based on phenol and carbonyl content at the 0.5% LS level. Forty-two ug protein were applied to each sample well of total proteins. For soluble proteins, the load amounts were variable, depending upon solubility (maximum load was 42 ug). Lane S was the molecular weight standard. Lane 1 contained total and soluble proteins of the control (SSP without LS treatment). Lanes 2-5 represent the total and soluble proteins from SSP treated with LS fractions (F, phenols; F, carbonyls; G, phenols and G, carbonyls, respectively).

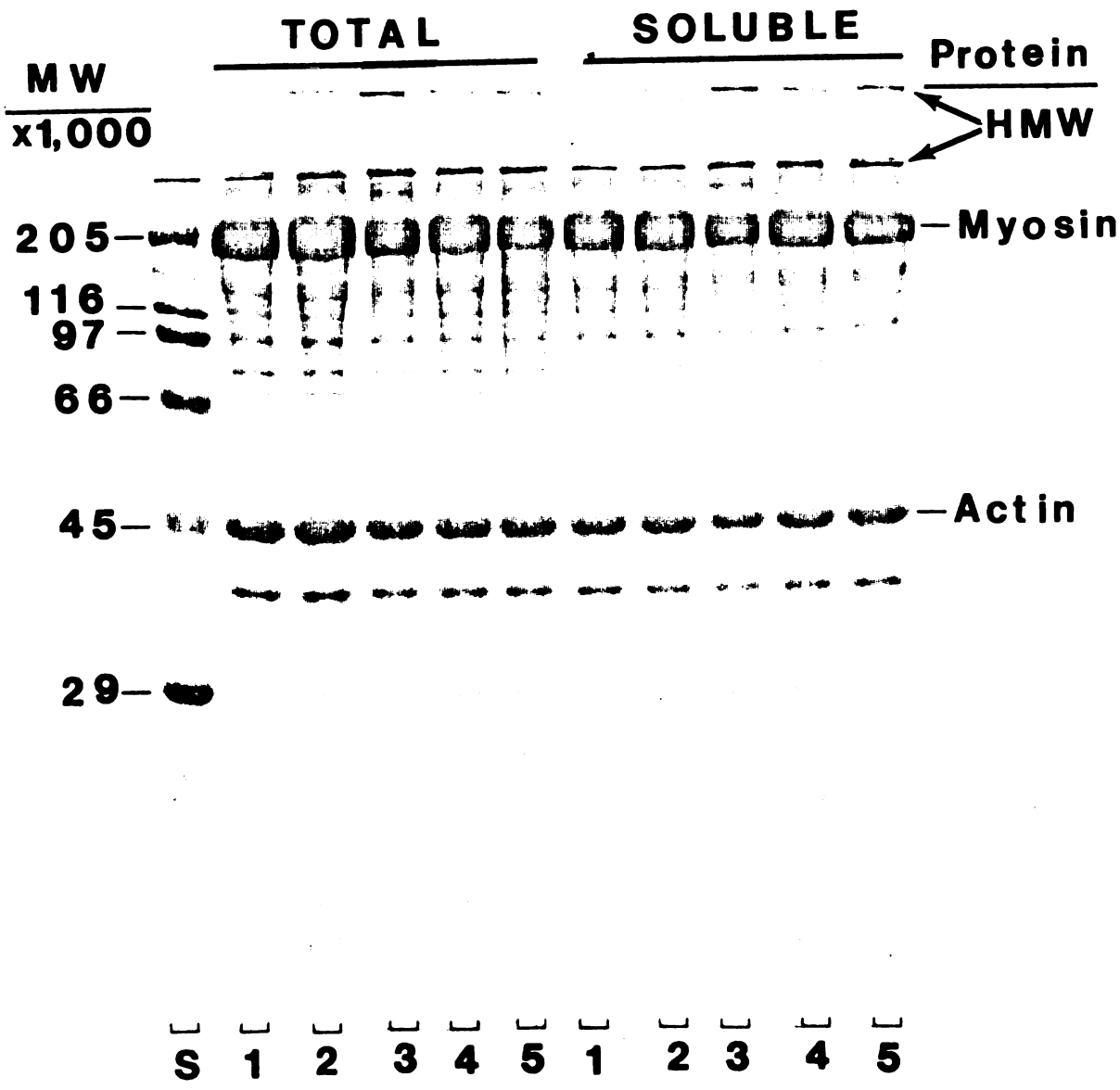
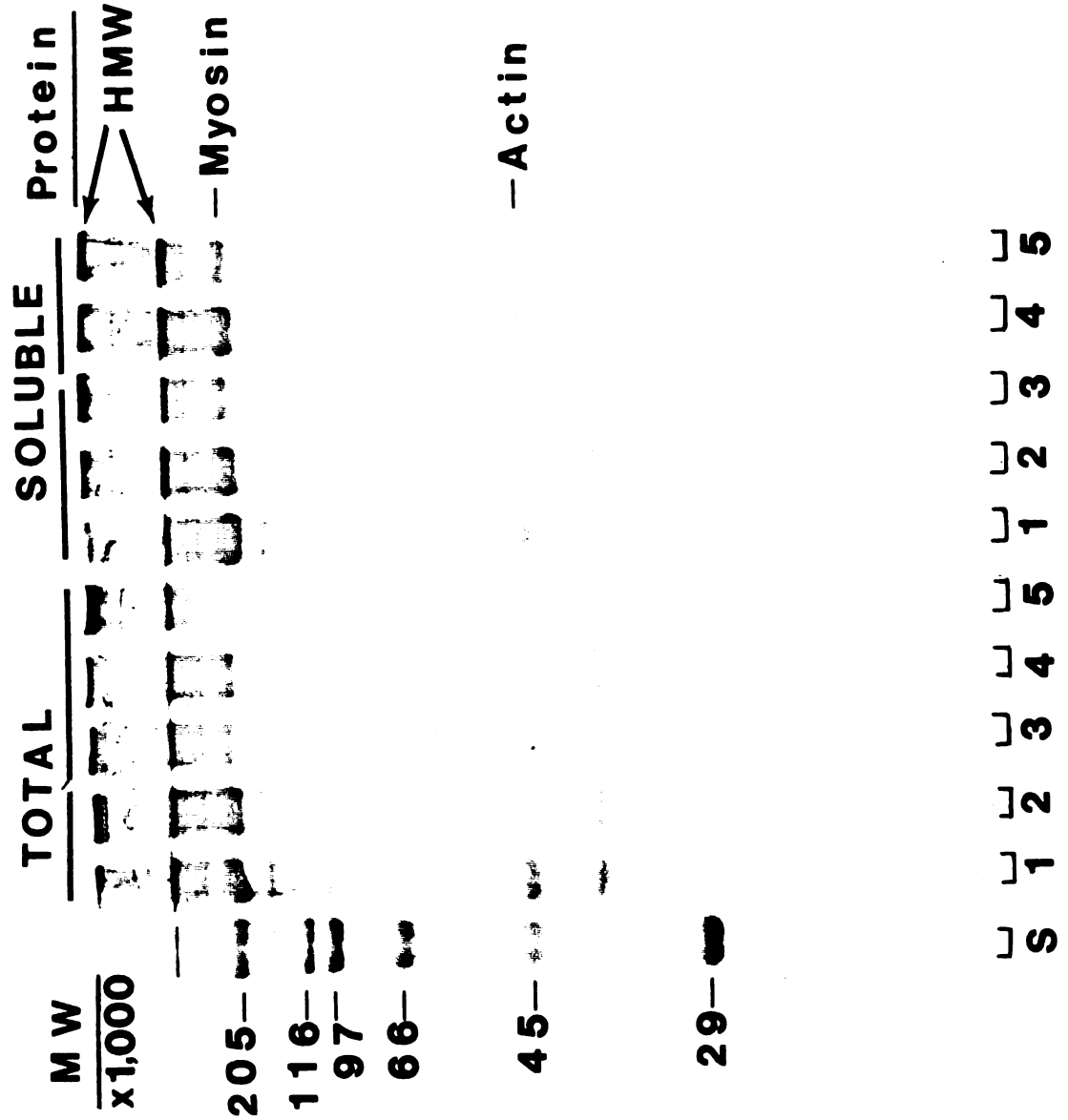

TOTAL
SOLUBLE
Protein
MW
x1,000
HMW
205
116
97
66
Myosin
45
Actin
29
S 1 2 3 4 5 1 2 3 4 5

Figure 13. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis without β-mercaptoethanol (βMCE) reaction of total and soluble protein fractions of 4% salt-soluble proteins (SSP) treated with liquid smoke (LS) fractions (phenols and carbonyls) in a buffered system (0.6M NaCl, 0.05M Na-phosphate buffer, pH 6.5). The concentration of smoke fractions was based on phenol and carbonyl content at the 0.5% LS level. Forty-two ug protein were applied to each sample well of total proteins. For soluble proteins, the load amounts were variable, depending upon solubility (maximum load was 42 ug). Lane S was the molecular weight standard. Lane 1 contained total and soluble proteins of the control (SSP without LS treatment). Lanes 2-5 represent the total and soluble proteins from SSP treated with LS fractions (F, phenols; F, carbonyls; G, phenols and G, carbonyls, respectively).


MW
x1,000
205
116
97
66
45
29
TOTAL
SOLUBLE
Protein
HMW
Myosin
Actin
S 1 2 3 4 5 1 2 3 4 5

about 45 KDa in the resolving gel when SSP were treated with the fractions (Lanes 2-5).

The microstructures of SSP gels in a buffered system treated with LS fractions are illustrated in Figs. 14, 15 and 16. The SSP gels (control) exhibited a dense protein network at both low (2,000 X, Fig. 14A) and high (6,000 X, Fig. 14B) magnifications as described in sec. 4.3.3. (Fig. 8A and 9A). Differences at both low (Fig. 15) and high (Fig. 16) magnifications in microstructure were observed among the four treatments. Globular particles and fine strands made up the microstructure of SSP gels treated with the phenol (Fig 15A) and carbonyl (Fig. 15B) fractions of LS F, and the carbonyl fraction (Fig. 15D) of LS G. The fine strands are made of small globular particles (Fig. 16A, 16B and 16D). The SSP gels treated with the phenolic fraction of LS G exhibited a homogenous and dense structure (Fig. 15C), which consisted of similar sized globular particles without fine strands (Fig. 16C). These gels were high in elasticity. The SSP gels treated with the carbonyl fraction from LS F (Fig. 16B) exhibited a distinct feature: they contained small sized particles and large voids existed within the gel. An interesting observation was that SSP gels treated with carbonyl fractions (Fig. 15B) generally produced smaller globular particles, large voids, non-homogenous and a fine filamentous interconnecting network, which was similar to microstructure of LS F treated SSP gels (Fig. 8B). This type of microstructure was consistent with

Figure 14. Scanning electron micrographs of salt-soluble protein (SSP) gels prepared with 4% SSP in a buffered system (0.6M NaCl, 0.05M Na-phosphate buffer, pH 6.5). The gels were heated at 70°C for 15 min.

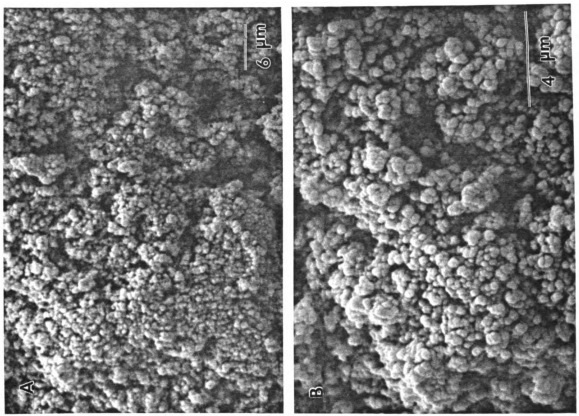

A
6 µm
B
4 µm

Figure 15. Scanning electron micrographs of 4% salt-soluble protein (SSP) gels treated by direct addition of F and G brands of liquid smoke (LS) fractions (phenols and carbonyls) in a buffered system (0.6M NaCl, 0.05M Na-phosphate buffer, pH 6.5), heated at 70°C for 15 min. The concentration of smoke fractions was based on phenol and carbonyl content at the 0.5% LS level. (A) F, phenols; (B) F, carbonyls; (C) G, phenols; (D) G, carbonyls.

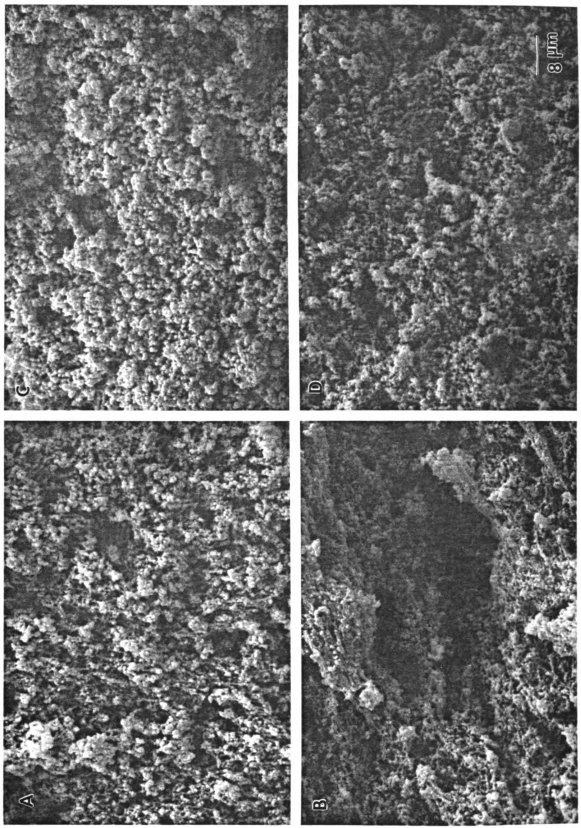
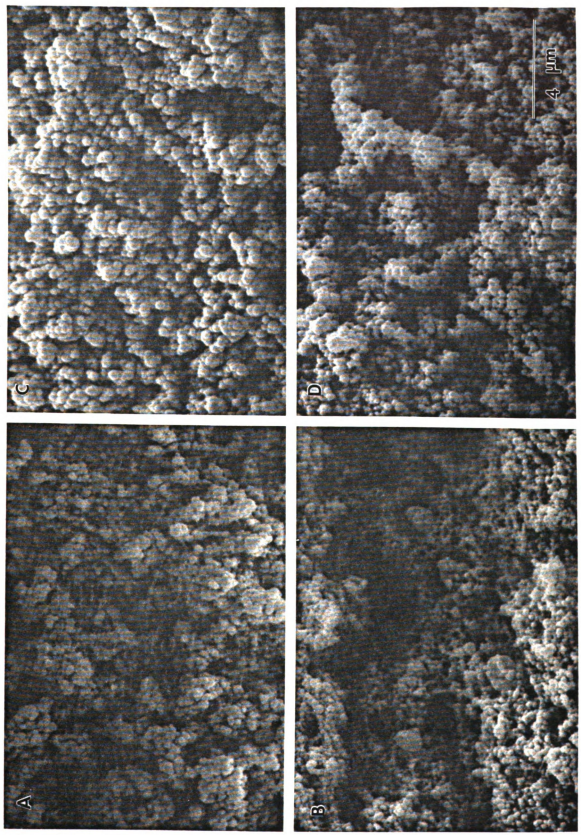

A
B
C
D
8 μm

Figure 16. Scanning electron micrographs of 4% salt-soluble protein (SSP) gels treated by direct addition of F and G brands of liquid smoke (LS) fractions (phenols and carbonyls) in a buffered system (0.6M NaCl, 0.05M Na-phosphate buffer, pH 6.5), heated at 70°C for 15 min. The concentration of smoke fractions was based on phenol and carbonyl content at the 0.5% LS level. (A) F, phenols; (B) F, carbonyls; (C) G, phenols; (D) G, carbonyls.


A
B
C
D
4 µm

lower WHC or moisture retention.

As discussed in sec. 4.1., LS F was high in carbonyls and low in phenols, while LS G had reversed concentrations. By choosing concentrations based upon the concentration of the constituents in the original LS, the results above demonstrated responses similar to those obtained for the original LS treatments. Further work is necessary to study the effect of constituent fractions with the concentration of the fraction held constant.

4.5.2. Liquid Smoke Fractions Added to Salt-Soluble Proteins at a 0.05% w/w Concentration (or 12.5 mg smoke fraction/g protein).

The phenol and carbonyl fractions of LS F and G were added to 4% SSP at 0.05%, w/w to further study the effects on SSP functionality in a buffered system. However, as mentioned earlier (sec. 3.5.1.), many compounds are expected to be present in each fraction because it is a crude fractionation method. Therefore, each fraction was analyzed for carbonyl and phenol contents using the same methods used for LS preparations, and the results are presented in Table 12. These data indicate that the fractionation procedure was not working as anticipated and the constituents were similar to the original commercial LS. The exception was the LS G phenol fraction, where a higher amount of phenols was fractionated. It should be mentioned that the amounts of phenols or carbonyls added to the 4% SSP were increased by a factor of at least two (and as high as six) when

Table 12. Concentration of phenols and carbonyls in liquid smoke (LS) fractions.

Liquid Smoke[1] Fractions	Carbonyls[2] (mg/ml)	Phenols[3] (mg/ml)
F (phenols)	34.9	17.7
F (carbonyls)	53.6	47.1
G (phenols)	32.6	109.3
G (carbonyls)	49.1	69.5

1: Liquid smoke F and G represents two brands of commercial LS. Phenols and carbonyls in parenthesis represents type of LS fractions.
2: Carbonyls measured as 2-butanone.
3: Phenols measured as 2-methoxyphenol.

compared to smoke fractions added based upon the original LS level as reported in previous section.

The results of fractions added at a 0.05% w/w concentration are presented in Table 13. The solubility (89.1%) of the SSP treated with carbonyl fraction from LS F was lower ($p < 0.05$) than other treatments. The SSP gels had the highest ($p < 0.05$) EM (33.1%) and yield (98.6%) values, followed by SSP gels treated with the phenol fraction from LS G. The SSP treated with phenol and carbonyl fractions from LS F and the carbonyl fraction of LS G were lower ($p < 0.05$) in EM and yield when compared with other treatments. Gels treated with smoke fractions from LS F at this concentration had higher ($p < 0.05$) apparent stress than the control. However, higher standard deviations were observed when concentration of smoke fractions increased. One may conclude that even though the fractionation procedure was not working, the constituents were altered causing greater variation. Gels treated with LS F fractions were firmer ($p < 0.05$) than the control. Gels treated with phenolic fractions of LS G had higher ($p < 0.05$) elasticity than the other treatments. The gel hardness patterns of all treatments were similar with their yield. This can be partially explained by the formation of a denser protein network due to the concentration effect of SSP during cooking.

One can conclude that smoke fractions caused changes in functional properties of SSP gels. Treatments with high

Table 13. Solubility, expressible moisture (EM), yield, apparent stress and apparent strain for 4% salt-soluble proteins (SSP) gels in 0.6M NaCl and 0.05M Na-phosphate buffer, pH 6.5 treated with liquid smoke (LS) fractions at a 0.05% w/w concentration.

Treatments[1,2]	Solubility[3,4] (%)	Expressible[3] Moisture(%)	Yield[3] (%)	Apparent[3] Stress (KPa)	Apparent[3] Strain
SSP	96.2±0.2^{a}	33.1±0.4^{a}	98.6±0.7^{a}	54.6± 1.4^{c}	1.54±0.02^{ab}
F (phenols)	95.5±0.7^{a}	20.1±0.8^{d}	80.4±0.8^{d}	105.3± 7.4^{a}	1.53±0.00^{b}
F (carbonyls)	89.1±2.4^{b}	20.9±1.0^{cd}	81.5±1.9^{d}	95.4±18.1^{ab}	1.53±0.01^{b}
G (phenols)	95.0±1.5^{a}	30.1±0.8^{b}	94.1±1.1^{b}	72.8± 9.8^{bc}	1.59±0.04^{a}
G (carbonyls)	94.2±0.6^{a}	22.7±0.4^{c}	84.4±0.6^{c}	81.5±15.0^{abc}	1.50±0.03^{b}

1: SSP represent control without the addition of LS fractions. Liquid smoke F and G represent two brands of commercial LS. Phenols and carbonyls in parenthesis represent the type of the LS fraction added to SSP.
2: Concentration of LS fraction (contain 10% Tween 20) on 4% SSP based on 0.05% w/w smoke fraction addition (or 12.5 mg LS fraction / g protein).
3: Means (n=3) within columns followed by the same letter do not differ significantly ($p > 0.05$).
4: ± = Standard deviation.

carbonyl content (phenol and carbonyl fractions of LS F and the carbonyl fraction of LS G) generally increased the protein-protein interaction by decreasing protein-solvent interaction as indicated by low moisture retention and high apparent stress. These observations agree with those discussed by Hermansson (1983). Further, a non-homogenous gel network made up of smaller globular particles and fine strands was produced. These observations agreed with those discussed by Means and Feeney (1971) that carbonyls can react with amino groups of proteins, causing crosslinking and conformational changes of proteins. One can postulate that when overcrosslinking occurs, protein-solvent interaction decreases and a non-homogenous gel network with large and irregular voids is produced. The mechanism for the formation smaller globular particles observed in the microstructure of treatments is not known and needs further investigation.

SSP treated with LS high in phenols or the phenolic fraction low in carbonyl content generally produced firm, elastic gels and with only a slight decrease in moisture retention. Higher molecular weight proteins remained soluble and formed a dense, homogenous gel microstructure. These results indicate that phenols and proteins may interact. This interaction may be through the thiol groups of proteins as discussed by Krylova et al. (1962). However, further study is needed to prove that components in LS cause changes in SSP through these mechanisms.

4.6. EFFECTS OF LIQUID SMOKE ON LOW FAT FRANKFURTERS

The raw batters or frankfurters contained 10.4% protein, 10.1% fat, 74% moisture and/or 0.5% w/w LS (F or G). After cooking, protein and fat content of the frankfurters differed among treatments ($p < 0.05$) (Table 14). Moisture content was not different ($p > 0.05$) from the control. Although lower protein values (LS G) and lower fat (LS F) were observed, the differences are small and within normal limits of frankfurter production.

The pH of frankfurters containing LS F was slightly lower ($p < 0.05$) than the control (Table 15). Cooked yield (Table 15) of the frankfurters with LS did not differ ($p > 0.05$) from the control. Reheat yield was significant ($p < 0.05$) among treatments. The control had highest ($p < 0.05$) reheat yield among treatments, and the lowest reheat yield was observed in frankfurters with LS G addition. These results agreed with those observed by Sink and Hsu (1977) where LS incorporated into the meat emulsion had significantly lower tenderness and juiciness scores than the control. Shear stress and shear strain at failure did not differ ($p > 0.05$) between treatments (Table 15). However, frankfurters containing LS F (23.0 KPa) were firmer than the control (19.4 KPa). Frankfurters with LS F had higher ($p < 0.05$) skin strength than G but each were similar to the control. It was an interesting trend that frankfurters containing LS retained less fat both after cooking and reheating. A thin layer of solid fat was observed on the

Table 14. Proximate composition of low fat frankfurters and reheated frankfurters before and after reheating.

Treatments[1]	Protein[2,3] (%)	Fat[2] (%)	Moisture[2] (%)
		Frankfurters	
Control	12.87±0.2[a]	12.36±0.0[a]	69.98±0.3[a]
Smoke F	12.73±0.2[ab]	11.21±0.4[b]	70.27±0.2[a]
Smoke G	12.54±0.0[b]	11.95±0.2[a]	70.14±0.2[a]
		Reheated Frankfurters[4]	
Control	14.36±0.1[c]	14.24±0.2[a]	67.15±0.1[a]
Smoke F	15.61±0.1[b]	13.57±0.8[a]	64.32±0.1[b]
Smoke G	16.07±0.1[a]	13.60±0.5[a]	63.26±0.1[c]

1: Control represents the frankfurters without LS addition. Smoke F and G represents two brands of commercial LS.
2: Means (n=3) within columns followed by the same letter do not differ significantly ($p > 0.05$).
3: ± = Standard deviation.
4: Reheated frankfurters represented approximate composition of cooked frankfurters after heating in boiling water for 10 min. The reheat yield of treatments were 89.3, 80.3 and 76.5 % for control, smoke F and G, respectively.

Table 15. pH, cooked yield, reheat yield, stress, strain and skin strength of low fat frankfurters with 0.5% (w/w) liquid smoke (LS) addition.

Treatments[1]	pH[2,3]	Yield[2,4] (%)	Reheat[2] Yield (%)	Stress[2] (KPa)	Strain[2]	Skin[2] Strength (N)
Control	6.60[a]	86.6±1.2[a]	90.5±0.9[a]	19.4±0.6[a]	1.30±0.07[a]	6.4±0.4[ab]
Smoke F	6.52[b]	88.7±0.1[a]	84.5±2.6[b]	23.0±2.2[a]	1.37±0.04[a]	7.2±0.6[a]
Smoke G	6.57[ab]	87.3±0.8[a]	77.1±1.1[c]	19.4±1.5[a]	1.26±0.07[a]	6.1±0.2[b]

1: Control represents the frankfurters without LS addition. Smoke F and G represent two brands of commercial LS.
2: Means (n=3) within columns followed by the same letter do not differ significantly ($p > 0.05$).
3: pH of treatments were measured from cooked frankfurters.
4: ± = Standard deviation.

surface of the products containing LS when stored in plastic bags at 2°C. This phenomenon was not observed with the controls. Further, frankfurters containing LS had lower ($p < 0.05$) reheat yield due to low moisture and fat retention capacity (Tables 15 vs 14). Only moisture loss was observed in reheated control. From these results, one can postulate that LS incorporated into meat emulsions decreased the ability of proteins to efficiently hold the moisture and fat. The exact mechanism of chemical reactions between smoke components and meat proteins is not known in a complex batter system and needs further study.

CONCLUSIONS

Acid, carbonyl and phenol content were measured in three commercial LS. Liquid smoke F contained high concentrations of carbonyls and acids while LS G was high in phenols. Liquid smoke treatments at three concentrations (0.125%, 0.25% and 0.50% w/w) were highly variable due to variability of SSP extracted from batch to batch of chicken. Most functional properties were not significantly different among treatments because of the variability. Studies were designed to eliminated batch variation of SSP.

In a buffered system, LS F decreased SSP solubility, and increased the amount of high molecular weight proteins which were observed using SDS-PAGE. The gels were low in yield and elasticity, but high in hardness values. Gel microstructure from these treatments contained a fine stranded interconnecting network, irregular grape-like clusters and voids. Liquid smoke H produced an uneven microstructure distribution and with protein gels low in elasticity. Liquid smoke G did not reduce solubility of SSP and produced a dense microstructure similar to the control. However, high molecular weight proteins were observed with all LS treatments. Liquid smoke G also produced firm gels without decreasing in elasticity. These results indicate

that LS caused changes in functional properties of SSP. Differences in carbonyl or phenol content of the LS may partially explain these observations. The decrease in moisture retention and increase in protein concentration of the gel may also be partially responsible for the increase in gel hardness.

In non-buffered systems, LS F which was high in acids and carbonyls caused tremendous changes in solubility and functionality of SSP. A loose and highly aggregated microstructure was evident in the subsequent gels. This observation was consistent with poor gel strength and moisture retention. Liquid smoke G did not decrease SSP solubility and produced a dense gel microstructure which was high in hardness and elasticity values. Liquid smoke H decreased the functional properties of SSP including solubility, moisture retention and elasticity of the proteins.

Effects of reaction time between SSP and LS on protein solubility and gelling properties in a non-buffered system indicate that most functional properties were changed during a short reaction time (6 hrs or less). Therefore, a 24 hr reaction time for LS treatments was adequate. SSP gels without LS addition improved in hardness over time indicating an ordered network structure may be produced upon standing.

The effects of LS constituents on characteristics of SSP at concentrations based on 0.5% w/w LS addition resulted

in the carbonyl fraction of LS F decreasing solubility of the SSP and moisture retention of the gel, but increasing in firmness of the gels. Smaller globular particle size and large voids existed in the microstructure of the gel. The phenol fraction of LS G had a response similar to the control and higher high molecular weight proteins were observed using SDS-PAGE. It was concluded that LS or smoke fractions high in carbonyls decreased the solubility and gelling properties of SSP. High molecular weight proteins were observed. Smaller globular particles, fine strands and large voids in gel microstructure were also documented. Liquid smoke or smoke fractions high in phenols produced a dense, homogenous gel microstructure with higher hardness and elasticity values.

Low fat frankfurters prepared containing 0.5% w/w LS had lower reheat yields when compared to the control. Lower fat and moisture retention was noted in frankfurters containing LS. Skin hardness was different in frankfurters treated with LS F.

FUTURE RESEARCH

Suggestions for future work include:

1. Determine the influence of pH on phenol-protein interaction when incorporating LS high in phenols directly into SSP.
2. Measure the changes of protein sulfhydryl groups before and after direct addition of LS into SSP and/or frankfurter.
3. Examine the microstructure of LS treated SSP by cryo scanning electron microscopy to determine the network structure of SSP before heating.
4. Characterize SSP before and after LS addition using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with carboxymethylate reagent and without β-mercaptoethanol.
5. Study the effect of pure phenols and carbonyls on SSP.
6. Evaluate the effect of direct addition of LS on commercial frankfurter formulations.

APPENDIX

Appendix A. Solubility, expressible moisture (EM) and yield for 4% salt-soluble proteins (SSP) gels in 0.6M NaCl and 0.05M Na-phosphate buffer, pH 6.5 treated with liquid smoke (LS) fractions based on a 0.5% w/w LS level.

Treatments[1,2]	Solubility[3] (%)	Expressible[3] Moisture(%)	Yield[3] (%)	Apparent[3] Stress (KPa)	Apparent[3] Strain
SSP	99.4±0.2[a]	31.2±0.8[a]	98.0±0.2[a]	45.3±6.5[a]	1.55±0.01[a]
F (phenols)	98.8±0.1[a]	33.7±0.9[b]	97.2±1.0[ab]	65.3±9.4[a]	1.45±0.03[a]
F (carbonyls)	88.1±3.9[b]	24.3±0.5[c]	86.4±0.6[c]	64.9±4.8[a]	1.42±0.03[a]
G (phenols)	98.0±0.6[a]	29.7±1.4[a]	97.2±1.2[ab]	61.2±6.2[a]	1.49±0.04[a]
G (carbonyls)	98.3±1.2[a]	29.1±0.4[a]	94.9±0.6[b]	55.0±7.5[a]	1.49±0.02[a]

1: SSP represents control without the addition of LS fractions. Liquid smoke F and G represents two brands of commercial LS. Phenols and carbonyls in parenthesis represent the type of the LS fraction added to SSP.
2: Phenol and carbonyl fractions were added to 4% SSP based on the constituent in the original 0.5% LS treatment level.
3: Means (n=3) within columns followed by the same letter do not differ significantly ($p > 0.05$).
4: ± = Standard deviation.

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