

THE EFFECTS OF THERMAL PROCESSING,
MIXING TEMPERATURES AND MIXING
TIMES ON TEXTURAL CHANGES
IN PEPPERONI

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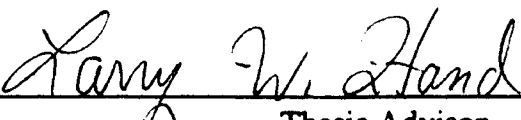
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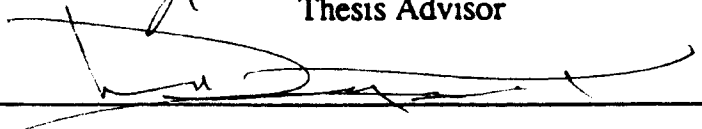
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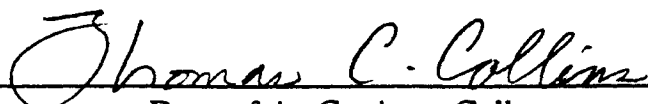
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CHAPTER I

INTRODUCTION

Approximately 390 million pounds of dry sausage are sold annually in the United States, with pepperoni being the largest volume variety produced (Nunes, 1991). The largest buyers of the pepperoni are pizza restaurant chains, where pepperoni reigns as the most requested pizza topping. The top three pizza chains in the United States (Pizza Hut, Domino's and Little Ceasars) combined for over \$9.4 billion in sales in 1990, with sales continuing to expand (Anonymous, 1991). One of the difficulties of producing a large volume of pepperoni or other dry sausages is that the process is capital and technology intensive. The manufacture of dry sausage takes large amounts of equipment such as grinders, mixers, stuffers and smokehouses, but unlike other sausage products, fermentation rooms and drying rooms are also needed (Deibel, 1974). In addition to the large amount of equipment necessary, the process of dry sausage manufacture from raw materials to a finished product can take up to two months for some specialty sausages such as San Francisco style pepperoni (Terrell et al., 1977). Since the drying of such products can take weeks, pepperoni manufacturers are faced with large, expensive inventories. With such a large amount of inventory on hand, product that does not meet the customer's specifications can be extremely costly to the manufacturer.

One of the major problems in the production of pepperoni is a textural change referred to as cupping, or the curling of pepperoni slices when they are cooked on pizzas

(Newkirk et al., 1993). Cupping is not acceptable to the consumer, therefore restaurant chains require manufacturers to produce pepperoni which lies flat when heated on pizzas. Although several studies have been done on the processing parameters and drying time of dry and semidry sausages (Acton and Keller, 1974; Townsend et al., 1974; Wardlaw et al., 1973; Palumbo et al., 1976), no research has been published on what factors cause textural changes in pepperoni. Therefore, this study investigates the manufacturing parameters of mixing time, mixing temperature and heating on textural changes and cupping in pepperoni.

CHAPTER II

REVIEW OF LITERATURE

Pepperoni Manufacture

Pepperoni is classified as a dry sausage with a moisture to protein ratio of 1.6:1 (USDA, 1983). In a review of dry sausage manufacturing practices, Terrell et al. (1977) categorized pepperoni to have a moisture content between 30 and 36 percent with a pH between 4.7 and 5.0 and a titratable acidity as expressed in lactic acid from 0.4 to 1.5 percent. Made from a combination of beef and pork, the manufacture of pepperoni begins with particle reduction by either grinding or chopping, then proceeds to mixing and regrounding (if a chopper is not used), stuffing, fermentation, and drying. This initial protein extraction step is necessary to provide binding between the meat particles, but should not be excessive so as to cause an increase in the product's ability to bind water, or to cause textural changes upon drying.

Meat proteins in dry sausages are not extracted to develop the same functional properties desired in emulsified products. Solubilization of the salt-soluble myofibrillar proteins is necessary but not to the extent found in comminution (Acton et al., 1977). In an emulsion system, proteins are extracted so as to maximize water binding (Rust and Olson, 1988), but dry sausage systems are not concerned with binding water. Rather,

excessive protein extraction is a major concern when considering the texture and drying time of dry sausages (Keller et al., 1974).

During grinding and mixing muscle fibrils are cut and the sarcolemma is disrupted, thus allowing myofibrillar proteins to be exposed (Hamm, 1975). These proteins then act to bind together pieces of meat when subjected to heating (Klement et al., 1973; MacFarlane, 1977; Siegel and Schmidt, 1979) or drying (Sokolov and Tchkovskaya, 1971). This binding of meat particles leads to firmness development and other textural changes in dry sausages. Two of the factors that affect protein extraction are mixing time and mixing temperatures, and these factors will be dealt with separately in this review.

Mixing Time

There are several reasons mixing time is important in the manufacture of dry sausage. One of the most important factors is controlling over mixing and the loss of particle size (Terrell, 1977). Yet, a certain degree of mixing is necessary to distribute ingredients throughout the batch and to extract some myofibrillar proteins which are important in the development of sausage texture. Mechanical action such as mixing shears muscle fibers and exposes myofibrillar proteins. These proteins can also function in the sausage system to contribute bind as the proteins denature or become insoluble (Sokolov and Tchkovskaya, 1971). Therefore, an increase in mechanical action by extending mixing time would be expected to expose more myofibrillar proteins and therefore alter the textural properties of dry sausages by allowing more proteins to contribute binding sites in the sausage system.

Although Acton et al., (1977) stated that research needed to be performed on the effect of mixing time on textural changes in dry sausages, no data has been published on the subject. Extensive work has been done on how mixing affects restructured meat products and boneless hams. Booren et al. (1981b), in a study on the effect of vacuum mixing on restructured beef steaks, found that adhesion between meat pieces increased

($P < 0.05$) as mixing time increased from 6 to 12 minutes. The same study also found that protein exudate increased as mixing time increased ($P < 0.05$), which indicates more protein extraction is occurring with increased mechanical action. In a similar study, Booren et al. (1981a) reported that as mixing time for sectioned and formed beef steaks increased from 0 to 18 minutes both Kramer shear values and Kramer shear work (area under the energy curve) decreased ($P < 0.05$), indicating increased mechanical action on the sectioned pieces, making the steak more tender. In 1981, Booren et al. characterized the exudate proteins involved in binding meat pieces in sectioned and formed beef steaks. They found that adhesion and sensory bind values increased linearly ($P < 0.01$) from 0 to 12 minutes of mixing, but showed no increase after 18 minutes (quadratic effect, $P < 0.01$). Booren et al. stated there was over 10 times as much exudate at the bond area after 12 minutes of mixing when compared with 0 minutes of mixing. In the same study, the researchers reported an increase in myosin as a percent of the total protein in the exudate from 0 to 12 minutes of mixing and stated that the maximum binding is achieved in sectioned and formed beef steaks after 12 minutes of mixing.

Booren et al. (1981c) also investigated the effects of blade tenderization, vacuum mixing, salt addition and mixing time on binding in sectioned and formed beef steaks. This study concluded that Instron adhesion measurements increased ($P < 0.05$) from 8 to 16 minutes of mixing, but showed no increase ($P > 0.05$) from 16 to 24 minutes while Kramer shear force decreased from 0 to 24 minutes of mixing ($P < 0.05$). This study found that mixing increased the protein at the bond area causing an increased binding effect after 8 minutes of mixing.

Temperature

Although it has been suggested that temperature control during the mixing and stuffing of dry sausages is critical, no research has been published on the subject. According to Terrell et al. (1977), one of the most critical factors in the manufacture of

dry sausage is temperature control during grinding and mixing to ensure product quality in terms of particle size and textural properties. Terrell stated that meats need to be kept between 28 and 34°F to reduce smearing, a condition where fat covers the meat particles upon mixing. Smearing can also occur during stuffing when a layer of fat coats the inside of the casing, inhibiting the drying of the product and leaving an unappealing coating on the sausage. Meat must be kept as cold as possible to prevent smearing during stuffing (Terrell, 1977). Mandigo (1974) in a discussion of flaking temperatures for restructured meat products stated: "... as you get up into the 25-26°F range, you start getting a lot of smearing, call it smearing of the flakes."

A significant amount of research has been done on chopping temperatures in emulsion systems. Many researchers have documented an increase in water-binding capacity in emulsion systems as chopping temperatures increase. Puolanne et al. (1985) found that water binding capacity increased as chopping temperatures increased from 12 to 16°C for a frankfurter-type sausage. However, increasing chopping temperatures past 15°C may actually decrease bind and water holding capacity. Sutton et al. (1994) reported that frankfurters chopped to 15°C were firmer, and had greater binding properties than those chopped to 30 or 45°C. Although the manufacture of emulsified products is affected greatly by chopping temperatures, those temperatures are much higher than the preferred grinding, mixing and stuffing temperatures for dry sausage products of less than 0°C.

Though little work has been done on the grinding and mixing temperatures in dry sausages, temperature has shown an effect in other non-emulsion systems. Mandigo (1974) investigated the effect of salt content and flaking temperature on flaked, formed and sectioned meat products. Consumer panelists in that study did not indicate a preference between products flaked at 2.5° or -5.6°C. Mandigo (1974) also states that cold flakes (24°F) are important in providing the desired texture of the finished product

for restructured steaks. Popenhagen and Mandigo (1978) found that flaking temperatures of -5.6°C are more desirable than flaking at 0.6°C in flaked and formed steak products.

Additionally, Hansen and Mandigo (1972) studied the use of warm prerigor pork combined with pork tempered to -4.4°C and manufactured into formed and sectioned steaks. They found that as the ratio of warm pork to cold pork in the steaks increased, fat stability (in milliliters released during cooking) increased and drip loss during cooking decreased. This effect could be attributed to the use of increasing amounts of prerigor pork, but flaking temperature may also have affected the amount of protein extraction.

Fermentation, thermal processing and drying

Acton et al. (1978) stated that firmness development in fermented, dried sausages is related to three events during processing: pH reduction during fermentation, heat input during thermal processing and moisture removal during drying. Each of these steps is critical in the production of pepperoni, and all serve to make dry sausage unique from other types of processed meats. Each will be dealt with in a separate section in this review.

Fermentation

Historically, fermentation originated with the addition of sugar and salt to ground meat and was followed with a holding period during which developed a desired flavor and texture in addition to enhanced preservation of the sausage. During this holding time, bacteria in the natural flora of the meat converted the sugar added to the sausage to lactic acid, thereby lowering the pH of the sausage and increasing the keeping qualities of the sausage due to the elimination or limited growth of spoilage and pathogenic bacteria as a result of the lower pH. These "wild" fermentations utilized the bacteria in the natural flora

of the meat. This fermentation technique often resulted in production failures such as the development of off flavors and casing breakage from the production of unwanted gas by the bacteria in the product (Diebel, 1974). A technique to control these fermentations by adding a portion of a freshly fermented meat batch back to a new batch of sausage was also common before the use of starter cultures. (Deibel, 1974; Everson et al., 1970). A version of this technique, called "backslopping" is still used by one major dry sausage manufacturer in the US. (Campano, 1993).

Numerous changes in the way fermented sausages have occurred since 1940 when Jensen and Paddock (1940) first introduced a starter culture utilizing various strains of lactobacilli to add to meat products. A major step forward in obtaining uniform fermentations occurred in 1957 with the introduction of lyophilized starter cultures consisting of the bacterial species *Pediococcus cerevisiae*. This technology was developed by the dairy industry, but many of the dairy-type starters did not grow in meat mixtures due to their lack of tolerance to salt and/or nitrite (Diebel, 1974).

There are three commercial forms of starter cultures currently used in the meat industry: (1) freeze dried concentrates, where the method of maintaining cell viability is lyophilization; (2) frozen concentrates, where the method of maintaining cell viability is by the use of temperatures less than 0°F; and (3) low temperature stabilized concentrates, where cells are maintained with high concentrates of sugars. Most of the commercial cultures sold in the United States are in the frozen concentrate form (Haymon, 1984), and commonly contain *Pediococcus acidilactici*, *Pediococcus* sp, *Micrococcus* sp, *Lactobacillus plantarum* and other genera and species (Anonymous, 1988; Everson, 1970; Haymon, 1984). Frozen concentrate cultures, introduced in 1968, were designed to have fermentation temperatures from 100-110°F at relative humidities of 90% with fermentation times of 15-20 hours (Everson et al., 1970). After fermentation, the sausage may be heated to a higher temperature, with 137°F being typical (to ensure trichina elimination), then moved to the drying room.

For these starter cultures to meet the needs of today's dry sausage processor and to ensure the safety of the product, the cultures need to meet these standards as described by Diebel (1974)

1. It must be tolerant of salt and grow vigorously in the presence of at least 6.0% sodium chloride.
2. It must grow in the presence of at least 100 micrograms/g of nitrite.
3. It must grow in the range of 80-110°F, preferably with an optimum around 90°F.
4. It must be homofermentative. Gas production and fermentation products other than lactic acid are not desirable.
5. It must not be proteolytic or lipolytic.
6. It must not produce any compounds that are associated with off-flavors such as amines or sulfides.
7. It must not be harmful to health.

These requirements are necessary for dry and semi-dry sausage products to meet the Good Manufacturing Practices published by the American Meat Institute (AMI, 1982). These GMP's stress that dry and semi-dry sausages must attain a pH of below 5.3, and set forth time-temperature relationships for fermentation. These guidelines were established for microbial control in dry and semi-dry sausages, with one of the major concerns being the control of coagulase positive *Staphylococcus aureus* (Wilson, 1982). Everson et al. (1970) reported that with the use of frozen concentrate starter cultures, fermentation times can be as short as 18-24 hours, a substantial reduction in the 3-5 days for fermentation required by early processors to obtain the same type of pH drop and lactic acid production (Wardlaw et al., 1973).

Besides providing extended shelf life and microbial protection due to low pH, fermentation causes textural and other changes in dried sausages. These changes can be attributed to the production of lactic acid in the sausage by the lactic acid bacteria in starter cultures. Wardlaw et al. (1973) in a study on the changes in meat components of a dried summer sausage, reported a decrease in pH from 6.05 to 4.85 during fermentation

with a corresponding increase in lactic acid from 0.0 to 0.47% after 36 hours of fermentation at 38°C. Acton and Dick (1976) found in a survey of commercial dry sausage products that pepperoni obtained at retail had pHs ranging from 4.95 to 5.77, with total acidity as expressed in percent lactic acid ranged from 0.94 to 1.68%.

This increase in lactic acid causes textural changes during the fermentation stage, namely the development of firmness in the sausage. One of the main concerns of pepperoni processors is the texture changing effects on the sausage with decreasing pH. Since lowering the pH has been shown to result in some measure of protein insolubility, fermentation becomes a step of major importance to processors. In a study on the association of protein solubility with physical properties in summer sausage, Klement et al. (1973) found that as the pH values for the sausages dropped during fermentation (from 5.3 to below 4.9), shear press values increased. All batches of sausage in that experiment that reached a final pH of below 5.0 were significantly different ($P < 0.01$) from the control sausages (pH 5.3). The data showed a significant drop ($P < 0.01$) of solubility for all samples as the internal temperature of the sausage changed from 6°C to about 37°C. They concluded that as pH declined at a constant temperature, the solubility of the myofibrillar proteins decreased and in turn the shear values (firmness) of the sausages increased.

Wardlaw et al. (1973) reported a 36% increase in the total insoluble protein nitrogen fraction of a fermenting sausage mix and stated that the sarcoplasmic fraction was more rapidly denatured during the 38°C fermentation than the myofibrillar protein fraction. Wardlaw stated that the low temperature heating (38°C) over the 36 hour fermentation apparently provided a continued thermal energy input which resulted in the substantial denaturation of muscle protein fractions. Similarly, Klement et al. (1973) reported decreases in the solubility of myofibrillar proteins of fermenting sausages ranging from 50-60% and sarcoplasmic proteins ranging from 21 to 47% as pH declined from 5.3 to either 4.9 or 4.6 at 37°C. Klement et al. (1974) also investigated the effect of bacterial fermentation on protein solubility in a sausage model system. In that study, the

researchers reported that as pH declined from 6.2 to 5.5, a decrease in solubility of about 35% occurred for the myofibrillar proteins. As pH dropped from 5.5 to 4.6, an additional reduction in solubility of 25% was noted. In the same study, Klement et al. (1974) reported that in unfermented control myofibrillar muscle fractions, no change in the solubility occurred during 46 hours of incubation at 37°C and only a 10% decrease in the solubility of the sarcoplasmic protein fraction occurred during the same incubation period.. This shows that a decrease in pH must accompany mild heating (37-38°C) to render protein fractions insoluble, whereas incubation alone to 37°C does not seem to affect myofibrillar protein solubility, and only slightly decreases sarcoplasmic protein solubility. The work of Brendall and Wismer-Pederson (1962) substantiates this by finding a combination of low pH and high temperature precipitates sarcoplasmic proteins into the myofibrils. In addition, Trautman (1964) reported that the effect of decreasing pH was linear on the solubility of both water soluble and salt soluble proteins, while Deng et al. (1976) found in solutions of mackerel and beef actomyosin, interactions between proteins increased as pH decreased from 6.8 to 5.8. A study on the manufacture of hams by Trautman (1964) also found that while myofibrillar proteins were completely soluble at pH 5.9 they were 90% insolubilized at pH 4.9.

Another factor researched by Klement et al. (1975) is the change over time in a protein solution at specific pHs and temperatures. In this study, Klement and researchers looked at the effect of direct acidification and heat on the solubility of proteins from a fermented sausage mix. This research added lactic acid to meat batches to drop the pH of protein mixtures to either 4.8 or 5.3 before placing the mixtures in a 37°C water bath for 24 hours. The group had two conclusions: 1) sarcoplasmic proteins in solution were insolubilized slightly by acid conditions alone, and 2) at pH values in the range 4.6 to 6.2, solubility was affected by holding time. The solutions reached 35 to 37°C after 1.5 hours and all samples showed a marked decrease in solubility after the first 1.5 hours of heating.

They also noted that myofibrillar proteins are more susceptible to irreversible changes in their solubility due to acid treatment than are sarcoplasmic proteins.

Since pepperoni is a dry sausage, a low pH is also desirable due to the reduction of water holding capacity (WHC) as pH decreases. This decrease in WHC makes the drying process more efficient since there is less water in the matrix to be removed. Hamm (1960) stated that the WHC minimum in fresh beef occurs near pH 5.0, corresponding to the approximate isoelectric point of actomyosin. In a study on the effect of fermented meat pH on summer sausage properties, Acton and Keller (1974) found that as sausage pH decreased, WHC decreased, reaching a minimum at pH 5.2. They suggested that some of the meat protein remained functional to bind moisture and was not completely denatured at this pH.

Thermal Processing

In traditional dry sausage manufacturing systems, pepperoni is taken directly from the fermentation stage to drying. This method relies on a combination of low pH, salt content and low water activity (A_w) to control pathogens. Using this method, processors must meet USDA regulations for drying time/temperature/salt content to ensure the control of trichinae. Trichinae are nematodes which enter the digestive system of swine and subsequently deposit larvae which then burrow into pork muscle and can get into the food supply through non-certified pork (Baccus, 1984). The time/temperature treatments consider casing diameter and are based on an initial salt content of 3.33 pounds per 100 pounds of meat and a maximum meat particle size of .75 inch (USDA, 1983).

Another method to control the incidence of trichinae is to heat products containing pork to 60°C (137°F) (USDA, 1983). At this temperature, the trichinae cysts are killed, thereby freeing processors from the USDA's time/temperature requirements, and allowing manufacturers to dry the pepperoni as quickly as possible to obtain an acceptable product at a moisture:protein ratio of 1.6:1. However, this is less than the 66°C to 71°C heat treatment generally applied to fully cooked sausages (Acton et al., 1978).

One of the major problems with heating dry sausages to 60°C is that textural changes occur, some of which may be unacceptable to consumers. Acton et al. (1978) states that while initial firmness develops during the fermentation stage, additional protein denaturation occurs in sausages which are subjected to subsequent thermal processing steps. Hamm and Deatherage (1960) showed that between 20°C and 40°C, a marked decrease of the solubility of structural (actin, myosin and actomyosin) proteins occurred, but the greatest decreased was observed when heating between the 40°C and 60°C range. Beyond 60°C the structural proteins become almost insoluble. Hamm and Deatherage also showed that meat heated to 45°C at a pH of 5.0 maintained 35 percent of its water holding capacity, while meat heated to 60°C at a pH of 5.0 only held 20 percent of its bound water. This fact is important when considering the drying of sausages, as products heated to 60°C will release water quicker than non-thermal processed products, thereby giving decreased drying times.

Of the muscle proteins, myosin has been shown to have the highest binding ability when compared with actomyosin and sarcoplasmic proteins (Macfarlane et al., 1977). In a study on the use of myosin fractions as meat binders in restructured steaks, Siegel and Schmidt (1979) reported that the ability of myosin to bind meat pieces was absent at final internal temperatures below 45°C. Increasing temperature to 80°C had a linear effect ($P<0.01$) on binding ability. In a study on the changes in soluble protein fractions in fermented dried sausages, Acton et al. (1978) reported a significant decrease ($P<0.05$) in the percent of extractable salt soluble and water soluble protein between the end of fermentation phase and the end of thermal processing. During the same time frame, percent moisture decreased ($P<0.05$), but the percent protein did not change ($P>0.05$). Shear values also increased ($P<0.05$) during the same period.

Palumbo et al. (1976) studied the specific effect of heat treatments on the percent yield of pepperoni dried for 42 days and found that unheated treatments (fermented at 35°C) displayed the lowest yield (46.05%) after 42 days, while treatments heated to 49°C

had increased yields ($P<0.05$) at 47.7%. Treatments thermal processed to 55.5 or 60°C showed higher yields (48.9% and 49.6% respectively) than the 49°C treatments ($P<0.05$), but those treatments were not different from each other ($P>0.05$). Acton et al. (1978) also found that upon heat processing to 60°C, fermented, dried sausages lost 7.68 percent of their initial weight. This loss was attributed to a significant ($P<0.05$) loss of moisture during the thermal processing phase. In contrast, Townsend et al. (1980) reported during the processing of non heat treated pork Genoa salami, that percent moisture content did not change ($P>0.05$) after from directly after fermentation to seven days of drying.

According to Acton and Dick (1975), heating a dry fermented turkey sausage to 71°C resulted in a product with an unacceptable dry texture. These researchers suggested heating this type of sausage to 46°C if it is to be fully dried to a moisture:protein ratio of 1.9:1.

Drying

Some of the unique properties of pepperoni and other dry sausages are imparted during the drying phase of sausage manufacture. Drying is performed to impart characteristics such as desired flavor and texture and meet UDSA requirements for the destruction of trichinae (Palumbo et al., 1976). It has been reported that non cooked sausage drying was performed at a temperature of 55 to 65°F with a relative humidity of 65 to 75% for up to 21 days (Terrell et al., 1974). With the current computerization of drying rooms resulting in more exacting control over the drying process, processors can dry pepperoni in 12 days without incurring detrimental textural changes. If sausages are dried too slowly, yeast and mold can grow on the surface producing an undesirable product. In contrast, if the product is dried too quickly, case hardening can result. Case hardening is the formation of a brown, hard ring on the outside of the sausage stick (Terrell, 1974; Palumbo et al., 1976). This case hardening can result in moisture being

trapped inside the product, reducing moisture migration to the surface and possibly incurring spoilage of the interior of the product.

As sausages lose moisture during drying, percent yields decrease while firmness of the product increases (Palumbo, 1976; Acton et al., 1978). Palumbo et al. (1976) studied the difference during drying between heated (60°C) and non heated pepperoni. They found that both treatments lost the same amount of weight on a percentage basis. However, in a subsequent study, Palumbo et al. (1976) reported that pepperoni that was not heated had a lower yield (46.05%) than pepperoni heated to 60°C (49.6%). They hypothesized that the fat in the sausage is melted by heating, thus coating the meat particles and hindering the movement of moisture from the heated sausages to the environment.

Several researchers have reported that shear force values for dry sausages increase as during time increases and percentage moisture decreases. Acton and Dick (1975) reported in a study on dry, fermented turkey sausage that shear values were highly and significantly correlated ($P < 0.05$) with moisture level ($r = -0.97$) and sausage weight loss ($r = -0.98$). They reported that shear force and weight loss displayed linear responses ($P < 0.01$) over the 12 day drying period. Wardlaw et al. (1973) also found an increase ($P < 0.05$) in shear force during the drying of 1:1 beef to pork ratio summer sausage. This increase was highly correlated ($r = -.90$) with a decrease in percentage moisture. In an investigation into the feasibility of adding freeze-dried meat to fermented sausage systems, Lu and Townsend (1973) stated that sausage firmness increased with drying time, the order of firmness was the same as that of the Warner-Bratzler Shear. They also found that Warner-Bratzler Shear results were irregular due to the pieces of connective tissue catching between the blades of the shear, thus giving higher shear values.

However, in investigating the effects of particle size on summer sausage properties during drying, Keller et al. (1974) reported no apparent relationship between sausage moisture content and sample shear force. They did find that particle size affects drying

rate, with sausages made from meat ground through a 6 mm plate reaching the dry sausage stage (35-40% shrink) after 30 days. Sausages manufactured with particle sizes of 3 mm and 6 mm reached the dry stage after 35 days, while products made from 6 mm and 9 mm particles did not reach 35% shrink at 45 days. The researchers concluded that meat packs differently upon mixing and stuffing, causing differences in drying times. In the second portion of this same study, Keller et al. studied the effect of casing diameter on drying rates. The group showed that after 30 days of drying, a 6.4% difference in moisture content existed between sausages stuffed in 52 mm and 73 mm diameter casings. Also, sausages stuffed in 52 mm casings displayed greater hardness as determined by shear force than products stuffed in 62 or 73 mm casings.

Although firmness development has been related to the loss of moisture in dry sausages, several researchers have pointed to protein aggregation during dry sausage structure development as the mechanism that provides firmness. Since heat processing, when applied, is generally less than that used for fully cooked products, some protein in the product remains soluble and maintains functional properties. Sokolov and Tchekhovskaya (1971) reported that during the drying of sausages, protein aggregation occurs. This protein aggregation is accompanied by electrostatic, hydrogen bonding and disulfide bond formation as moisture is lost from the meat system. These other types of bonding also increase interaction between protein strands, thereby increasing the firmness of the sausage. However, Sandholm et al. (1972) showed the number of sulfhydryl groups increased as sausages dried from manufacture to day 20, then decreased to near the initial level. They stated that the increase is due to the reduction of S-S bonds of the proteins of the sausages rather than to the unfolding of the peptide chains. This increase was attributed in part to the drying of the sausages, which decreased in weight 30% during the drying phase. After day 20, Sandholm and workers stated that the reduction of SH groups is possibly due to the increase in the rate of oxidation of the SH groups to disulfide bonds.

Acton et al. (1978) also studied the changes in sulfhydryl groups of soluble protein fractions in fermented dried sausage heated to 60°C. This study reported significant decreases in the free sulfhydryl content of the sausages after heat processing and again after 10 days of drying for the salt soluble protein fraction. The water soluble proteins displayed no change ($P>0.05$) after heat processing, but showed a significant decrease ($P<0.05$) after 10 days of drying. Extractable protein of both fractions decreased upon heat processing, and myofibrillar extractable protein decreased during the drying phase. These findings were not in agreement with Sandholm et al. (1972), but did agree with the findings of Sokolov and Tchekovskaya (1971). Acton et al. hypothesized that the free sulfhydryl content of soluble proteins of the sausage decrease while an increase of free sulfhydryls occurs in the nonprotein nitrogen fraction. Dierick et al. (1974) and Wardlaw et al. (1973) have reported that nonprotein nitrogen compounds increase during sausage drying. Acton et al. (1978) attributed the firmness development on sausage dehydration to the loss of protein solubility and the conversion of free sulfhydryls to form disulfide bonds.

Cyclic compression texture analysis

Firmness development as sausages dry are an important parameter for processors and researchers to consider, and there are many different mechanical deformation tests available to food scientists to evaluate texture parameters of foods. These tests were developed to not only provide comparisons between organoleptic qualities of foods, but to also be able to assess the textural characteristics of products, and to compare the textural parameters of an "unknown product" to a known food (Szczesniak et al., 1963)

One of the simplest and most useful tests for evaluating texture is the compression test (Voisey et al., 1977). This test requires flat parallel surfaces moving towards each other at a constant speed, such as the flat metal plates utilized on the Instron Universal Testing machine. These plates are used to compress the sample from 50-85% of its original height in two successive measurements, thereby giving rise to the term cyclic compression. The

first compression cycle provides a measure of firmness, while the second compressive cycle is used to establish cohesiveness of the product (Voisey, 1977) and Szczesniak et al. (1963) defined the textural parameters associated with compression analysis in the following ways:

1. Firmness - organoleptically, the force necessary to penetrate a substance with molar teeth. Objectively, the force necessary to attain a given deformation.
2. Elasticity - the rate at which a deformed material goes back to its undeformed condition after the force is removed.
3. Cohesiveness - the strength of the internal bonds in the product.
4. Guminess - organoleptically, the denseness that persists throughout mastication. It is a product of a low degree of hardness and a high degree of cohesiveness.
5. Chewiness - organoleptically, the time required to masticate a sample. It encompasses the parameters of hardness, cohesiveness and elasticity.

Bourne (1968) defined firmness as hardness and elasticity as springiness. He stated that hardness was the peak of the first compression force curve, while cohesiveness was calculated as the ratio of the two areas under the compression curves. Although sensory panelist evaluations correlations for hardness were considered good by Szczesniak et al. (1963), these researchers stated that it is difficult for panelists to determine cohesiveness and elasticity.

Much work has been performed using cyclic compression on frankfurter-type products to determine the textural properties of the sausages. Singh et al. (1985) stated that the firmness of a product is related to the heat treatment it undergoes. This heat treatment causes the denaturation or coagulation of proteins which give the product its firmness. They found that as cooking temperature increased for 50 to 70°C, cohesiveness of the product decreased while hardness increased. In a study on preblending in coarse-ground sausages, Hand et al (1992) reported that cohesiveness, or the amount of bind

between meat particles, increased linearly between 0-16 hours of preblending. This was attributed to increased protein interactions due to the preblending treatments.

Summary

The manufacture of dry sausages includes several critical stages which must be tightly controlled by processors to produce an acceptable product. The mixing times and temperatures are an important control point due to the detrimental effects noted for dry sausages when excess protein extraction occurs. In addition, the fermentation stage should provide an adequate pH drop to at least 5.2, using a starter culture that produces only lactic acid, and not undesirable fermentation products such as CO₂. Thermal processing and the drying of pepperoni can also cause substantial changes in the texture of the sausage which might render it unacceptable to the intended customer. Cooking of the product denatures proteins and dries the product, thereby providing firmness to the product and giving it different drying parameters than noncooked dry sausage. Cooking also can melt the fats in the pepperoni, which then coat the meat particles and can limit the rate of moisture loss upon drying. Processors also must control the rate of drying to avoid adverse textural changes such as case hardening around the outside edge of the sausage which occurs when sausages are dried too fast.

Overall, the manufacture of dry sausages like pepperoni is one of the most complex processes undertaken in the meat industry. Although many processors have considered the manufacture of these sausages an art, it is clear that science controls the process. But until these scientific principles become better understood, the art of dry sausage manufacture will live on.

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CHAPTER III

CHANGES IN PEPPERONI DURING DRYING AS INFLUENCED BY HEAT TREATMENT, MIXING TEMPERATURE AND MIXING TIME

Abstract

Pepperoni was manufactured using three different mixing temperatures (-5, 0 and 5°C), three different mixing times (2, 8 and 14 minutes) and two cooking treatments (noncooked or 60°C) to evaluate the effect of these parameters on the drying of pepperoni. Regression analysis showed uncooked pepperoni to have lower yields ($P < 0.05$) than cooked treatments, while no difference was noted for percent change of diameters between treatments over the drying time. Cohesiveness values were highest for the cooked treatments, and for the -5°C treatments and 14 minute mix treatments.

Introduction

Dry sausages such as pepperoni are dried from 12 to 60 days (Hoogenkamp, 1989; Terrell et al., 1977) to develop characteristic texture and flavor (Everson et al., 1970), provide an extended shelf-life (Townsend et al, 1975), provide control of trichinae and meet U.S.D.A. standard of identities. Although several researchers have investigated some of the changes in sausages during drying, little work has been published on how different manufacturing parameters affect the characteristics of sausages during the drying phase.

Drying of sausages also results in textural changes, some of which may be detrimental to the desirability of the product. Drying the product has been shown to increase shear values (Acton and Keller, 1974; Keller et al., 1974; Wardlaw et al., 1973). However, drying the product too rapidly can cause case hardening, in which a hard ring forms on the outer edge of the sausage, preventing adequate drying of the sausage interior (Keller et al., 1974; Terrell et al., 1977).

Over the drying period, decreases in percent moisture (Wardlaw, et al., 1973) and sausage diameter (Keller et al., 1974) have been reported. The pepperoni manufacturing system includes a fermentation step, usually aided by the addition of a lactic acid-producing starter culture (Wardlaw et al., 1973; Klement et al., 1973; Acton et al., 1977), followed by either drying, or thermal processing to 60°C and subsequent drying (Palumbo et al., 1976a). These different manufacturing systems result in products with different drying characteristics. Palumbo et al. (1976) reported pepperoni heated to 60°C resulted in higher yields than non-thermal processed pepperoni. A better understanding of the changes during drying could help pepperoni processors establish new critical control points during the drying phase for the texture of the product to ensure proper endpoint characteristics.

Due to the competitive nature of the pepperoni business especially with the growth in the pizza topping market (Anonymous, 1991), it is desirable for manufacturers to reduce drying time to increase profitability. It is well known that increased mixing can affect the textural changes of meat products (Booren et al., 1981 a, b, c; Gillett et al., 1977). Increased temperature has been shown to affect protein extraction (Hamm and Deatherage, 1960; Gillett et al. 1977; Gadea de Lopez and Hand, 1993) and increased mixing temperatures have proven to affect the properties of meat products (Popenhagen and Mandigo, 1978). However, no research has been published on the processing parameters of mixing time and mixing temperature and their affect on dry sausage.

Therefore, the purpose of this study was to investigate heat treatment, mixing

temperature and mixing time and their affect on drying time and other physical and textural changes during drying in pepperoni.

Materials and Methods

Pepperoni preparation

Frozen boneless cow trim was obtained from the Oklahoma State University meat laboratory and fresh boneless pork shoulders were purchased from local suppliers. Beef trim was thawed at 2°C. Beef and pork portions were then ground (Biro Mfg., Marblehead, OH) separately through a 9.5 mm plate, mixed (Leland ribbon-paddle mixer) one minute for homogeneity and sampled for proximate analysis. Portions were then stored 48 hours at 2°C. Nine 15-kg batches were prepared at 1:1 beef to pork ratio, each portion consisting of 22% fat. Meat batches were then tempered overnight to 5°C before pepperoni manufacture.

Pepperoni batches (Table 4.1) were mixed in a twin-shaft paddle mixer (U-Mec model 320, Hayward, CA) with paddles rotating at 25 rpm. Batches were mixed at either -5, 0 or 5°C for 2, 8 or 14 minutes. Prior to mixing, the meat batch temperature was equilibrated to the appropriate mixing temperature by the addition of CO₂ snow. The spice mixture (1.56%, A.C. Legg, Birmingham, AL) and dextrose (0.50%) were added during the first minute of mixing, followed by NaNO₂ (0.0156%), salt (2.25%) and antioxidant mixture (0.006%, Tenox 6, Eastman Kodak). The starter culture, *Pedicoccus acidilactici* (Diversitech HP, Diversitech, Gainesville, FL) was added last according to the manufacturer's directions.

After mixing, pepperoni mixtures were reground through a 4.8 mm plate, then were vacuum stuffed (Vemag Robot 500, Robert Reiser) into 45 mm fibrous cellulose casings (1R-60, Viskase, Chicago, IL) into 0.46 kg sticks. During stuffing, samples were taken for pH determination and proximate analysis. Three replications of the experimental batches were manufactured in different weeks using different lots of beef and pork.

Pepperoni Fermentation and Processing

Sticks were placed into a computer controlled one-truck smokehouse (Alkar, Lodi, WI) and fermented 10 hours at 38°C and 85% relative humidity. After fermentation, each treatment combination (consisting of a mixing time and mixing temperature) was split into two thermal processing treatments: 1) fermented-noncooked and 2) fermented-cooked. The noncooked sticks were then transferred to a one-truck drying chamber (Alkar, Lodi, WI) with an initial dry-bulb setting of 15.6°C and wet-bulb setting of 14.4°C. Wet bulb setting was slowly reduced as the product dried to a final setting of 13.3°C. The cooked sticks were subsequently thermal processed in a smokehouse controlled by a step program to an endpoint temperature of 60°C (Table 4.2). After thermal processing, the cooked sticks were also transferred to the drying chamber.

Sausages were dried to reach an endpoint moisture:protein ratio of 1.6:1 as specified by the USDA. Previous trials using the same system indicated drying times of 11 days were required for fermented-cooked treatments and 13 days for fermented-noncooked treatments.

pH Determination

Each treatment was analyzed for pH at these stages: raw, after fermentation, after thermal processing and on day 3, 7, 11 and 13 of drying. A modified method for pH determination as described by Keller et al. (1974) was used. Modifications included blending the 10-g samples of meat in 100 ml of distilled water with a Polytron Laboratory Blender (Kinematica, Luzerne, Switzerland) for 60 seconds.

Percent yield and diameter change measurements

Six sausage sticks per treatment were identified and weighed immediately after stuffing. The same sticks were subsequently weighed after fermentation, after thermal

processing and on day 3, 7, 11 and 13 of drying. The same six pepperoni sticks per treatment were measured for stick diameter at the same sampling periods. Sticks were measured at the midpoint longitudinally with a stuffing diameter tape measure.

Texture profile analysis

Hardness and cohesiveness measurements were conducted on an Instron Universal Texture Profile Machine (Model 4500, Instron Corp., MA) using a modified method as described by Voisey (1977). Modifications included cyclic compression of a 3 cm segment of the sausage to 65% of its original height. Compression speed was 50 mm per minute performed with a 10-kg load cell. No dwell time at the bottom of the compression stroke was employed. The height of the first compression peak force represented the hardness measurement and the ratio of peak 2:peak 1 force was used as the cohesiveness measure. Pepperoni stick samples (in duplicate) were measured after stuffing, after fermentation, after thermal processing and at days 3, 7, 11 and 13 of drying. Samples were held overnight at 4°C in plastic bags, then allowed to warm to room temperature before analysis.

Proximate analysis

Moisture (oven drying), fat (ether extraction) and protein (Kjeldahl nitrogen) were performed according to AOAC procedures (AOAC, 1985). Raw meat batches were analyzed and pepperoni sticks (in duplicate) were measured at each subsequent stage of processing.

Statistical analysis

Statistical analysis was performed using the Statistical Analysis System (SAS Institute, 1985). The 2x3x3 split plot design with randomized complete blocks was analyzed by one-way analysis of variance using a significance level of $P < 0.05$. Where appropriate, regression equations were developed using the General Linear Models

procedure of SAS.

Results and Discussion

pH

Regression lines for the changes in pH over drying days are displayed in Figure 1. Cooked pepperoni showed a quadratic change in pH ($P < 0.05$), with a slight peak occurring between days 4 and 6, and a decrease toward the end of the drying period to a final mean pH of 4.61. The noncooked pepperoni developed a final pH of 4.62. Although regression equations were significant for both cooked and noncooked treatments, overall there were only slight changes in product pH over the drying time. These slight changes agree with the findings of DeKetelare et al. (1974) and Keller et al. (1974), who reported that fermented sausage pH changed little after the fermentation process is completed. However, Wardlaw et al. (1973) reported that pH increased from 4.85 at the end of fermentation to 5.05 after 10 days of drying, suggesting the increase is due to the accumulation of basic nonprotein nitrogen compounds. The final pH values observed in this study are lower than those reported by Wardlaw et al. (1973), who noted final pH values of 4.85. However, Acton et al (1977) reported pH values of sausage fermented with dextrose to have a pH of 4.71 after a 24 hour fermentation period.

Moisture:protein ratio

Cooked and noncooked treatment data and regression lines for moisture:protein ratio are shown in Figure 3.2. Over the drying time, cooked pepperoni moisture:protein ratios decreased ($P < 0.05$), with the most rapid decrease over the first week of the drying phase. The rate of drying for cooked product then leveled out over the remainder of the period to a final moisture:protein ratio of 1.61:1. This data tends to agree with Keller et al. (1974) that showed summer sausage stuffed into 52 mm casings, heated to an internal temperature of 62°C and dried for 15 days achieved a moisture:protein ratio of 1.40:1.

Our data indicated that noncooked pepperoni displayed a decrease ($P<0.05$) in moisture:protein ratio over the drying period to a final ratio of 1.40:1. After 11 days of drying, mean moisture:protein ratios for noncooked pepperoni were 1.76:1, and decreased to 1.42:1 on day 13. Palumbo et al. (1976) stated that when comparing heated and nonheated pepperoni, heated pepperoni retained more moisture than the noncooked product. This difference was attributed to the fat being melted by heating, thus coating the meat particles and hindering the movement of moisture from the heated sausage. In contrast, the cooked pepperoni in this study reached the desired moisture:protein ratio sooner than the noncooked product. Heating of pepperoni tends to drive off water during the thermal processing stage, thereby lowering the moisture:protein ratio before the product is placed into the drying chamber.

Yields

The effect of drying days on yields is shown in Figure 3.3. Both cooked and noncooked pepperoni displayed yields which decreased ($P<0.05$) over the drying period. The cooked pepperoni treatment yield decreased more rapidly at the initiation of the drying phase than the noncooked product. Final product yield for the cooked pepperoni was 69.0%. Noncooked pepperoni showed a more gradual decrease initially, but also displayed a more rapid decrease in yield over the last two days of the drying period to a final yield of 64.6%.

Decreases in yields tended to closely match decreases in moisture:protein ratios for the noncooked pepperoni, with corresponding decreases to approximately day 5 of drying, followed by a lag phase over when little decrease was observed until the end of the drying phase. For M:P ratios and yields of noncooked pepperoni, a greater rate of decrease was observed toward the end of the drying period. The initial decrease in yield to approximately day 6 of drying for cooked pepperoni closely resembles the regression line plotted for moisture:protein ratios. However, cooked pepperoni yields tend to decrease

toward the end of the drying time, while only a slight decrease is noted for M:P ratios after day 6. Palumbo et al. (1976a) reported that pepperoni fermented to different pH values, heated to 60°C or not heated, all appeared to lose the same amount of weight on a percentage basis during the drying period. However, our data tends to agree with the findings in a subsequent study by Palumbo et al. (1976), where it was reported that unheated pepperoni displayed a lower ($P<0.05$) percent yield after 42 days of drying than pepperoni heated to 60°C (46.06% to 49.6%). Keller et al. (1974) reported that summer sausage heated to 62°C then dried appeared to lose weight in a curvilinear-manner, with approximately a 77% yield after 10 days drying and 69% yield after 15 days drying. Additionally, Palumbo et al. (1977) predicted a curvilinear decrease in yields over 98 days drying for fermented and heated pepperoni. The curvilinear trend reported by Keller et al. (1974) and predicted by the equation of Palumbo et al. (1977) does not agree with the trend of the quartic regression equation generated from this data for cooked pepperoni, but it does tend to agree with the curvilinear regression line generated for the decrease in cooked pepperoni moisture:protein ratios. This difference could be related to the higher drying room temperatures and different humidities in this study than those of Palumbo et al. (1977) and Keller et al. (1974). Also, measurements in this study were conducted every 4 days (excluding the 2 day period at the end of drying for noncooked pepperoni), thereby better characterizing the early stages of drying. In contrast, only 3 drying time measurements plotted by Palumbo et al. (1977) for days 0 to 14 of drying.

Diameter

There was no difference ($P>0.05$) for the percent of original diameter measurements between cooked treatments (Figure 3.4). Diameter decreased a final percent diameter of 86.00%. Diameter decreases tend to follow the decreasing trends for moisture:protein ratios and yields over the first week of drying. However, diameter decreases more closely resemble the decreasing trend for the noncooked product when

considering pepperoni yields.

Texture profile analysis

Instron cyclic compression tests were used to test the hardness (force required for first bite) and cohesiveness (binding between meat particles). There was no significant ($P < 0.05$) interaction or main effect for the texture parameter of hardness (mean = 0.531kn). Cohesiveness values showed significant interactions ($P < 0.05$) for cook treatment by drying days (Figure 3.5), mixing temperature by drying days (Figure 3.6) and mixing time by drying days (Figure 3.7). All of these interactions displayed cubic responses ($P < 0.05$) for cohesiveness measurements over the drying time. The noncooked treatments showed higher initial cohesiveness values than the cooked treatments, and also displayed a more rapid decrease in cohesiveness values through the first three days of drying. The noncooked pepperoni tended to have higher cohesiveness values than the cooked treatments at the end of the drying time.

Many researchers have reported that as moisture decreases and drying time increases for sausages, the texture of dry sausages changes. Shear force value increases have been noted by Lu and Townsend (1973), Wardlaw et al. (1973), Keller et al. (1974), and Townsend et al. (1980). Wardlaw et al. (1973) also reported that shear force values and moisture loss were highly correlated ($r = -.90$). However, Kramer shear measures the rupture forces inherent in the meat particles, not the binding of particles measured by cyclic compression.

The initial decrease in cohesiveness measurements corresponds with the decrease moisture:protein ratios, showing that as the product dried over the first four days, bond strength of the product decreased. In contrast, Sokolov and Tchekhovskaya, (1971) and Acton et al. (1978) described an increase in protein aggregation during sausage drying, which was attributed to the formation on disulfide bonds between myofibrillar proteins. Decrease of cohesiveness seems to be closely related to the loss of moisture from

pepperoni. Cohesiveness values are a ratio of compression force curves (peak 2:peak 1), with the first peak representing the measurement for hardness. Since there was no change in hardness over the drying time ($P>0.05$), differences seem to be related to changes in peak 2 of compression force. Visual cursory observation showed compression samples taken later during the drying phase to display more massive deformation after the first compression cycle and a greater degree of internal fracture. This propensity to fracture as the product dries led to a decrease in peak 2 of the compression cycle, resulting in lower cohesiveness values, overcoming the increasing attraction between proteins due to aggregation.

Conclusion

The drying of pepperoni is characterized by decreasing moisture:protein ratios, yields and cohesiveness, regardless of cooking treatment, mixing temperature or mixing time. Only slight changes in pH were noted over the drying periods for both cooked and noncooked pepperoni. The changes in texture observed during the drying of pepperoni closely follow the decreasing trends for moisture:protein ratios. Cooking treatment significantly affects the rate of drying of pepperoni ($P<0.05$), with cooked pepperoni drying faster than noncooked pepperoni when mean moisture:protein ratios are considered. Decreasing trends for yields, percent of original diameter and cohesiveness tend to follow decreases in moisture:protein ratios.

Cooked pepperoni displayed more gradual decreases in moisture:protein ratios and lower initial cohesiveness values than noncooked pepperoni. Noncooked pepperoni showed more gradual decreases in yields, while there was no difference between cooking treatments for changes in diameter. Pepperoni mixed at lower temperatures displayed higher cohesiveness values, while pepperoni manufactured with longer mixing times tended to also show higher cohesiveness trends. Depending upon the production process,

this data suggests that processors could monitor the manufacturing parameters of yields, diameters or cohesiveness to remove pepperoni from the drying rooms at the appropriate time to maximize yields and still meet the moisture:protein ratio of 1.6:1.

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Figure 3.1. Effect of days drying and cooking on pH of pepperoni

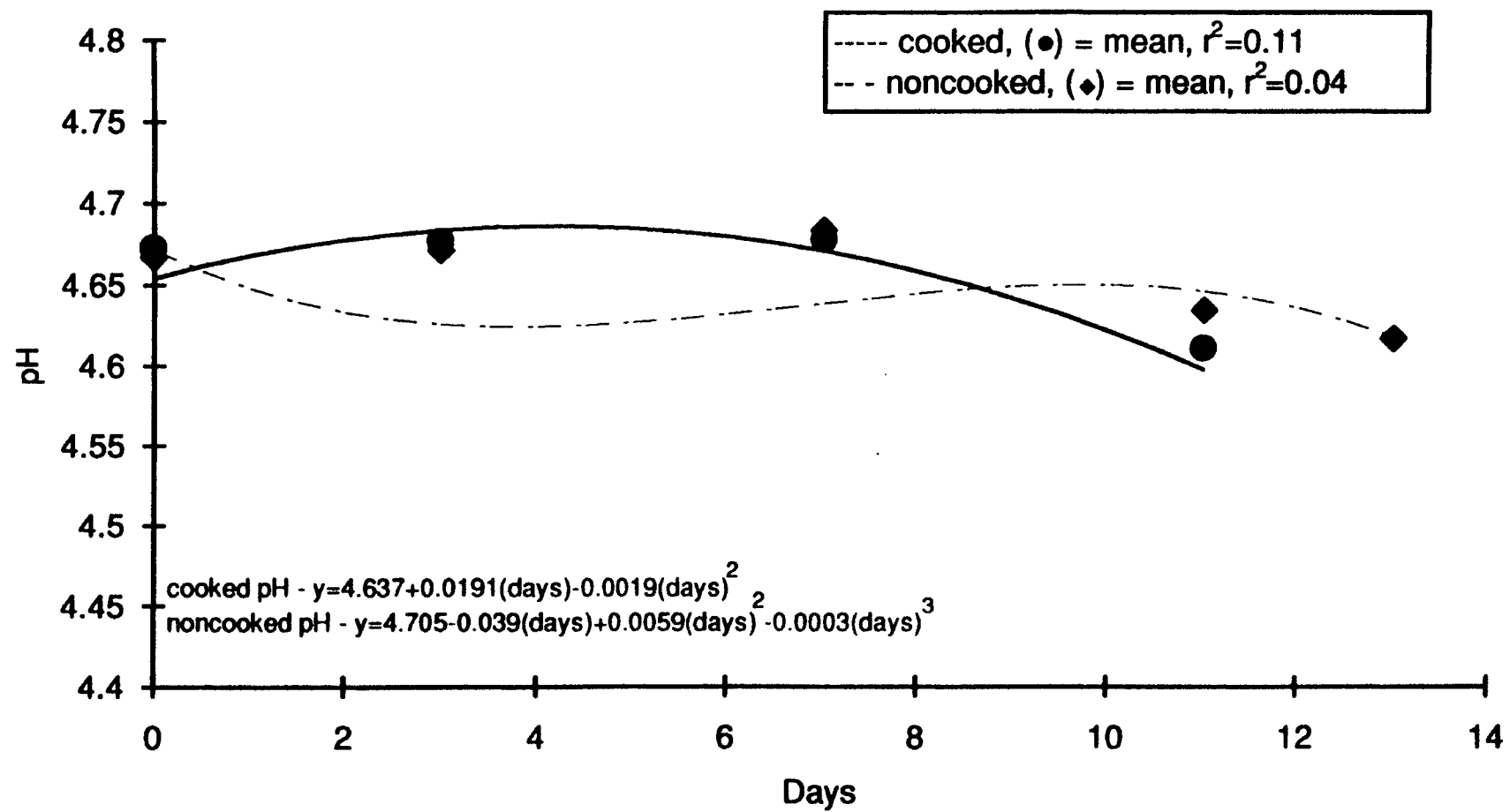


Figure 3.2. Moisture:protein ratio as affected by cooking treatment and days drying

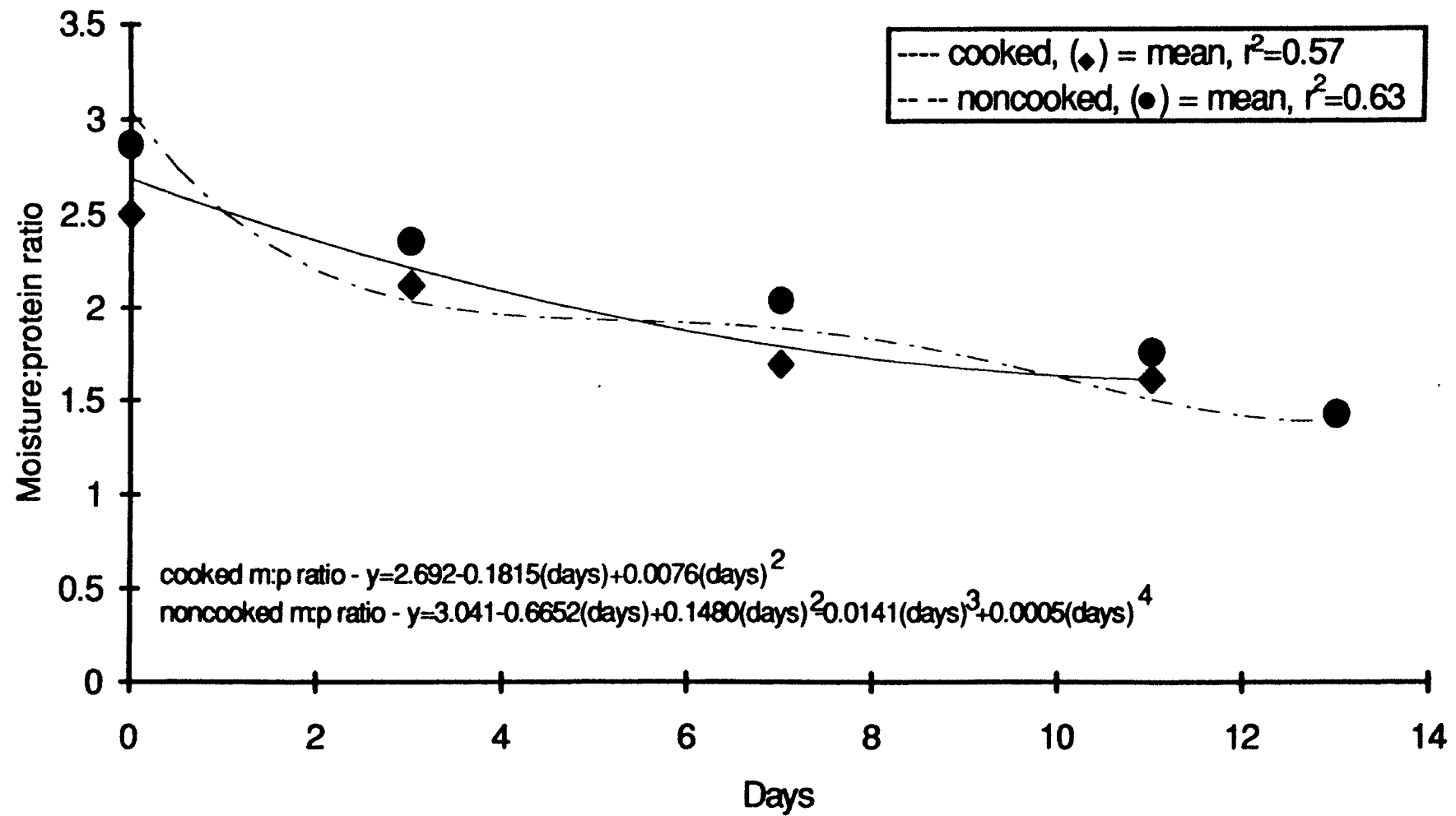


Figure 3.3. Yields of pepperoni as affected by cooking treatment and drying days.

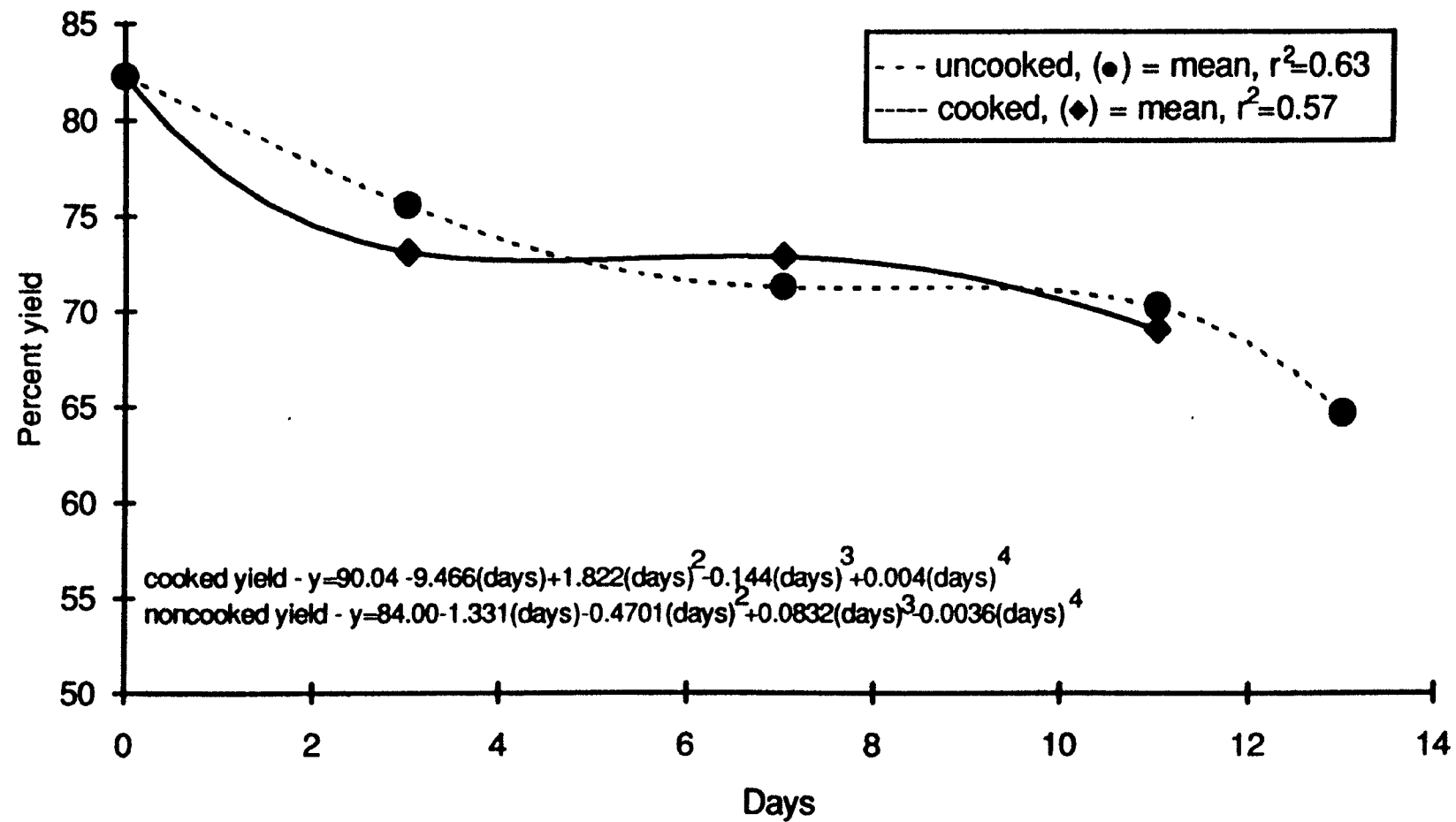


Figure 3.4. Percent of original diameter as affected by days drying for combined cooking treatments

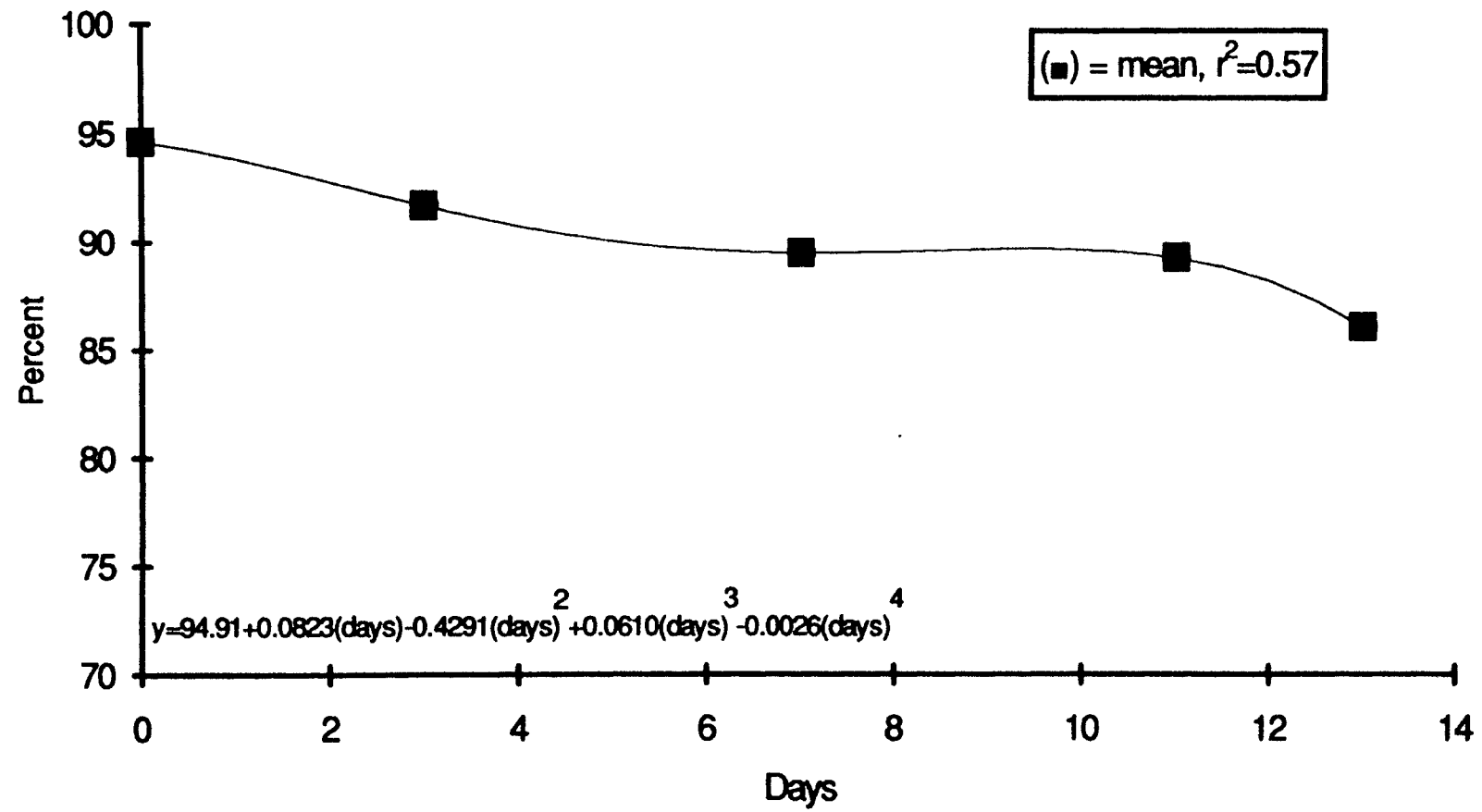


Figure 3.5. Cohesiveness values (compression force peak 2:peak 1) as affected by cooking treatment and drying days.

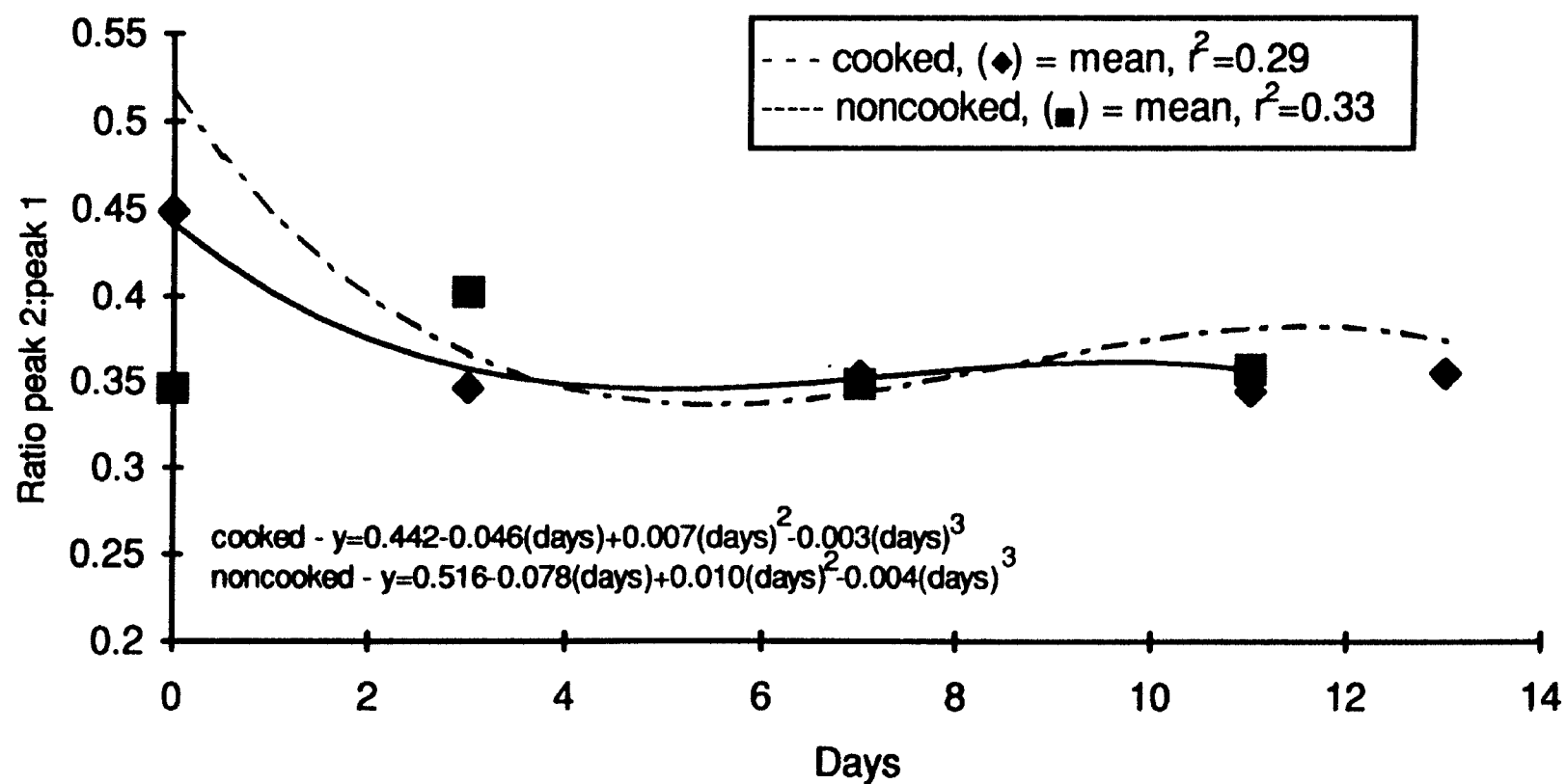


Figure 3.6. Cohesiveness values (compression force peak 2:peak 1) of pepperoni as affected by mixing temperature and days drying.

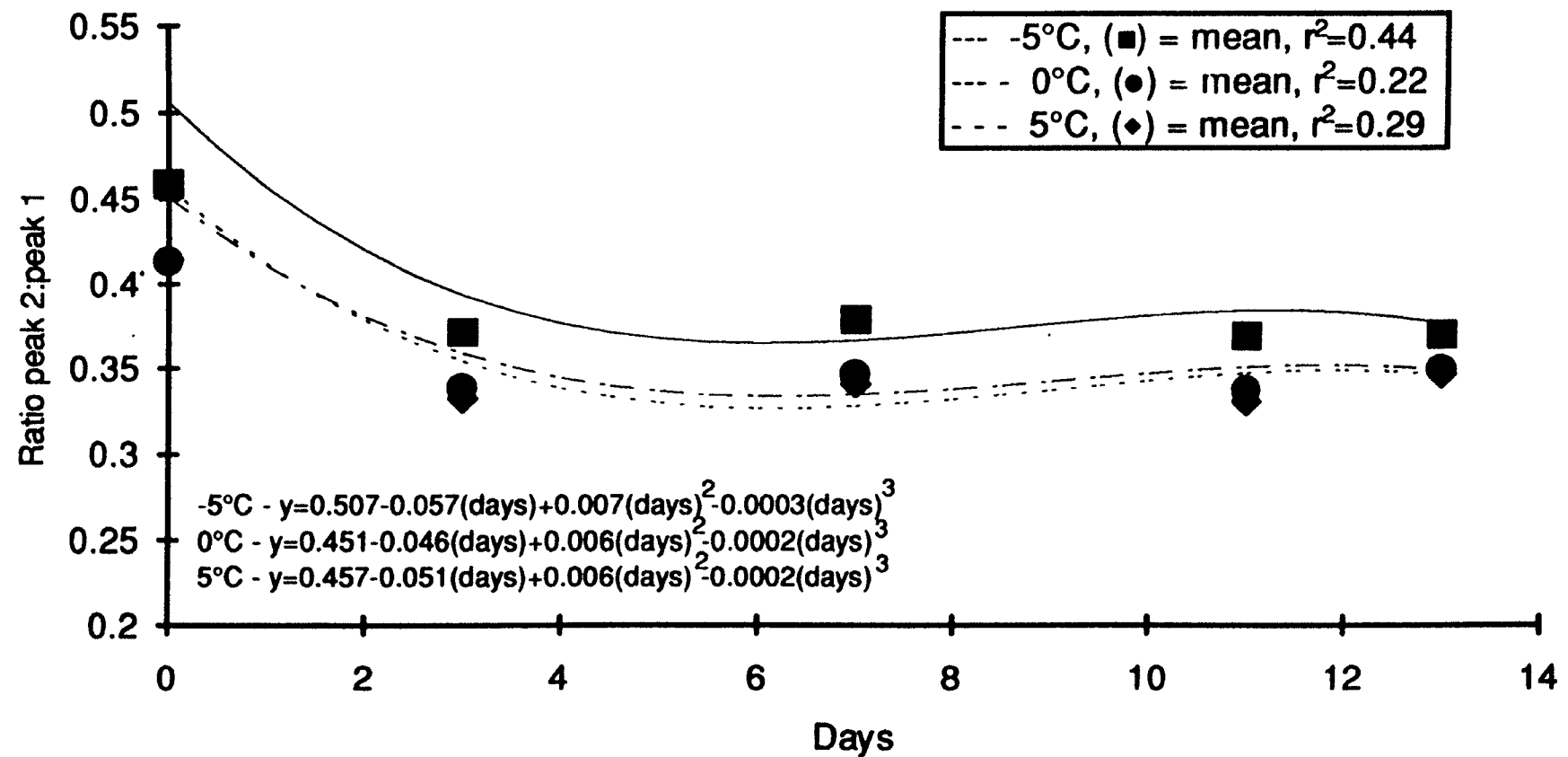
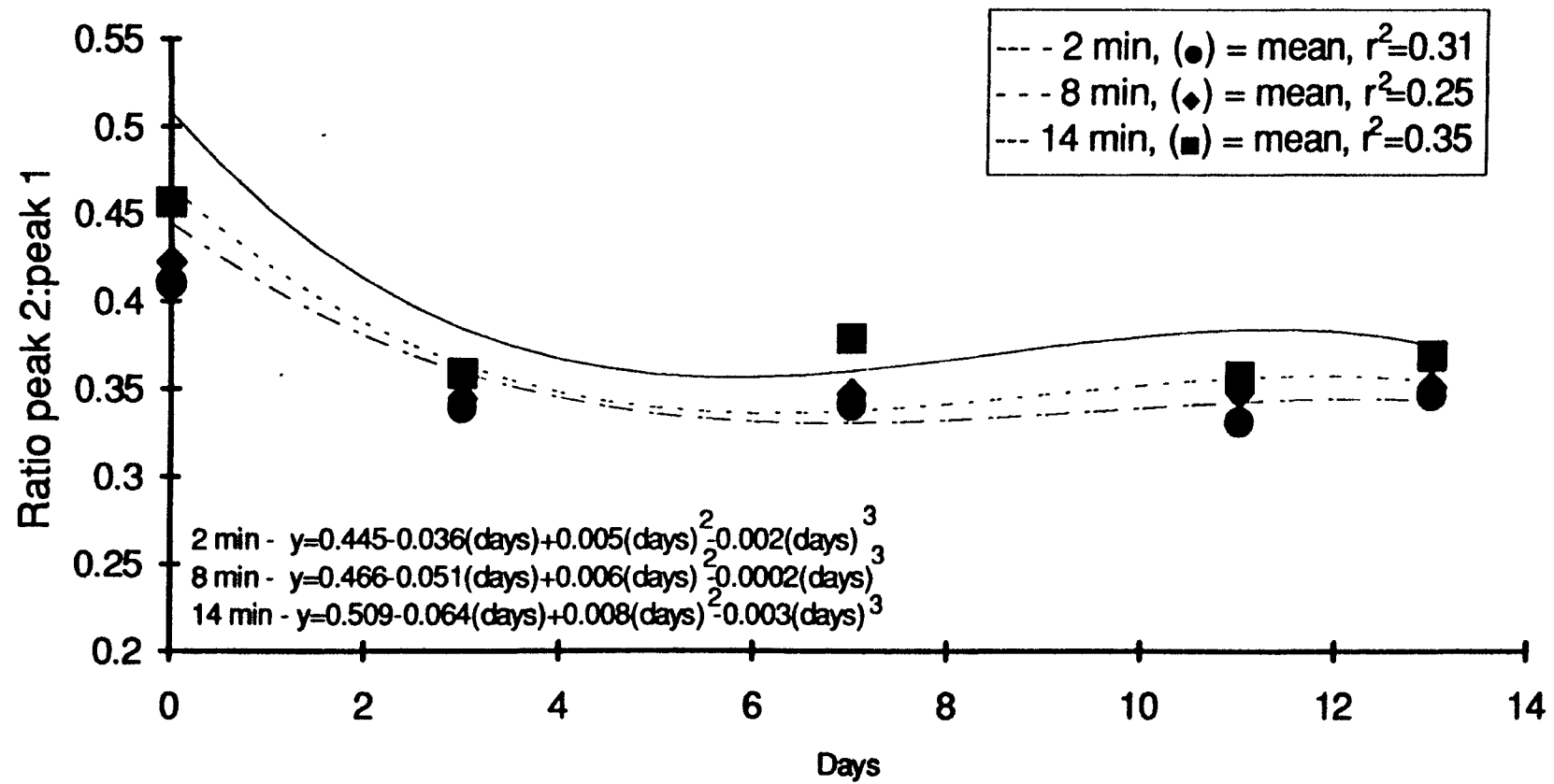


Figure 3.7. Cohesiveness value (compression force peak 2:peak 1 ratio) of pepperoni as affected by mixing time and days drying.



CHAPTER IV

THE EFFECTS OF THERMAL PROCESSING, MIXING TEMPERATURE AND MIXING TIME ON ENDPOINT TEXTURAL CHANGES AND CUPPING IN PEPPERONI.

Abstract

Pepperoni was manufactured using three different mixing temperatures (-5, 0 and 5°C), three different mixing times (2, 8 and 14 minutes) and two cooking treatments (noncooked or 60°C) to evaluate the effect of these parameters on the endpoint textural parameters and cupping of pepperoni. Covariance analysis higher cohesiveness values and lower cupping scores ($P < 0.05$) for the -5°C treatments than for the 0 or 5°C mixed treatments. Cooked pepperoni displayed less percent diameter shrink ($P < 0.05$) and higher cupping scores ($P < 0.05$) than the noncooked treatments. Path analysis (standard partial regression coefficients) showed that diameter and cohesiveness have the greatest direct effect on cupping in pepperoni.

Introduction

It has been documented that increased mixing can affect the textural changes of meat products (Booren et al., 1981 a, b, c). Increased temperature has been shown to affect protein extraction (Hamm, 1960; Gadea de Lopez and Hand, 1993) and increased mixing temperatures have proven to affect the properties of restructured steaks (Popenhagen and Mandigo, 1978). Additionally, the heating of sausages causes textural

changes (Palumbo, 1976a). Although these manufacturing parameters have been studied in numerous types of meat systems, no research has been published on the effects of these parameters on endpoint textural parameters in dry sausage.

Dry sausages such as pepperoni are dried from 12 to 60 days (Hoogenkamp, 1989; Terrell et al., 1977) to develop characteristic texture and flavor (Everson et al., 1970), provide an extended shelf-life (Townsend et al., 1980), provide control of trichinae and meet U.S.D.A. standard of identities. However, drying also results in textural changes, some of which may be detrimental to the desirability of the product. Over the drying period, decreases in percent moisture (Wardlaw, et al., 1973) and sausage diameter (Keller et al., 1974) have been reported. These changes also result in increasing shear values (Acton and Keller, 1974; Keller et al., 1974; Wardlaw et al., 1973).

One of the properties unique to pepperoni manufacturing referred to as cupping (Hoogenkamp, 1989; Newkirk et al., 1993). Cupping is the curling of pepperoni slices when they are cooked on a pizza, and is objectionable to consumers. Therefore, the objective of this research was to examine the manufacturing parameters of cooking, mixing temperature and mixing time on the endpoint textural aspects and cupping in pepperoni.

Materials and Methods

Pepperoni preparation

Frozen boneless cow trim was obtained from the Oklahoma State University meat laboratory and fresh boneless pork shoulders were purchased from local suppliers. Beef trim was thawed at 2°C. Beef and pork portions were then ground (Biro Mfg., Marblehead, OH) separately through a 12.7 mm plate, mixed (Leland ribbon-paddle mixer) one minute for homogeneity and sampled for proximate analysis. Portions were then stored 48 hours at 2°C. Nine 15-kg batches were prepared at 1:1 beef to pork ratio, both consisting of 22% fat. Meat batches were then tempered overnight to 5°C before

pepperoni manufacture.

Pepperoni batches (Table 4.1) were mixed in a twin-shaft paddle mixer (U-Mec model 320, Hayward, CA) with paddles rotating at 25 rpm. Batches were mixed at either -5, 0 or 5°C for 2, 8 or 14 minutes. Prior to mixing, the meat batch temperature was equilibrated to the appropriate mixing temperature by the addition of CO₂ snow. The spice mixture (A.C. Legg, Birmingham, AL) and dextrose was added during the first minute of mixing, followed by NaNO₂, salt and antioxidant mixture (Tenox 6, Eastman Kodak). The starter culture, *Pedococcus acidilactici* (Diversitech HP, Diversitech) was added last according to the manufacturer's directions.

After mixing, pepperoni mixtures were reground through a 4.7 mm plate, then were vacuum stuffed (Vernag Robot 500, Robert Reiser) into 45 mm fibrous cellulose casings (1R-60, Viskase, Chicago, IL) into 0.46 kg sticks. During stuffing, samples were taken for pH determination and proximate analysis. Three replications of the experimental batches were manufactured in different weeks using different lots of beef and pork.

Pepperoni Fermentation and Processing

Pepperoni was placed into a computer controlled one-truck smokehouse (Alkar; Lodi, WI) and fermented 10 hours at 38°C and 85% relative humidity. After fermentation, each treatment combination (consisting of a mixing time and mixing temperature) was divided into two thermal processing treatments: 1) fermented-noncooked and 2) fermented-cooked. The noncooked sticks were then transferred to a one-truck drying chamber (Alkar, Lodi, WI) with an initial dry-bulb setting of 15.6°C and wet-bulb setting of 13.3°C and an airspeed of 12 meters per minute. During drying, the wet-bulb setting was decreased slightly until moisture:protein ratios reached 1.6:1. The cooked sticks were subsequently thermal processed in a smokehouse controlled by a step program to an endpoint temperature of 60°C (Table 4.2). After thermal processing, the cooked sticks were also transferred to the drying chamber.

Sausages were dried to reach an endpoint moisture:protein ratio of approximately 1.6:1. Previous trials using the same system indicated drying times of 11 days were required for fermented-cooked treatments and 13 days for fermented-noncooked treatments.

pH Determination

Samples were analyzed for pH at the following stages: raw, after fermentation, after thermal processing and on day 3, 7, 11 and 13 of drying. A modified method for pH determination as described by Keller et al. (1974) was used. Modifications included blending the 10-g samples of meat in 100 ml of distilled water with a Polytron Laboratory Blender (Kinematica, Luzerne, Switzerland) for 60 seconds.

Percent yield and diameter change measurements

Six sausage sticks per treatment were weighed immediately after stuffing. The same sticks were subsequently weighed after fermentation, after thermal processing and on day 3, 7, 11, 13 of drying. The same six pepperoni sticks per treatment were measured for stick diameter at the same time period. Sticks were measured at the midpoint longitudinally with a stuffing diameter tape measure.

Texture profile analysis

Hardness and cohesiveness measurements were conducted on an Instron Universal Texture Profile Machine (Model 4500, Instron Corp., MA) using a modified method as described by Voisey (1977). Modifications included cyclic compression of a 3 cm segment of the sausage to 65% of its original height. Compression speed was 50 mm per minute performed with a 10-kg load cell. No dwell time at the bottom of the compression stroke was employed. The height of the first compression peak force represented the hardness measurement and the ratio of peak 2:peak 1 force was used as the cohesiveness measure. Pepperoni stick samples (in duplicate) were measured after stuffing, after

fermentation, after thermal processing and at days 3, 7, 11 and 13 of drying. Samples were held overnight at 4°C in plastic bags, then allowed to warm to room temperature before analysis.

Proximate analysis

Moisture (oven drying), fat (ether extraction) and protein (Kjeldahl nitrogen) were performed according to AOAC procedures (AOAC, 1985). Raw meat batches were analyzed and pepperoni sticks (in duplicate) were measured at each subsequent stage of processing.

Cupping Evaluation

Ten pepperoni slices were arranged in three rows on 25.4 cm frozen cheese pizzas (Jeno's, Kansas City, MO). Slices were placed on pizzas in a specific pattern so as to assign a location number for each slice on each pizza. Three pizzas per replication were manufactured for each of the 18 mixing time-mixing temperature-cook treatment combinations (162 total pizzas). Pizzas were then cooked in a conveyer-fed impingement oven (Model 1022, Lincoln, Ft. Wayne, IN) for 3.5 minutes at 260°C. Pizzas for each separate replication were cooked on different days. Pizzas were evaluated for cupping by two methods: 1) subjectively - a trained panel evaluation and 2) objectively - cooked slice height.

Panelist Evaluation

Ten panelists were trained in two 30-minute sessions to evaluate cupping of pepperoni slices on a 4-point scale (1 = flat, 2 = wrinkled, 3 = moderately cupped, 4 = fully cupped). Training was performed using sample pizzas which displayed the various ranges of pepperoni cupping. Panelists were then presented pizzas in a random order and asked to assign a cupping score to each slice (1-10) on each pizza.

Pepperoni measurement

Pepperoni slices from locations 1, 5, 6 and 10 were removed from each pizza and were measured for cupping height (mm) by two evaluators. Slices were removed from the pizzas, excess cheese removed and placed upside down on a Plexiglas board. A depth measurement from the highest point of the pepperoni slice to the bottom of the Plexiglas was taken with a micrometer. The depth of the Plexiglas was then subtracted from each slice height measurement.

Statistical analysis

Statistical analysis was performed using the Statistical Analysis System (SAS Institute, 1985). The 2x3x3 split plot design with randomized complete replications (n=3) was analyzed by one-way analysis of variance and where appropriate, means were differentiated by least squares means. Covariance using the mean endpoint moisture:protein ratio as the covariate was employed to analyze differences between parameters. Additionally, path coefficients (standard partial regression coefficients) were computed to evaluate the relative importance of various characteristics that influence cupping (Wright, 1934; May et al., 1992).

Results and Discussion

Analysis of the final moisture:protein ratios (Table 4.3) showed a three way interaction between cooking treatment, mixing time and mixing temperature. There was no difference within treatment for any of the noncooked batches. Cooked treatments showed, in general, the highest endpoint moisture:protein ratios for the treatments that underwent the longest mixing times (8 and 14 minutes) at the highest temperatures (5°C), with the exception of the 2 minute at 0°C treatment. In general, the noncooked pepperoni displayed lower moisture:protein ratios than the cooked treatments, which can be attributed due to an additional 2 days of drying. In order to more appropriately compare and analyze the treatments, covariance using the mean endpoint moisture:protein ratio

(1.53:1) as the covariate was used.

Covariance analysis showed no difference ($P>0.05$) between treatments for the parameters of pH (mean = 4.61), yield (mean = 66.14%) or texture profile hardness (mean = .531). These pH values agree with those found by Townsend et al. (1980), but are slightly lower than those reported in the range of 4.7-4.9 by Palumbo et al. (1976) in studying a pilot plant process for pepperoni. Mean yields are slightly lower than those reported by Keller et al. (1974) of approximately 77% at 10 days of drying and 69% at 15 days of drying, but are slightly higher than the approximately 60% yield after 14 days of drying predicted by Palumbo et al. (1977).

Cooking treatment percent of original diameter values differed significantly ($P<0.05$). Cooked pepperoni displayed larger (mean = 88.38) percent diameter values than noncooked pepperoni (mean = 86.12). The higher mean percent diameter for the cooked treatments could be attributed to firmness development of the sausage due to the 60°C heat treatment. Palumbo et al. (1976) reported that sausages fermented and heated to 60°C, and sausages only fermented developed a firm texture upon drying, while nonfermented, nonheated sausage developed poor texture. However, no objective data on texture parameters was presented by Palumbo et al. (1976). Palumbo et al. (1977) stated that heating increases the homogeneity of the mixture as melted fat is redistributed in the product. This redistribution of fat resulted in higher percent yields for heated pepperoni when compared with nonheated pepperoni after 42 days of drying. This research shows no difference in the percent yield between cooked and noncooked pepperoni at the same moisture:protein ratio, but the noncooked sausage took an additional 2 days of drying to reach the required moisture:protein ratio of 1.6:1.

Several researchers has shown that sausages become firmer as they dry (Townsend et al.; 1980; Lu and Townsend, 1973; Keller et al., 1973). Although shear values have been evaluated, the texture parameter of cohesiveness has not been reported for dry sausage. Shear values measure the rupture force of the particles in the sausage,

while cyclic compression measures the binding strength between particles. There was no difference between the cooked and noncooked pepperoni ($P>0.05$), but differences were displayed for different mixing temperatures ($P<0.05$). The -5°C mixed treatment (mean = 0.372) proved to have the higher cohesiveness values than the 0° (mean = 0.343) or 5°C (mean = 0.333) treatments. Cohesiveness values are calculated by dividing the force at the height of the second peak of the cyclic compression cycle by the force at the height of the first peak (Voisey, 1976). Since there was no difference in the hardness of the product which is related to the height of the first peak (Bourne, 1968), differences in cohesiveness occurring in the products appeared in the second deformation curve. Possibly, the -5°C treatments displayed a higher degree of elasticity between compression cycles, giving higher cohesiveness values. Visual cursory observation showed less fracturing of the -5°C product upon the first compression cycle, which would have aided the elasticity of the product.

Cupping Evaluation

Panelist scores and cupping height measurements are shown in tables 4.4. Subjective and objective evaluations were highly correlated ($r^2 = 0.79$). Both subjective and objective measurements displayed lower cupping values for the noncooked pepperoni ($P<0.05$). Hamm and Detherage (1960) showed that heating of meat to 60°C at a pH of 5.0 renders many of the structural proteins insoluble. Therefore, while heating should be considered beneficial to increasing the drying rate of sausages, it also tended to promote textural changes causing the cooked product to cup more extensively than noncooked pepperoni.

Panelist and cupping height measurements both showed the -5°C treatments to demonstrate the least amount of cupping ($P<0.05$). There was no difference ($P>0.05$) between the 0 and 5°C treatments. These higher mixing temperatures tended to extract more protein in the sausages, as evidenced by their increased water binding capacities and

higher endpoint moisture protein ratios. However, moisture:protein ratio was held constant for the covariance analysis. Popenhagen and Mandigo (1974) found that as temperatures for flaked and formed steak products was increased from -5.6°C to 0.6°C, adhesion between meat particles increased. This increase in protein extraction could also lead to a greater moisture amount of retained moisture in the 0 and 5°C mixed treatments due to their increased water binding ability. Hoogenkamp (1987) stated that cupping was caused by a differential in moisture between the outer edge of the slice and the inner edge. Therefore, increased mixing temperatures from -5°C to 0 or 5°C could lead to a more severe moisture gradient in pepperoni. During drying, moisture would be most easily lost from the outer edge of the product, leaving the interior of the sausage with a greater percentage of water. Keller et al. (1974) showed that there was approximately a 5% difference in percent moisture between the inner 2/3 and outer 1/3 of a summer sausage after 10 days of drying. The gradient increased to approximately 7% after 30 days of drying. This difference in interior moisture could have led to the increase in cupping between the treatments.

To determine which textural parameters could possibly serve as indicators of cupping in pepperoni, the relationships between various processing parameters and measurements were used to construct a path analysis for cupping height measurement (Figure 4.1). Path analysis ($P < 0.05$) was also conducted for evaluator scores, but only the height measurement path is presented for discussion. The path coefficients (standard partial regression coefficients) are shown in parentheses on the straight single-headed arrows. Squaring the path coefficient gives the percentage of variation in cupping accounted for by the direct effect of that parameter. For example, the direct effect of diameter on height measurement accounts for 79% of the variation in height ($0.89^2 = 0.79$). For height measurements, the paths in the diagram account for 70% of the variation in height. Diameter (79%) and cohesiveness (55%) had the largest direct effect on height measure.

Additionally, each variable has an indirect effect through its correlation with other variables. These effects can be found by multiplying the correlation between the variables and the path coefficient between the second variable and the cupping measure. For example, the indirect effect of diameter through cohesiveness on height measurement is $-0.39 \times 0.74 = -0.29$. All additional numbers located on the arrows (single and double headed) represent the simple correlations between those parameters. Since diameter and cohesiveness had the greatest direct effect on cupping, the indirect effects with the largest impact on height measure are those routed through either diameter or cohesiveness. The order of importance for indirect paths through diameter was cohesiveness (-0.35), yield (0.27), hardness (0.20), moisture:protein ratio (0.14) and pH (-0.05). The order of importance for indirect paths through cohesiveness was pH (0.41), diameter (-0.29), moisture:protein ratio (0.22), hardness (-0.03) and yield (-0.01). These correlations were used to calculate the path coefficients, and they account for all the direct and indirect paths and are the sum of all paths.

Changes in diameter and cohesiveness brought about by drying seem to impact the degree of cupping in pepperoni. When the negative indirect path of cohesiveness through diameter is considered (-0.35), it appears that a decrease in a combination of those parameters increases the incidence of cupping. This tends to agree with the covariance analysis for cohesiveness, which showed that as cohesiveness decreased ($P < 0.05$) between the -5°C and the 0 and 5°C treatments, height measurements tended to increase. The relatively high indirect path coefficient for pH through cohesiveness (0.41) is mainly due to the simple correlation between cohesiveness and pH ($r = 0.55$). Interestingly, pH alone accounts for only 3% of the variation in cupping, and is not significant ($P > 0.05$) when analyzed by both covariance or analysis of variance.

Conclusions

In conclusion, this study indicates that noncooked pepperoni tends to cup less

than cooked pepperoni, and pepperoni mixed at -5°C also displayed the least amount of cupping. Path analysis showed diameter and cohesiveness were the best predictors of cupping in pepperoni. Therefore, processors may be able to evaluate the change in diameter and cohesiveness of pepperoni as it dries as a measure to predict cupping in the finished product.

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Table 4.1. Pepperoni formulation on a per batch basis.

Ingredient	Quantity	Percent (of meat block)
Pork	7.5 kg	
Beef	7.5kg	
Spice mix	234g	1.56
Salt	337.5g	2.25
Sodium nitrite	2.34g	0.0156
Dextrose	75g	0.50
Starter culture	75g	0.50
Antioxidant mixture	0.9g	0.006

Table 4.2. Smokehouse schedule for pepperoni fermentation and cooking.

Method	Dry Bulb (°C)	Wet Bulb (°C)	Time
Cook	101	97	10 hr
Smoke and cook	120	113	1 hr
Smoke and cook	130	121	1 hr
Smoke and cook	148	139	Until internal temp. ≥ 60°C
Hot shower			3 min
Cold shower			7 min

Table 4.3. Means and standard errors for endpoint moisture:protein ratios.

Temp (°C)	Time (min.)	Cooked	Noncooked
-5	2	1.59 ^a (0.21)	1.48 ^a (0.15)
	8	1.57 ^a (0.21)	1.32 ^a (0.15)
	14	1.44 ^a (0.21)	1.32 ^a (0.12)
0	2	1.80 ^b (0.12)	1.35 ^a (0.21)
	8	1.51 ^a (0.21)	1.51 ^a (0.21)
	14	1.52 ^a (0.21)	1.33 ^a (0.21)
5	2	1.40 ^a (0.12)	1.48 ^a (0.21)
	8	1.79 ^b (0.12)	1.44 ^a (0.15)
	14	1.76 ^b (0.11)	1.59 ^a (0.15)

^{ab} Means within same column with same superscripts not different at the $P < 0.05$ level.

Table 4.4. Measurements for cupping evaluation and cupping height measurements for pepperoni mixed at different temperatures or subjected to different cooking treatments.^a

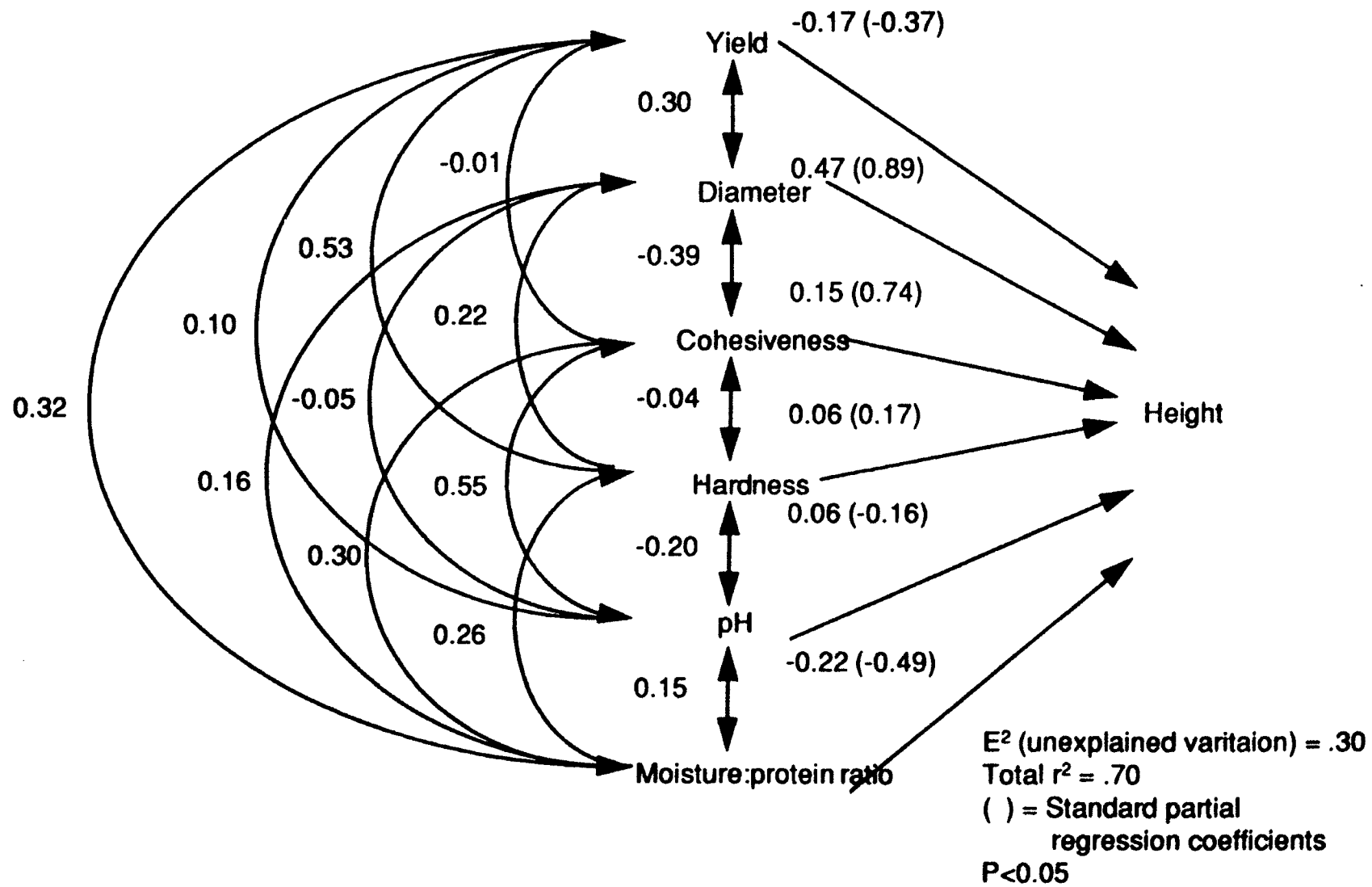
Parameter	Temperature (°C)	Measurement
Evaluator score	0	3.04 (0.12) ^c
	5	3.37 (0.10) ^c
	-5	8.63 (0.25) ^b
Height measurement (mm)	0	9.59 (0.30) ^c
	5	10.34 (0.25) ^c

Parameter	Temperature (°C)	Measurement
Evaluator scores	cooked	3.21 (0.10) ^c
	noncooked	2.74 (0.08) ^b
Height measurement (mm)	cooked	9.83 (0.23) ^c
	noncooked	9.20 (0.19) ^b

^a Parameters adjusted by covariance to a mean moisture:protein ratio of 1.53:1.

^{bc} Means for the same parameter and treatment with the same superscript not different at the $P < 0.05$ level.

Figure 4.1. Path coefficient diagram for selected constituents of height measurement.



APPENDIX A
REGRESSION EQUATIONS FOR SAUSAGE DRYING

Appendix A: Regression equations, standard error of estimates and R^2 for significant ($P < 0.05$) response variables.

Measurement	Variable	Regression	Equation	S.E.E.	R^2
pH	Cooked	Intercept	4.636795172	0.01720612	0.112430
		Days	0.019163202	0.00049520	
		Days ²	-0.001873637	0.00665928	
	Noncooked	Intercept	4.704682519	0.03059889	0.044044
		Days	-0.039196039	0.00012479	
		Days ²	0.005876321	0.00284629	
		Days ³	-0.000251690	0.01784660	
M:P ratio	Cooked	Intercept	2.690063276	0.06870450	0.668240
		Days	-0.181512423	0.02701128	
		Days ²	0.007574356	0.00201971	
	Noncooked	Intercept	3.040898976	0.09792117	0.729346
		Days	-0.66524615	0.13545971	
		Days ²	0.14802926	0.04132587	
		Days ³	-0.014120103	0.00439112	
Yields	Cooked	Intercept	90.03999377	1.09397898	0.570689
		Days	-9.46548403	0.00104608	
		Days ²	1.82168434	0.03160433	
		Days ³	-0.14350528	0.31851134	
		Days ⁴	0.00377076	1.17844887	
	Noncooked	Intercept	83.9949400	1.04091395	0.632475
		Days	-1.33084102	0.00118565	
		Days ²	-0.47018271	0.03441810	
		Days ³	0.08317343	0.32596972	
		Days ⁴	-0.00356077	1.12545989	
% diameter	All	Intercept	94.90904556	0.34927344	0.571317
		Days	0.08242721	0.36676281	
		Days ²	-0.4286768	0.10296599	
		Days ³	0.061240789	0.01056451	
		Days ⁴	-0.00244895	0.00035721	

Appendix A cont.

Measurement	Variable	Regression	Equation	S.E.E.	R ²
Cohesiveness	Cooked	Intercept	0.4415179203	0.01038321	0.286678
		Days	-0.0462571713	0.00006814	
		Days ²	0.0070015818	0.00134987	
		Days ³	-0.0003178865	0.00739167	
	Noncooked	Intercept	0.516106927	0.01589988	0.330559
		Days	-0.0776684804	0.00008921	
		Days ²	0.0104192512	0.00197888	
		Days ³	-0.0004067174	0.01151741	
	-5°C mix	Intercept	0.5065392533	0.01296584	0.436029
		Days	-0.0567632128	0.00006356	
		Days ²	0.0071651754	0.00140765	
		Days ³	0.0002744277	0.00854118	
	0°C mix	Intercept	0.4509222297	0.01761291	0.218214
		Days	-0.0456222437	0.00008412	
		Days ²	0.0055779003	0.00186422	
		Days ³	-0.0002054991	0.01136294	
	5°C mix	Intercept	0.456663468	0.01585979	0.288479
		Days	-0.05825242	0.00007644	
		Days ²	0.0062291094	0.00169461	
		Days ³	-0.0002286455	0.01031480	
	2 minute mix	Intercept	0.4451462025	0.01409409	0.306333
		Days	-0.0413939337	0.00006871	
		Days ²	0.0047372775	0.00152159	
		Days ³	-0.0001658411	0.00923917	
	8 minute mix	Intercept	0.4654613391	0.01752115	0.250148
		Days	-0.05640872	0.00008467	
		Days ²	0.0062377082	0.00187450	
		Days ³	-0.000230833	0.01139624	
	14 minute mix	Intercept	0.5085699371	0.01597453	0.353818
		Days	-0.063451963	0.00007653	
		Days ²	0.0082894132	0.00169816	
		Days ³	-0.0003230806	0.01035490	

APPENDIX B

METHOD FOR PEPPERONI HEIGHT MEASUREMENT

METHOD FOR THE MEASUREMENT OF PEPPERONI HEIGHT

1. Set Plexiglas square (15 cm^2) in laboratory stand clamp and adjust to approximately eye level.
2. Measure depth of Plexiglas with micrometer.
3. Remove pepperoni slices from pizza and scrape off excess cheese.
4. Place slice upside down on Plexiglas and measure the depth of both the Plexiglas and pepperoni height with a micrometer.
5. Record height measurement and subtract off the depth of the Plexiglas measurement taken in step 2.

APPENDIX C
ANALYSIS OF VARIANCE TABLES

ANALYSIS OF VARIANCE TABLES

TABLE FOR CHAPTER 3

Total

Mixing time
 Mixing temperature
 Replication
 Mixing time x mixing temperature

Error a (Mixing time x mixing temperature replication)

Cooking treatment
 Cooking treatment x mixing time
 Cooking treatment x mixing temperature
 Cooking treatment x mixing time x mixing temperature

Error b (Cooking treatment x mixing time x mixing temperature x replication)

TABLE FOR CHAPTER 4

Total

Mixing time
 Mixing temperature
 Replication
 Mixing time x mixing temperature

Error a (Mixing time x mixing temperature x replication)

Cooking treatment
 Pizza(slice)
 Cooking treatment x pizza (slice)
 Cooking treatment x mixing time
 Cooking treatment x mixing temperature
 Cooking treatment x mixing time x mixing temperature
 Cooking treatment x mixing time x mixing temperature x pizza (slice)

Error b (Cooking treatment x mixing time x mixing temperature x pizza (slice) x replication)

VITA

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Master of Science

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