This is to certify that the

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COLOR AND FLAVOR STABILITY IN FROZEN PIZZA

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VANEE KOMOLPRASERT

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M.S. degree in PACKAGING

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# COLOR AND FLAVOR STABILITY IN FROZEN PIZZA

By

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## ABSTRACT

COLOR AND FLAVOR STABILITY OF FROZEN PIZZA

By

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The effect of storage temperature, freeze-thaw cycling, exposure to display light, and packaging material and method on color and flavor changes in frozen pizzas during storage were investigated. Chemical and sensory methods were utilized to assess deteriorative changes at monthly intervals for a period of five months. Lipid oxidation by TBA test, nitric-oxide myoglobin and color by the Hunter Colorimeter were determined on pork sausage, pepperoni and tomato sauce, and compared with data obtained by sensory evaluation using an untrained panel.

An increase in storage temperature from -18°C to -7°C accelerated color and flavor change in the three components. Within two months, pizzas held at -7°C were judged undesirable by the panelists. A freeze-thaw cycle (5°C, 7 hrs.) did not significantly affect color or flavor. Exposure to display light (200 ft-c fluorescent light, 8 hrs.) resulted in discoloration of pepperoni but had no effect on tomato sauce; such expose did not influence the development of rancidity in the pork sausage. Using a

Nylon/PE laminated pouch and packing under a vacuum of 24 in.Hg significantly decreased off-color and off-flavor in frozen pizza stored at -18 °C. There was no advantage in using the Nylon/PE package and sealing under atmospheric conditions as there was no reduction in rancidity of pork sausage in such packages. There was low but significant correlation between results from chemical and sensory analyses. Storage temperature and amount of available oxygen in the package were the most important factors influencing color and flavor change in frozen pizza.

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# INTRODUCTION

Frozen pizza is a ready-to-heat product which has gained much in popularity. Maintenance of quality of the frozen product during storage is critical to successful marketing, as evidenced by customer acceptance and satisfaction. Frozen pizzas, as with other frozen foods, may undergo physico-chemical changes which result in quality deterioration. The factors affecting shelf life of frozen pizza become complex because of the numerous components, in the pizza which vary in their susceptibility to deteriorative changes. However, certain components which show marked change in storage may serve as quality indicator(s) in determining shelf life of frozen pizza.

The conventional basic components of a pizza are crust (mainly wheat flour), mozzarella cheese and various condiments such as pepperoni, pork sausage, tomato sauce, ham, onions, olives and other less frequently used items. In frozen storage environment, oxygen, light and temperature are the major environmental factors affecting color and flavor. Desiccation, autoxidation and photoinitiated oxidation can cause deterioration of lipids and other components during frozen storage. Pork sausage, pepperoni and tomato sauce, can all be affected by these reactions

which result in the development of objectionable flavors, odors and colors. The color and flavor stability of frozen pizzas can be moderated by employing appropriate protective packaging. Most commercial frozen pizzas are heat-sealed in a Polyolefin pouch which may or may not be placed in a paperboard folding carton for good light protection and protection against physical damage. Polyolefins are poor oxygen barriers; hence autoxidation is not inhibited. Because loss of moisture in frozen foods can result in desiccation and freezer burn which is unattractive and may also promote lipid oxidation, the package material should be a good moisture barrier as well as a good oxygen barrier. Packaging techniques which can potentially reduce lipid oxidation include headspace control (vacuum, nitrogen flush and modified atmosphere), the use of oxygen scavengers and antioxidant impregnated films.

Little or no data are available in the literature concerning color and flavor deterioration in frozen pizzas due to environmental abuse. Therefore, the objectives of this study are: (1) to quantify the quality stability of frozen pizzas as influenced by effects of storage temperature, freeze-thaw cycling and light exposure during a five month period, and (2) to determine package design criteria which influence color and flavor change in frozen pizzas.

The experiments were undertaken on frozen pizzas supplied by The Pillsbury Co. Minneapolis, Minnesota. The

packaged pizzas were stored at -18±5 °C and -7±3 °C in the dark. Prior to evaluation the pizzas were exposed to light. Vacuum and atmospheric packs using good moisture and oxygen barrier packages were prepared and stored at -18±5 °C. Lighting consisted of exposing pizzas to 200 ft-c fluorescent light for 8 hours under the designed storage temperature. Freeze-thaw cycling consisted of storing pizzas at 5 °C for 7 hours.

# LITERATURE REVIEW

## 4.1 Lipid oxidation of pork sausage and pepperoni

Animal fats are composed chiefly of triglycerides and phospholipids in ratios which vary with the type and species of animal. Hornstein et al. (1961) observed that beef muscle contained 2-4% triglycerides while pork muscle contained 5-7%. The phospholipid content was 0.8-1.0% in beef and 0.7-0.9% in pork. The ratio of triglycerides to phospholipids was about 4:1 in beef and about 8:1 in pork.

In frozen meat, deteriorative changes are often not well understood. Greene (1969) and Watts (1954) pointed out that the storage stability and quality of frozen meat depends crucially on the composition of constituent lipids and on the degree of unsaturation. Igene et al. (1979b) reported that cooking increased the percentage of phospholipids in relation to total lipids and accounted for a significant increase in rate of lipid oxidation during frozen storage of cooked meat. These results were supported by Campbell and Turkki (1967) and Fooladi (1977).

Igene et al. (1980) suggested that both triglycerides and phospholipids contributed to development of rancidity in meats, though phospholipids made the greatest contribution. The role of triglycerides in the development of rancidity

depended upon the degree of unsaturation and the length of time in frozen storage. These results are in agreement with those of other investigators (Cadwell et al., 1960; Greene, 1969; Hornstein et al.,1961; Igene and Pearson, 1978; Younathan and Watts, 1960).

The phospholipids are closely associated with the muscle proteins and pigments. Bratzler et al. (1977) reported that heating meat promoted phospholipid oxidation via a heme-catalyzed reaction.

4.1.1 Lipid autoxidation

Autoxidation is the most recognized mechanism which promotes lipid oxidation in meat products. The reaction of oxygen with unsaturated fatty acids (RH) involves free radical initiation,propagation and termination. The mechanism of autoxidation has been reviewed by Frankel (1980). Initiation takes places by loss of a hydrogen radical in the presence of trace metals,light or heat. The resulting lipid free radicals (R.) directly absorb oxygen to form peroxy radicals (ROO.) which are reactive,they then react with more RH to form lipid hydroperoxides (ROOH) in the propagation step. Lipid hydroperoxides are the primary products of autoxidation.

$$RH \xrightarrow{\text{initiator}} R. + H.$$

$$R. + O_2 \longrightarrow ROO.$$

$$ROO. + RH \longrightarrow ROOH + R.$$

Decomposition of lipid hydroperoxides is complicated and produces several compounds that cause flavor change. The breakdown products formed include aldehydes, ketones, alcohols, hydrocarbons, esters, furans and lactones (Forss, 1972; Frankel, 1983). In addition to the formation of breakdown products, lipid hydroperoxides can further react with oxygen to form secondary products or condense into dimers and polymers which can break down and produce volatile materials.

The mechanism of hydroperoxide formation varies because of the different unsaturated fatty acids incorporated into triglycerides and phospholipids. In Table 1 is shown the composition of cooked pork sausage and pepperoni. The total lipid in cooked sausage is approximately 31.1% and 44.0% in pepperoni. Approximately 60% of the total lipids are unsaturated fatty acids. In Table 2 is shown the fatty acid breakdown of the lipid fraction. Oleic and linoleic are the fatty acids present in the greatest amount. Therefore, in these products oleate and linoleate autoxidation is to be expected. A mechanism for oleate and linoleate autoxidation has been proposed by Frankel (1979), and is shown in Figure 1.

However, the actual oleate autoxidation mechanism was found more complicated than that proposed according to analysis based on GC-MS (Frankel et al., 1977a) and HPLC (Chan and Levett, 1977).

Table 1 Composition of cooked pork sausage and pepperoni(a)

| Description | Content(%) | |
|---|---|---|
| | Pork sausage | Pepperoni |
| Water | 44.57 | 27.06 |
| Protein | 19.57 | 20.97 |
| Total lipid | 31.16 | 43.97 |
| Total carbohydrate | 1.03 | 2.84 |
| Fibre | 0.00 | 0.00 |
| Ash | 3.60 | 5.17 |

(a) From Richardson et al.(1980)

Table 2 Lipid composition of cooked pork sausage and pepperoni(b)

| Fatty acid | Content(%) | |
|---|---|---|
| | Pork sausage | Pepperoni |
| Saturated | 37.90 | 38.76 |
| Unsaturated | 62.10 | 61.24 |
| C 18:1 (oleic) | 44.92 | 45.47 |
| C 18:2 (linoleic) | 11.50 | 8.99 |
| C 18:3 (linolenic) | 1.89 | 0.99 |
| C 20:4 (arachidonic) | - | 0.34 |

(b) Calculated from Richardson et al.(1980)

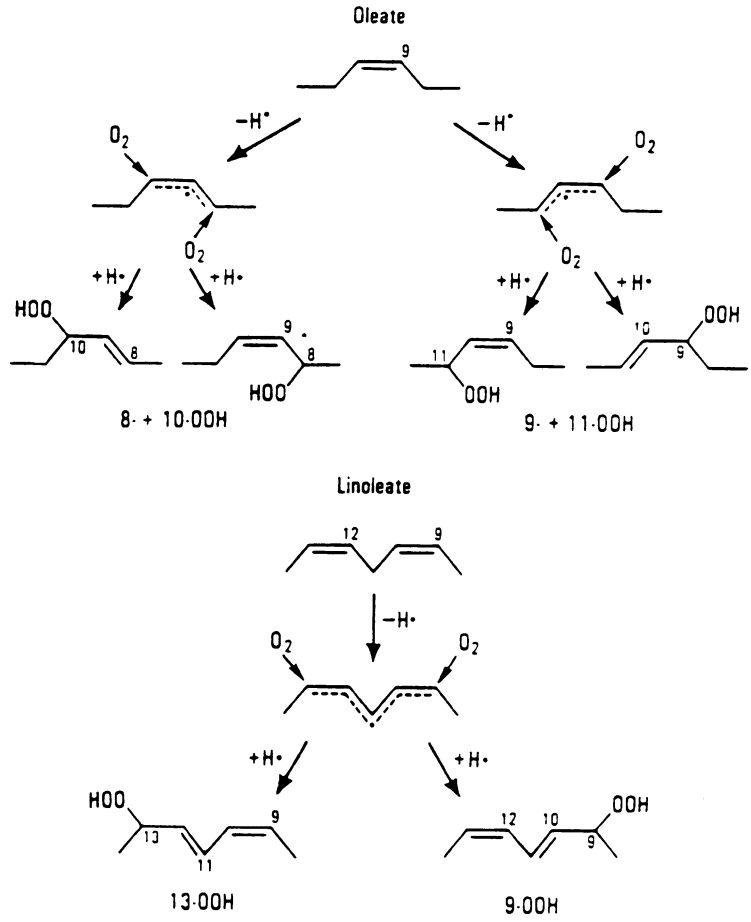



Figure 1 Mechanism of oleate and linoleate autoxidation (Frankel, 1979)

Kimoto and Gaddis (1969) discovered that alk-2,4-dienals are the major aldehydes formed, from linoleic acid, in autoxidized lard and pork adipose tissue. Alk-2,4-dienals contribute significantly to undesirable odors and flavors.

Although only small amounts of linolenic and arachidonic fatty acids are found in pork sausage and pepperoni, they can produce oxidation products. The oxidation of linolenate and arachidonate may take place according to a mechanism suggested by Frankel et al. (1961), and shown in Figures 2 and 3.

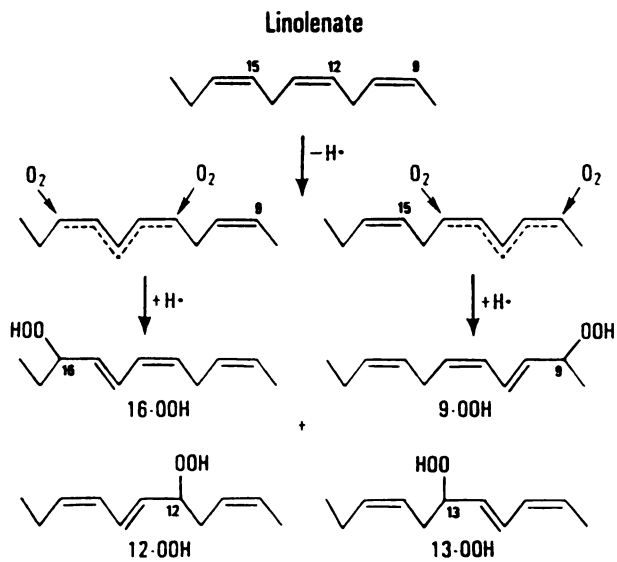



Figure 2 Mechanism of linolenate autoxidation (Frankel, 1961)

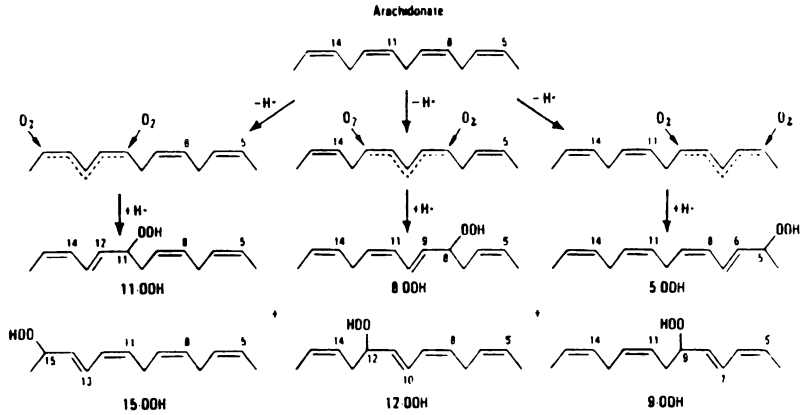



Figure 3 Mechanism of arachidonate autoxidation (Frankel, 1961)

Cyclic peroxides are secondary oxidation products formed from poly-unsaturated fatty acids such as linolenic by autoxidation, enzyme oxidation and/or photosensitized oxidation. Pryor et al. (1976) proposed a mechanism for the cyclization of peroxides from linolenate (Figure 4). Mono- and bicycloendoperoxides are precursors of malonaldehyde which can react with TBA.

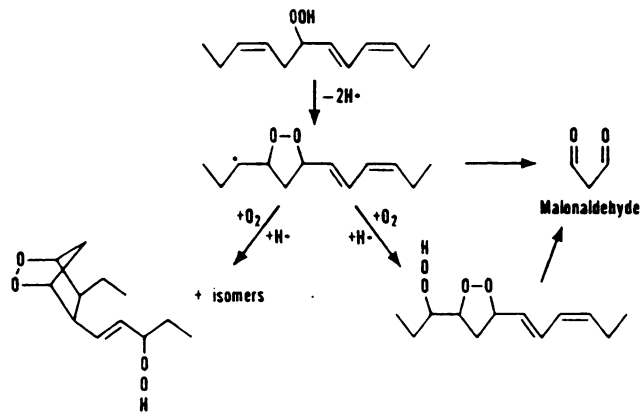



Figure 4 Mechanism of 1,3-cyclization of 12- and 13 hydroperoxides of linolenate and formation of malonaldehyde

### 4.1.2 Photo-sensitized oxidation

Unsaturated lipids can undergo oxidation via photo-oxidation which results from exposure to light and a sensitizer such as a pigment which is able to absorb light. Gollick (1968) proposed two pathways for photo-sensitized oxidation. In type 1, following light absorption the sensitizer becomes activated and reacts with lipid to form intermediates which then react with triplet oxygen (ground state) to yield oxidation products. In type 2, the activated sensitizer directly reacts with oxygen to form singlet oxygen (generally regarded as the reactive species) which then reacts with lipid to form oxidation products. Chan (1977) suggested that riboflavin sensitization involves type 1 mechanism while erythrosine involves type 2 sensitization mechanism.

Frankel (1984) reported that photo-sensitized oxidation is a non free radical process. He proposed that triplet oxygen ($^3O_2$) is activated to the singlet state ($^1O_2$) by transfer of energy from the photosensitizer. The resulting singlet oxygen is very active, at least 1500 times faster than triplet oxygen (Rawls and van Santen, 1970). Singlet oxygen then directly reacts with lipid (RH) to form lipid hydroperoxides (ROOH). It is believed that singlet oxygen may play an important role in initiating the normal free radical autoxidation of unsaturated fats.

$$\text{Sens} + h\nu \xrightarrow{\quad} {}^{1}\text{Sens}^{*} \text{ (excited)}$$

$$^{1}\text{Sens}^{*} + {}^{3}\text{O}_2 \xrightarrow{\quad} \text{Sens} + {}^{1}\text{O}_2$$

$${}^{1}\text{O}_2 + \text{RH} \xrightarrow{\quad} \text{ROOH}$$

Decomposition of lipid hydroperoxides (ROOH) produces oxidation products which can result in undesirable flavors and odors. Lipid hydroperoxides produced via photo-oxidation can be different from those from non photo-sensitized autoxidation. Thus different isomers and distribution levels may result (Frankel et al., 1977a, 1977b, 1977c, 1979).

Frankel (1980) proposed the following mechanism for the photo-sensitized oxidation of oleate and linoleate via reaction with singlet oxygen (Figure 5).

In the presence of natural quenchers such as carotenoids, photo-sensitized oxidation can be disrupted (Foote, 1976).

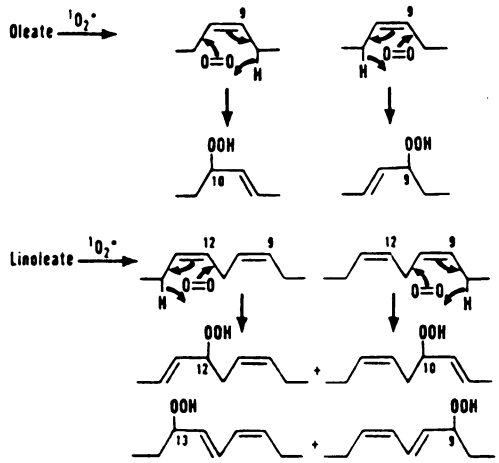
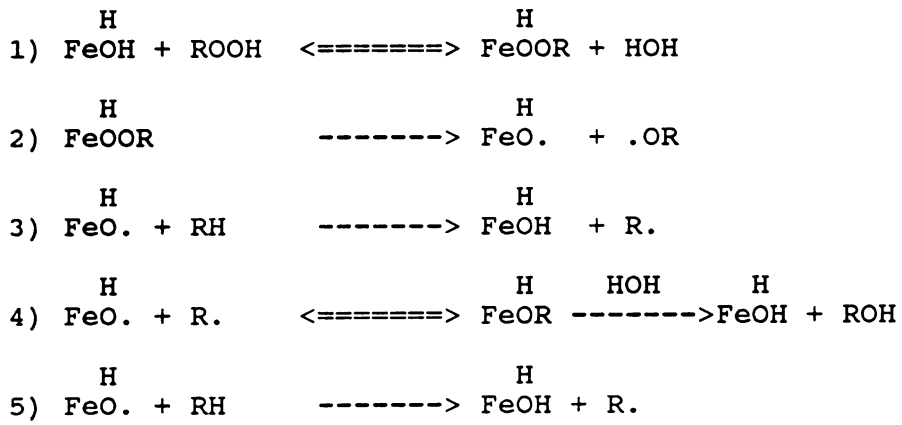



Figure 5 Mechanism of photo-sensitized oxidation (Frankel, 1980)

### 4.1.3 Heme-catalyzed lipid oxidation

The catalyzed oxidation by hematin compounds has been investigated by many researchers (Love and Pearson, 1974; Sato and Hegarty, 1971; Tappel, 1953,1955; Younathan and Watts, 1959). Most workers believe that meat pigments accelerate lipid oxidation and give rise to the development of warmed-over flavor (WOF) and rancid odor. Maier and Tappel (1959) proposed that hemes ($FeOH_2$) as active catalysts form unstable compounds with fat peroxides (ROOH) which then decompose to give two free radicals (FeOH., .OR), each of which is capable of initiating an oxidation propagation.




Greene and Price (1975); Liu and Watts (1970) reported that heme ($Fe^{3+}$) and nonheme ($Fe^{2+}$) were active catalysts of lipid oxidation in model systems and cooked meats; and that the ferric state was more active (Greene and Price, 1975). This agreed with work done by Tarladgis (1961) and Younathan and Watts (1959). Tarladgis (1961) suggested that

the ferric compounds initiated lipid oxidation while ferrous heme compounds were inactive.

Younathan and Watts (1959) found that uncured meat containing ferric denatured globin hemochromogen became rancid faster than cured meat containing pigments with ferrous iron. Increased rancidity was observed in cured meats apparently associated with the brown colored pigments, during storage.

Hirano and Olcott (1971),Kendrick and Watts (1969) and Nakamura and Nichida (1971) postulated that heme compounds could act as either accelerators or inhibitors of lipid oxidation depending upon the ratio of heme to unsaturated fatty acids. They suggested that heme and heme-proteins act as catalysts at low concentration and as inhibitors at high concentration.

It was suggested by Igene et al. (1979a); Love and Pearson, 1974; and Sato and Hegarty, 1971) that nonheme iron ($Fe^{2+}$) is the major prooxidant in cooked meat. Using a model system, Love and Pearson (1974) found that the addition of metmyoglobin to meat at levels of 1-10 mg/g of meat did not promote lipid oxidation but as low as 1 ppm $Fe^{2+}$ did. Ledward (1971) explained that porphyrins in denatured heme compounds possed low spin characteristic and were less effective as catalysts of lipid oxidation. Oxidation catalyzed by nonheme iron was pH dependent. Liu (1970) pointed out that the maximal activity of prooxidant

$Fe^{2+}$ was in the range of pH 5.0-5.5.

Igene et al. (1979a) have illustrated that nonheme iron is released from bound heme pigments during the cooking of meat. This explained the results of Erickson (1975) who reported that heat had the greatest effect on heme catalyzed lipid oxidation because of the increased iron exposure to unsaturated fatty acids.

Schaich (1980) postulated that photoeffects may have been involved in many experiments reported upon in the literature. Therefore, heme iron may act as a photosensitizer of lipid oxidation.

The addition of sodium chloride to meats can act as a pro-oxidant in oxidation reactions. The effect of freezing on oxidation is due to marked changes in pH. Ang and Young (1986) have recently shown that NaCl acted as a pro-oxidant in raw and freshly cooked chicken patties, but as an anti-oxidant in cooked stored meat. They explained that addition of NaCl increased ionic strength (IS) and polyphosphates increased pH and IS which reduced oxidation rate.

Igene et al. (1979a), Sato and Hegarty (1971), and Zipser et al. (1964) proposed that the addition of nitrite to meats reduced lipid oxidation, with a fivefold to a ninefold decrease occurring according to the type of meat (Igene et al., 1979a). The role of nitrite as an antioxidant in cured meat has been widely studied. Goutefongea et al. (1977), and Woolford and Cassens (1977) indicated that up to 35% of the nitrite added during curing was localized in the

adipose tissue and generally remained in the free state (unbound). Therefore, nitrite was available to react with unsaturated fatty acids (Frouin et al., 1975) which could result in the inhibition of lipid oxidation. Pearson et al. (1977) postulated that nitrite may either stabilize the lipid components or may inhibit the action of prooxidants normally present in muscle tissue. Zipser et al. (1964) proposed that nitrite reacted with heme-containing proteins to form catalytically inactive species. However, it was pointed out by MacDonald et al. (1980) that nitrite can act as either a prooxidant or antioxidant. He showed that nitrite in concentrations exceeding 25 ppm acted as a prooxidant. Nitrite acted as an antioxidant in model systems containing a low concentration of nitrite or $Fe^{2+}$ as prooxidants. It was proposed that nitrite may act as a metal chelator to tie up $Fe^{2+}$. Furthermore, heme-catalyzed oxidation of cooked meats can be prevented by the addition of polyphosphates and vegetable extracts (Ramsey and Watts, 1963).

The terms "nonheme" and "heme protein" were used by Love (1983). He referred to nonheme as free inorganic iron compounds. Nonheme iron in tissues can be found to associate with a variety of proteins. Heme protein has been generally used to refer to myoglobin or hemoglobin type compounds. However, the effect of heme and nonheme iron on lipid oxidation is not always clearly describable,

especially when heme containing proteins exist as part of lipids.

## 4.2 Tomato color

The color associated with tomatoes and tomato products is a result of the presence of carotenoid pigments which contribute a yellow to red color. Many different types of pigments are present but the most abundant carotenoid is lycopene which comprises approximately 83% of the total pigment in tomatoes (Gould, 1983). The general structure of chromophoric compounds such as carotenoids consists of alternative double and single bonds in conjugation. At least seven double bonds in conjugation are necessary to generate perceptible yellow color. The red color results from the large number of conjugated double bonds in the lycopene structure which usually are extended in an all trans configuration (Mackinney and Little, 1962).

Lycopene

Although carotenoids are much more stable than many animal and plant pigments, they may be partially destroyed due to light, heat, and acid (Mackinney and Little, 1962), and in the presence of metallic ions or oxygen (Gould, 1983). Isomerization of the trans-form to cis-form due to exposure to light, by heat and by acid results in loss of

color. The all trans-form results in the deepest red color while the all cis-form is the weakest. Because the carotenoids are highly unsaturated, they are susceptible to oxidation in the presence of oxygen and catalysts. Therefore, low storage temperature and exclusion of oxygen will reduce color loss.

## 4.3 Meat pigments

In meat muscle, myoglobin and hemoglobin are the major pigments. Both are complex proteins that undergo similar reaction, though myoglobin is much more responsible for the development of cured color. A molecule of myoglobin is composed of a protein known as globin, complexed to a nonprotein fraction containing iron. The iron-containing fraction is known as heme and is composed of two parts,an iron atom (at the center) and porphyrin which is made up of four heterocyclic pyrole rings linked together by methene bridges. The nature of the group attached to the iron atom of the heme determines the pigment color (Figure 6).

Dependent upon the group attached to the iron, it can exist in the ferrous ($Fe^{2+}$) or ferric ($Fe^{3+}$) forms. The ferrous form contributes to the bright red color found in both fresh and cured meat. On the contrary, the ferric form contributes to the brown color in the oxidized myoglobin which is considered to be an undesirable color. All three forms of myoglobin are reversible when subjected to favorable conditions.

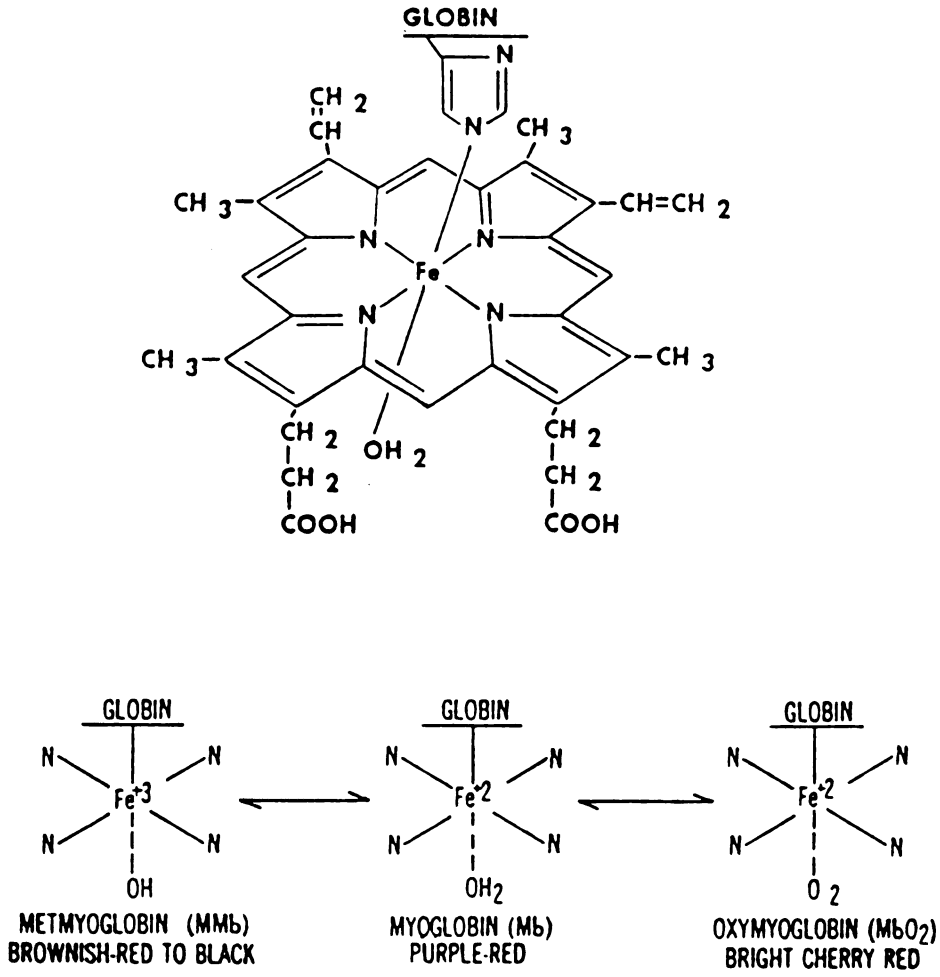



Figure 6 The structure of myoglobin and its reversible forms (Pearson and Tauber, 1984)

## 4.4 Cured meat pigments

Cured meat color results due to a reaction between nitrite and myoglobin. The mechanism by which cured color is developed in meat has been investigated by Fox and coworkers (1963, 1966 and 1968). They proposed that the cured meat chromophore was developed through oxidation-reduction reactions where nitrite acts as a strong oxidant and ascorbate and/or cysteine as reductants. The resultant cured color is due to nitrosylmyoglobin which then denatures to form nitrosylhemochrome if cooked to 150°F. The proposed reactions involved in cured meat color formation are shown in Figure 7.

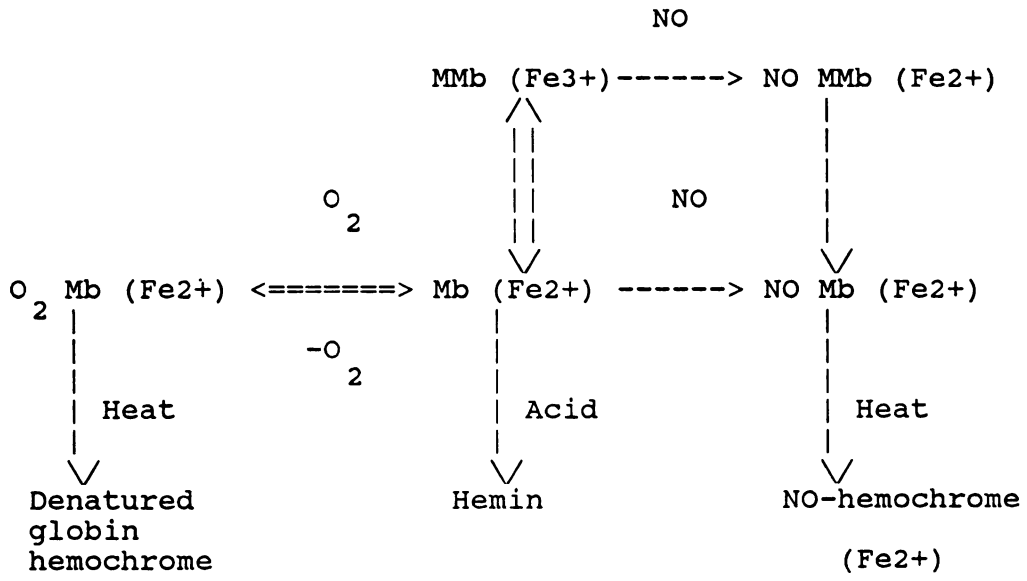



Figure 7 Heme pigment reactions of cured meat products (Fox, 1966), [$O_2$ Mb, oxymyoglobin; MMb, metmyoglobin; NO MMb, nitrosylmetmyoglobin; NO Mb, nitrosylmyoglobin ]

However, Tarladgis (1962b) stated that the cured pigment was dinitrosylhemochrome, which is composed of two nitric oxide molecules complexed to the heme. This theory was based on correlation of the position of the maximal absorption to the bond type in iron-porphyrin compounds. His suggestion was strongly supported by Lee and Cassens (1976). Although the reaction of NO with the Fe atom of myoglobin is widely accepted, the series of reactions leading to the cured color complex is not clearly understood (Cassens et al., 1979).

Cured meat pigments are stable, though they can be effected by chemical reaction, including lipid oxidation or environmental conditions such as light and temperature and packaging. Watts (1954) stated that color fading can result from either lipid oxidation or by irradiation with fluorescent light which accelerates the dissociation of nitric oxide from the ferrous heme complex. Tarladgis (1961) suggested that both mechanisms involved electron removal from iron which then becomes susceptible to oxidation, resulting in formation of oxidized pigments.

Walsh and Rose (1956) observed the effect of light and temperature on oxidation of nitric oxide myoglobin (MbNO) and concluded it was a first order reaction in the dark and in exposure to light. MbNO was destroyed in the dark by an autoxidation reaction.

$$MbNO + 1/2\ O_2 \longrightarrow MMb + NO_2^-$$

In the light, photo-oxidation was involved.

$$MbNO \underset{}{\overset{light}{\Longleftrightarrow}} MbNO^{*} \xrightarrow{1/2\ O_2} MMb + NO_2^-$$

The reaction was greater at elevated temperatures and light intensities. Fox (1966) suggested that excluding oxygen from the product delayed discoloration.

To avoid the effect of light on dissociation of nitric oxide, the product should be stored in the dark or the product should contain a sufficiently high concentration of nitrite and a reducing agent such as ascorbic acid (Watts, 1954).

Tarladgis (1962b) pointed out that his proposed mechanism (1961) for heme-catalyzed lipid oxidation was supported by the structure associated with cured meat pigments.

## 4.5 Measurement of tomato color

Among several techniques developed to measure color in tomato products, the Tristimulus colorimeter or color difference meter is most widely used. This instrument evaluates sample color using three photo-cells connected to a very sensitive galvanometer which records in numerical terms; hue, saturation and luminosity viewed in reflected light from a standard source (Goose and Binsted, 1973). The Hunter lab color meter (Hunterlab D-25 model) operates on the same basis and measures whiteness and blackness, redness and greenness, and yellowness and blueness, which are

represented as L, a and b on a scale, respectively. In actual usage, it must be standardized against permanent color standards, usually affixed to ceramic tiles. The color characteristics of the selected standard should not be far different from the sample under examination.

In practice, tomato color represented as a ratio of a/b has become the norm for most control purposes (Goose and Binsted, 1973). The a/b ratio was suggested by Yeatman (1969) because it provided high correlation with visual judgement. Other numerical notation can also be used with similarly high correlation, including b/a, ab and $aL^2$ (Francis and Clydesdale, 1970). The color function $a/L[1/(a^2+b^2)^{1/2}]$ was found to produce high correlation for use in grading tomatoes based on all three parameters (Hunter and Yeatman, 1961).

4.6 Measurement of cured meat color

A widely accepted procedure used to quantify cured meat pigments is the extraction method using 80% acetone in water, developed by Hornsey (1956). He indicated that only nitric oxide heme pigments or nitrosohemochrome were extracted producing an acetone-nitric oxide heme complex. The extract can be measured spectrophotometrically at 540 nm. Using this technique, only 80% of the total meat pigments are extracted with the other 20% possibly present as metmyochromogen (Tarladgis, 1962a). The uncombined or unreduced pigment in cured meat can be extracted using a

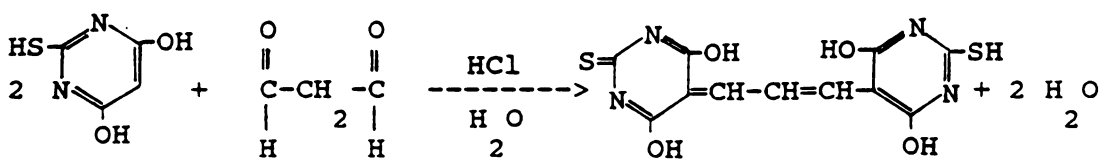
modified technique (Hornsey, 1956) and measured spectrophotometrically at 640 nm.

The bright red acetone extract is stable at least one hour (Hornsey, 1956) gradually fading to a yellow-brown color. Hornsey (1957) found that the color fading of cured meat pigments due to light catalyzation, is a first order reaction. He stated that the original concentration of pigment was of primary importance, because a highly pigmented zone, even after 50% loss, may still appear to be red whereas a pale area undergoing the same absolute rate of loss, appeared to be much less intense.

In addition to the extraction method, the color intensity associated with the cured meat surface may be directly measured using a spectrophotometric method (Erdman and Watts, 1957) where the extinction ratio of absorbance at 570/650 nm is used to characterize changes in the pigments. The higher the ratio the more red pigments are present. Iversen (1984) used the Hunterlab Labscan II sphere spectrocolorimeter to measure color of cured meat in terms of L, a and b. Rikert et al. (1956a) also used the Hunter Color and Color-Difference Meter to assess the color change on the meat surface. They presented the change in terms of AE where $AE = (AL^2 - Aa^2 - Ab^2)^{1/2}$ ; AL, Aa and Ab represent differences between readings on the sample surface and those of the standard. The decrease in AE or increase in Aa reflected the increase in redness of the cured pigments.

## 4.7 TBA method for following lipid oxidation

The 2-thiobarbituric acid (TBA) test is widely used to measure the extent of oxidative deterioration of lipids in muscle foods (Gray, 1978). The test is based on the formation of complex colored compounds derived from the reaction of TBA and malonaldehyde. Malonaldehyde is a water soluble substance formed or released upon heating oxidized samples in an acid medium. Sinnhuber et al. (1958) proposed that condensation of two moles of TBA with one mole of malonaldehyde results in formation of TBA chromagens, which can be measured spectrophotometrically , normally at 540 nm.




TBA Malonaldehyde TBA chromagen

Rhee (1978) stated that the TBA test can be used on muscle foods in three ways: (1) directly on the food product, followed by extraction of the colored complex (Sinnhuber and Yu, 1958; and Yu and Sinnhuber, 1957); (2) on an extract of the food (Witte et al.,1970); and (3) on a portion of the steam distillate of the food (Tarladgis et al.,1960). He concluded that the steam distillate method is the most popular one for measuring the TBA number in muscle foods. The TBA number is reported as mg of malonaldehyde per 1000 gm of sample.

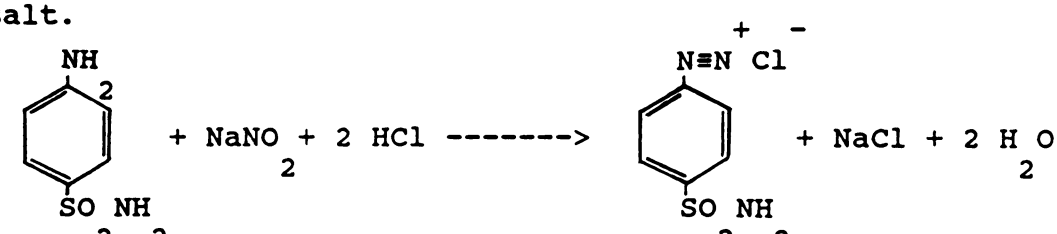
Although the distillate method (Tarladgis et al., 1960) is used by most researchers, it does not necessarily mean that it is the most accurate or reproducible method (Melton,1983). It has been reported that alk-2,4-dienals also react with TBA to form a red complex with the same absorption maximum as the malonaldehyde TBA complex at 532 nm (Jacobson et al.,1964; and Marcuse and Johansson, 1973).

Witte et al. (1970) found that TBA values recorded for meat by the distillation method were twice as large as those done by extraction. The heat involved in distillation may have stimulated release of malonaldehyde from carbonyl addition compounds and/or have produced increased quantities of aldehydes from lipid precursors.

The distillate method has been modified by several investigators. The addition of antioxidant at the distillation stage to prevent oxidation has been studied (Moerck and Ball, 1974; and Rhee, 1978). An increase in the TBA number was found for some of the trials instead of a decrease. Consequently, the advantage of using antioxidants to prevent oxidation during testing is not clear. Rhee (1978) found that chilled blending and addition of propyl gallate (PG) and disodium ethylenediamine tetraacetate (EDTA) at the distillation or blending stage reduced the TBA number of catfish steaks, but had no effect for ground beef.

For cured meat products containing nitrite, the addition of sulfanilamide at the blending stage in the distillate method was advised (Zipser and Watts, 1962) to

prevent nitrite from reacting with malonaldehyde which could give lower TBA numbers. Barnes and Folkard (1951) described the reaction of sulfanilamide with nitrite to form diazonium salt.




Sulfanilamide Diazonium salt

Younathan and Watts (1959) observed that a high level of nitrite (200 ppm) interfered with the TBA reaction but a low level (100 ppm or less) had no effect. In contrast, Shahidi et al. (1985) have recently reported that the TBA values for cured meat containing 100-200 ppm of nitrite were larger in the presence of sulfanilamide than in the absence thereof, but the opposite was found with cured meat containing 0-50 ppm of nitrite. This led them to conclude that the use of sulfanilamide in the distillate method may cause the underestimation of TBA values when residual nitrite is very low or not present. The residual nitrite in cured meats was reported in the range of a few ppm to 50 ppm (Cassens et al., 1974).

Tarladgis et al. (1960, 1962) stated that in addition to malonaldehyde TBA reagent can react with other compounds in oxidized foods. Impurities in the reagent may react which could result in color interference (Yu and Sinnhuber, 1967). Baumgartner et al. (1975) postulated that TBA may

react with certain carbohydrates to form a red color. To avoid this problem, direct measurement of malonaldehyde in the distillate has been investigated by Kakuda et al. (1981) using high pressure liquid chromatography (HPLC). They reported high correlation between the HPLC and colorimetric methods and suggested that the HPLC method was faster and not affected by the presence of other compounds.

TBA values do not always continue to increase throughout storage of muscle foods. Benedict et al. (1975); Igene et al. (1979b); Seo (1976); and Tarladgis and Watts (1960) observed a decline in TBA values during frozen storage of cooked meats. The decreased TBA values found during frozen storage may be due to reaction(s) between malonaldehyde and proteins (Buttkus and Bose, 1972; Gardner, 1979; and Karel, 1973). Gokalp et al. (1983) suggested that the lower TBA values found in cooked beef patties during frozen storage was a result of the slow rate of autoxidation of unsaturated fatty acids at low temperatures and the instability of malonaldehyde. They observed a reduction in total unsaturated fatty acids in triglycerides and phospholipids. The reduction in total unsaturated fatty acids may have resulted in the decreased malonaldehyde content and in turn lower TBA values. In triglycerides C18:1 and C18:2, and in phospholipids C18:3 and C20:4 changed significantly (Gokalp et al., 1983).

Dahle et al. (1962) observed that the TBA test was a meaningful tool for comparison of samples of a single material at different stages of oxidation.

4.8 TBA value and undesirable flavor

Several attempts have been made to establish a relation between the TBA value and undesirable flavor development in fat tissues. Zipser et al. (1964) obtained high correlation between rancid odor and TBA numbers for both cured and uncured meat samples. In contrast, poor correlation was observed by Wyatt and Day (1965). Gray (1978) postulated that the poor result reported by Wyatt and Day was due to the type of flavor evaluation used, which was based on flavor threshold. The average flavor threshold is derived from the level of oxidized sample at which 50% of the judges detect rancidity. Pohle et al. (1964) found that flavor score could not relate to any given fat from TBA data since the relative level varied from product to product. The threshold range for detection of rancid off-flavor in cooked pork was approximately a TBA number of 0.5-1.0 (Tarladgis et al., 1960). This range was in agreement with observation made by Turner et al. (1954), who reported a value of 0.46. Turner et al. (1954) found that pork with a TBA value over 1.2 was unacceptable according to the results of a taste panel.

The TBA number can serve as an indicator of oxidized odor or flavor in meat as shown by trained or experienced

sensory panelists (Gokalp et al., 1983; Tarladgis et al, 1960; Turner et al, 1954; and Zipser et al., 1964). The assessment of oxidized flavor in cooked meat by untrained panelists was investigated by Greene and Cumuze (1983). They observed low correlation between TBA values and flavor scores which they felt was due to the result of the variability in individual panelist scoring and inconsistencies in the TBA test.

## 4.9 Factors affecting storage life of frozen pizza

Frozen pizzas are a precooked frozen food. Martin and Schoch (1977) stated that the quality of precooked frozen foods can be affected by a number of factors, including the nature of food components, the condition of product entering storage, method of cooking employed, degree of doneness, flavoring ingredients, packaging material and method of packaging, and storage temperature.

### 4.9.1 Effect of storage temperature

It is generally accepted that quality loss in frozen meat is less if stored at -17.7 °C (commercial storage temperature). Fluctuating storage temperature results in change in the rate of deterioration. The speed of chemical reactions in frozen food is increased two and one half times when the temperature is raised 18 °F or 10 °C (Martin and Schoch, 1977). The oxidation of meat pigments are temperature dependent. Tressler and Evers (1947); and Ramsbottom and Koonz (1941) found that the higher the

storage temperature, the greater the change in color from red to brown. Simard et al. (1983) observed that frankfurters stored at -4 and 0 °C had better keeping quality than those held at 3 and 7 °C. Klose et al. (1959) found that storage life of poultry meats at -6.7 °C was one half of that held at -17.7 °C. Hanson and Fletcher (1958) reported that turkey dinners developed rancid flavors in 1.5 to 3 months in samples held at 20 °F but not till 6 months at 10 °F.

4.9.2 Effect of fluctuating temperature

When a frozen food is stored under fluctuating temperature, an increased rate of quality deterioration is usually expected. However, if the maximum temperature during temperature fluctuation is sufficiently low so that the product remains solidly frozen and no liquid separates, the deterioration rate is approximately equivalent to that of the product held constantly at the mean temperature (Martin and Schoch, 1977). This was also reported by Emerson et al. (1951) and Gortner et al. (1948).

During frozen storage, desiccation and freezer burn due to surface dehydration are likely to take place in highly fluctuating storage conditions. Freezer burn is an extensive desiccation resulting in an irreversible protein denaturation. Some workers have studied the effect of freezer burn on quality of frozen foods. Kaess and Weideman (1967) found that freezer burn caused ice cavities to form

due to sublimation of ice crystals. Cook and White (1940) reported that freezer burn accelerated rancidity development in frozen poultry meat because in the dried surface condition the fat is not protected from oxygen by an ice barrier, therefore, the greater contact between oxygen and fat enhanced an increased oxidation rate. Freezer burn also results in deterioration in appearance and color. Surface desiccation is prevented by packaging with a low water vapor permeability film and reducing the headspace between product and package.

Winter et al. (1952) reported that fluctuating temperature in the range of 0-13 $^{o}$F did not cause change in flavor or desiccation of ground meats in comparison with the product stored at a constant temperature of 0 $^{o}$F. The choice of packaging material and the shape of the package were the major factors contributing to flavor change. Hustrulid et al. (1949) found that the temperature fluctuation below 0 $^{o}$F did not affect the quality of well packaged ground beef and ground pork, as compared to storage at a constant temperature of 0 $^{o}$F. Townsend and Bratzler (1958) found that alternatively thawing (24 hr , 36 $^{o}$F) and freezing (24 hr, -20 $^{o}$F) caused fluctuations in percent metmyoglobin formation in steaks packaged in cry-o-vac bags. Surface darkening occurred with samples thawed more than once.

4.9.3 Effect of light

The effect of light on lipid oxidation and color fading of meat products has been widely recognized. The reaction rate is dependent upon light intensity and spectral distribution, distance between light source and product, light absorptability of active chromophores in the food, presence of sensitizers, temperature and available oxygen (Spikes, 1981).

Cool white fluorescent lamps (40 watts) are used most in display cabinets. The emitted light spectrum from the lamp falls in the range of 350-750 nm where the maximum emission is at 580 nm (GE, 1978). Most of the emitted light from cool white fluorescent lamps fall in the blue, green and yellow range (Figure 8). Much research has been conducted on the influence of light on discoloration of meat pigments.

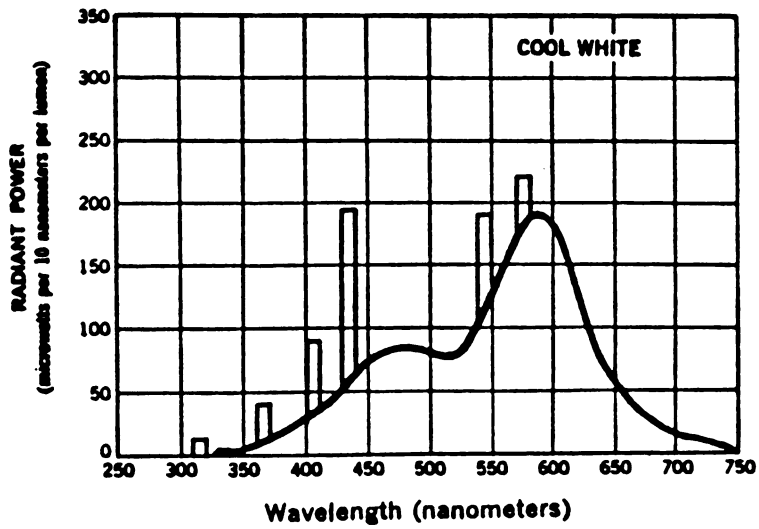



Figure 8 The spectral distribution of a 40 watt cool white fluorescent lamp (GE, 1978)

Sacharow (1970) stated that the blue and green light stimulated the dissociation of nitric oxide. Townsend and Bratzler (1958) found that light between 560 nm and 630 nm contributed to the greatest color fading in packaged frozen meat. Bard and Townsend (1971) reported that the fading of cured meat pigments was due to oxidation of nitric oxide hemochromogen (pink) to metmyoglobin (brown), in the presence of oxygen and light (Watts, 1954). Iversen (1984) suggested that exclusion of light below 600 nm slowed the degradation of cured meat pigments.

Light intensity plays an important role in extent of color change of meat and has been investigated by many workers. Kraft and Ayres (1954) reported that soft white fluorescent light of 50-60 footcandles promoted color fading of bologna faster than that of 30-35 footcandles. Townsend and Bratzler (1958) observed that 56 footcandles of fluorescent light did not much affect discoloration of meat stored at 0 °F. Sacharow (1970) suggested that the light intensity in display cabinets should not exceed 25 footcandles for cured meats, cold cuts and not over 15 footcandles for ham.

Igbinedion et al. (1983) found that 100 footcandles of cool white fluorescent light significantly increased the total aerobic psychrotroph count of fresh pork stored at 2+1 °C but had no effect on TBA number and rancid odor.

Simard et al. (1983) reported that 100 footcandles of fluorescent light had a significant effect on color fading

of vacuum packed frankfurters. Vacuum packed samples had more light fading than on nitrogen packed samples. However, the total effect of light on cured pigments was very little for both vacuum and nitrogen packed. This supported the work by Fox (1966) who suggested that exclusion of oxygen by vacuum or nitrogen packaging remarkably inhibited oxidation of nitric oxide hemochromogen.

Even after color fading, cured pigments can recover some of their original color upon storage in the dark (Sacharow, 1970). Rikert et al. (1956a) postulated that use of vacuum packaging might accelerate the return of red color in meat after long storage though no explanation was clearly made.

Hunt et al. (1975) found that incandescent light caused less color degradation than did fluorescent light because it was poorly absorbed by meat pigments. Sacharow (1969) stated that fluorescent light has a stronger bleaching effect than ordinary incandescent light bulbs. An important factor is that incandescent bulbs radiate heat which may cause discoloration due to an increase in temperature (Sacharow, 1969).

Distance between the products and the light source is also a crucial factor in discoloration. If the distance is reduced by half, the effect of the lighting is four times increased (Sacharow, 1969).

### 4.9.4 Effect of packaging

Packaging materials and packaging techniques are tools which can provide protection to frozen foods during storage. Among the packaging materials being used, plastic packaging has expanded most rapidly because of their light weight,wide available and different varieties. Plastics may be used as flexible wraps and semirigid and rigid packages.

Feinberg and Hartzell (1968) suggested that when selecting a package for frozen foods, several factors must be taken into account, these include: (1) atmospheric oxygen, (2) loss of moisture, (3) flavor contamination, (4) entry of microorganisms, (5) mechanical damage and (6) exposure to light. They stated that desired properties include impermeability to moisture and oxygen, transparency, heat sealability, heat shrinkable, toughness and flexibility at low temperature. An effective package reduces deterioration and prolongs storage life of frozen foods.

Plastic materials

Different plastic films have different properties. Coatings and laminations may be used to obtain a required property which a single material does not provide. de Fremery and Sayre (1968) suggested that for frozen poultry, packaging materials should have at least two most important characteristics of impermeability to oxygen and to moisture. The ideal method of protection is vacuum or inert gas packing in cans where temperature of freezer storage is

no longer critical unless it is higher than 15°F (Sulzbacher and Gaddis, 1968). Moisture loss is not only a direct loss of product weight but also causes loss of appearance due to freezer burn on the product surface.

Polyethylene (PE) is a good moisture barrier, heat sealable and pliable at low temperature but is a poor barrier to oxygen. It is often combined with a Polyester film (Mylar or PET) and then it provides a good barrier to both water and oxygen as well as being heat sealable and flexible at low temperature ( down to -95°C).

Rubber hydrochloride film (Pliofilm) was used in the early years to provide transparency and heat sealability. Today PE has replaced it in most applications. Cellophane is usually coated with other resins such as PE or Saran for a specific use. Cellophane is a fairly good oxygen barrier when dry, but when wet it loses that barrier property. Its sensitivity to moisture can be protected by using a coating.

Oriented polypropylene (OPP) film is often used in combination with PE to provide clarity and improved barrier properties.

Polyvinylidene chloride (Saran® or PVDC) is a transparent, heat shrinkable film which provides a good barrier to both moisture and oxygen. Shrink packaging has been supplied by The Cryovac company since 1948 to protect against freezer burn and desiccation in frozen poultry. Saran also is frequently used as a coating resin for paper substrates. Ethylene vinyl alcohol copolymer (EVAL®) has

been increasingly used to replace Saran because of its superior oxygen barrier property.

Polyamide (Nylon) films have excellent toughness, tear and break strength properties. They are used by themselves and in laminates.

Klose et al. (1959) reported that use of a low oxygen permeability film maintained better quality chicken than did a high oxygen permeability film. They found that moisture loss had little effect on organoleptic deterioration but storage temperature and partial pressure of oxygen associated with chemical changes were more important. They noted that oxygen contributed to deterioration as much as a substantial rise in storage temperature.

Ground beef and hamburger are somewhat sensitive to oxidation and surface dehydration. Dalhoff and Jul (1965) found that a vacuum pack in a low oxygen and water vapor permeability film increased storage life of these meats to 270 days whereas in a poor barrier film the shelf life was 120 days.

Packaging techniques

In addition to the packaging material, the packaging method is a critical criterion to be considered in protecting frozen foods from environmental factors such as light, oxygen and temperature. Light accelerates the development of rancidity in fats and oils and also causes discoloration in cured meat products. Sacharow (1969)

stated that ultraviolet light accelerates discoloration in fresh meat due to an increased rate of desiccation and subsequent oxidation of myoglobin. In cured meats, the pink nitroso-myoglobin turns to brown metmyoglobin due to an oxidation reaction catalyzed by display light. Frozen cured meat also is susceptible to color fading with the degree of fading proportional to the exposure time. Opaque packaging materials reduce color fading in cured meats. Exclusion of oxygen and limiting the amount of light exposure also reduces fading.

Many investigators have attempted to employ vacuum techniques to protect meat products from oxidation reactions. Sacharow (1969) reported that vacuum packed ham in a package made from Cellophane/Pliofilm and packed under a vacuum of 29 inches faded after exposure to 115 footcandle-light for 60 hours. The degree of exposure to light is an important factor affecting the shelf life of vacuum packed-cured meats. The commonly used laminate films are PVDC/PE, PVDC/Surlyn and Nylon6/PE. Surlyn® is an ionomer which is resistant to oil and an excellent heat sealing material. It has been found that transparent laminate films in combination with vacuum did not provide significant protection from discoloration in cured meats when exposed to light (Amundson et al., 1982; Igbinedion et al., 1983; Kraft and Ayres, 1954; and Lin and Sebranek, 1979). The presence of a small amount of residual oxygen in

the package after the vacuum process still causes discoloration (Kraft and Ayres, 1954).

THe effect of vacuum level on color with different barrier films was examined by Amundson et al. (1982). In their experiment, Nylon/Saran/Surlyn and Nylon/Surlyn structures served as a high barrier and an intermediate barrier, respectively. They observed that nitroso-pigment conversion was higher in bacon packaged in the high barrier film. The results agreed with those reported by Lin and Sebranek (1979). Hunt et al. (1975) reported that the use of Saran film resulted in a higher retention of meat color than Cryovac L-300 bags (poor oxygen barrier).

The vacuum level required to maintain color of cured meat pigments in packaged bologna was 27 in.Hg (Kraft and Ayres, 1954). Sacharow (1970) suggested that 28 in.Hg provided good protection from discoloration.

The role of barrier packaging on lipid oxidation has been investigated. Lindsay (1977) reported that Surlyn/PVDC/Surlyn provided better protection from lipid oxidation of fish fillets than did Nylon/Surlyn and PE or Surlyn singly. They suggested that a vacuum of at least 20 in.Hg should be employed to achieve required protection.

Iversen (1984) suggested that the protective ability of the packaging material may be increased by selecting materials that are nontransparent or exhibit selective absorption in the region where the product is most sensitive. He proposed the use of colored packaging

materials to prevent pigment degradation in cured meats. He also observed that colored films: light brown, dark brown and dark red absorbed light below 400, 500 and 600 nm, respectively, and provided increased protection from discoloration of cured meat held at 4±1 °C.

Other than vacuum packing, headspace control by inert gas flushes can be applied and has been investigated by researchers (Ordonez and Ledward, 1977; Simard et al., 1983 and Jantawat and Dawson, 1977). The use of UV light absorbers in the film is another possible technique. Also, the use of oxygen scavengers such as palladium or platinum pallets can function to remove oxygen from a closed package (King, 1955). The effectiveness of a scavenger pouch (PET/Foil/Ionomer/Catalyst/Ionomer) in reducing oxygen headspace levels was demonstrated by Karel (1974). Hoojjat et al. (1986) have recently reported that the use of 3,5-Di(T-butyl)-4-Hydroxy Toluene (BHT) impregnated in HDPE pouches can reduce oxidation of oatmeal cereal. The diffusion of BHT from the pouch into the cereal resulted in the antioxidant activity.

# MATERIALS AND METHODS

## 5.1 Frozen pizzas

All pizzas were supplied by the Pillsbury Company, Minneapolis, MN. They were of the microwave, deluxe, combination pork sausage and pepperoni type. The pizzas were circular, seven inches in diameter. A crust was made from enriched wheat flour, fortified vitamins and other compounds. The topping was composed of Mozzarella cheese substitute (preservative added), cooked pork sausage, pepperoni ($NaNO_2$, BHA, BHT, citric acid, and color added), green pepper, red pepper and so on. The sauce was made from tomato puree, modified corn starch, artificial color, and other compounds.

The pizzas were originally packaged in a pouch made of Polyolefin, having a material thickness of 0.001 inches. The pouch had no barrier properties with respect to oxygen and water vapor because the pouch contained preformed holes. The pouched pizzas were contained in paperboard folding cartons and packed twelve in a corrugated shipper. Pizzas were shipped frozen via air transport and received at the School of Packaging, MSU. Each shipment (2 total) contained enough pizzas for a five month study.

## 5.2 Design of experimental treatments

The experimental design for the study is shown in Table 3. Fourteen treatments were selected from combination of factors, including packaging type and method, storage temperature, freeze-thaw cycling, and exposure to display light.

Upon arrival at the School, all pizzas were immediately placed in a -18 °C chamber prior to assignment to a specific treatment. The two shipments of pizzas were divided into four groups of samples, one and two from the first shipment and three and four from the second shipment. An equivalent number of pizzas for each treatment were randomly assigned.

At least three pizzas were randomly drawn for initial evaluation from each shipment. At monthly intervals, three pizzas from each treatment were evaluated. The study lasted for five months.

## 5.3 Sample preparation

On the day of evaluation, pizzas from the same group were removed from storage and individually analysed for quality change of the to pork sausage, pepperoni, and tomato sauce. Three measurements were obtained from each ingredient if a sufficient amount of that component was present for testing. To avoid the effect of room light on sample degradation during testing, the three components were isolated from the pizzas in a darkened room where the

Table 3 The design of experimental treatments

| Shipment | Gr. | Trt. | Packaging | | Temp. | Freeze-thaw | Light exposure |
|---|---|---|---|---|---|---|---|
| | | | Type | Method | (oC) | | |
| 1 | 1 | 1 | B | Atm | -18 | N | N |
| | | 2 | B | Atm | -18 | N | Y |
| | | 3 | B | Atm | -18 | Y | N |
| | | 4 | B | Atm | -18 | Y | Y |
| | 2 | 5 | B | Atm | -7 | - | N |
| | | 6 | B | Atm | -7 | - | Y |
| 2 | 3 | 7 | G | Vac | -18 | N | N |
| | | 8 | G | Vac | -18 | N | Y |
| | | 9 | G | Vac | -18 | Y | N |
| | | 10 | G | Vac | -18 | Y | Y |
| | 4 | 11 | G | Atm | -18 | N | N |
| | | 12 | G | Atm | -18 | N | Y |
| | | 13 | G | Atm | -18 | Y | N |
| | | 14 | G | Atm | -18 | Y | Y |

B = Poor barrier (original package)

G = Good barrier (test package)

N = Without F/T cycling or light exposure

Y = With F/T cycling or light exposure

Atm = Atmospheric pack

Vac = Vacuum pack

intensity of fluorescent light was reduced to as low as 2 footcandles (ft-c). At this intensity, the experimenter was still able to work with no problem. However, 20-25 ft-c of room light was required to give enough brightness to allow scraping of tomato sauce from pizzas. Light intensity was measured using the Gossen light meter (Panlux Electronic model).

Prior to sampling, vacuum packages generally required thawing in cold water for 5 min. to loosen the components squeezed under vacuum, while other packages were thawed at room temperature for 30 min. A longer time was required for thawing tomato sauce, normally 3 hrs at room temperature. Preparation was carried out in a darkened room as well.

5.4 Test package

The test package was a three side sealed pouch made from a good barrier film laminate (Nylon/PE) with dimensions of 11 in.x 10 in.x 0.003 in. (length, width and material thickness, respectively). The test packages were supplied by the Koch company, Kansas city, MO. The outer layer of the package material was Nylon, about 0.001 in. in thickness and the inner layer was PE, about 0.002 in. in thickness (see Appendix A for the film identification). The barrier properties, oxygen and moisture vapor, were determined by The American Society for Testing and Materials (ASTM) procedures,D-3585 (1981) and E-96(1980).

Upon arrival at the School, some of the pizzas were repacked into the test packages. For vacuum packs, a vacuum of 610 mm Hg was drawn on the unsealed pouches using a Multivac vacuum chamber machine (model AGW) , the pouches were then heat sealed under vacuum to close the packaged pizzas. These vacuum packed pizzas were placed into group three. The vacuum level inside the test packages was determined by reading from a pressure gauge (Kennedy Enterprises) inserted in a barrier pouch and heat sealed under vacuum in the same manner as the samples. An average vacuum level of 610 mm Hg was obtained from several readings. For atmospheric packs, the pizzas were inserted into the test packages and heat sealed under atmosphere conditions. These pizzas were placed into group four. The repackaging operation was carried out in a -12 $^{o}$C room, with light supplied by an incandescent lamp.

## 5.5 Storage condition

### 5.5.1 Temperature

Pizzas were stored either in a -18 $^{o}$C or in a -7 $^{o}$C chamber. Cold air in the chamber was circulated by an electric fan which automatically stopped blowing when the set temperature was reached. Therefore, both moving air and static air were involved. The temperature in the chamber fluctuated, mainly due to a loss of cold air which resulted when the chamber door was opened. Also, the temperature in the chamber varied with the amount of samples being stored,

the smaller amount the samples the lower temperature the chamber was. It was observed that the temperature varied from -25 °C to -13 °C for the -18 °C chamber and -8.3 °C to -3.8 °C for the -7 °C chamber.

In the -18 °C chamber, Pizzas were left in the corrugated shipping boxes and stacked five high on the chamber floor. In the -7 °C chamber, pizzas in corrugated boxes were placed on shelves without stacking.

### 5.5.2 Freeze-thaw cycle

One freeze-thaw cycle was selected to simulate a cumulative effect of several short periods of temperature fluctuation during transportation (private communication with Michael J. Gabriel, scientist, Pillsbury company). The freeze-thaw cycle consisted of placing boxes of pizzas on shelves without stacking in a 5 °C chamber for seven hours and then returning them to the -18 °C chamber. The temperature profile of pizzas during thawing was determined at three positions in the box (top, middle and bottom), using three probe thermometers which were inserted through the box into the pizza crust. Three simultaneous readings were taken at approximately hourly intervals, from 0 to 7 hours. Two boxes of pizzas were used to determine the temperature profile. The freeze-thaw treatment was performed within the first three days after the pizzas were received.

5.5.3 Light condition

In order to simulate display case lighting in grocery stores, a survey was performed to measure light intensity at several stores with different freezer displays. Therefore, the type of light and intensity were determined. It was found that most stores used cool white fluorescent lamps with varied wattages. There was wide variability from store to store, in the type of display case (chest freezers, cabinet freezers with doors, and cabinet freezers with curtains), light position and position of pizzas in the case. Measurements were taken in four grocery stores in East lansing, Michigan, and are expressed in Table 4. Two hundred footcandles was selected as a rough average and was considered to be a severe condition.

One day prior to testing, pizzas were exposed to display light for eight hours (average life of pizzas on shelves before being purchased) under storage conditions. Two 40 watt, white cool fluorescent lamps, 40 inches in length, were set at a predetermined height above the pizzas to give an average of 200 ft-cs at the sample surface.

A problem which occurred during light exposure was that a decrease in intensity output of the lamps at both storage temperatures ($-18^{o}C$ and $-7^{o}C$) occurred. The reduction was due to the limitation of the lamps which function properly at an optimum temperature of $37.7^{o}C$ (GE, 1978). It was found that by lowering the lamps down to a distance of 6 inches and 14 inches above the pizza surface, 200 ft-c of

Table 4 Light intensity readings* in four grocery stores in East Lansing, Michigan

| Freezer type | Light type (all fluorescent) | Light intensity (footcandles) |
|---|---|---|
| Chest freezer | Store's overhead lighting | 85 |
| | | 50 |
| | | 65 |
| | | 90 |
| | Rear lighting enclosed within chest | 110 |
| | | 190 |
| | | 140 |
| Cabinet freezer | At each partition division, enclosed | 35 |
| | | 400 |
| | | 20 |
| | | 320 |
| | | 80 |
| | | 120 |
| | | 100 |
| | | 240 |
| | | 220 |
| | | 260 |
| | | 480 |
| | | Average = 210 |

* obtained from survey by Ann Lameka

light exposure could be maintained at both -18 °C and -7 °C conditions, respectively. These distances were used from the beginning of the exposure time.

## 5.6 Analytical methods

### 5.6.1 Lipid oxidation

The extent of lipid oxidation in pork sausage and pepperoni was determined by the distillation TBA method as described by Tarladgis et al. (1960). Cut samples removed from pizzas were chilled before grinding using a William Polytron homogenizer (model 125-C) for 15 sec. Distilled, deionized water was used where necessary. Triplicate samples from each treatment analysis and a blank (no sample) were evaluated in exactly the same manner. Five ml of distillate were mixed with five ml of TBA reagent and heated under boiling water. The pink color which developed was measured in % transmission against the blank at a wavelength of 532 nm using a Beckman DU Spectrophotometer. The absorbance was then calculated. The TBA value is presented using the direct absorbance method instead of an actual TBA number (mg of malonaldehyde/1000 gm of sample). The higher the TBA value the greater the extent of oxidation in the sample.

Using the same procedure, pepperoni isolated from the pizzas for the first treatment was evaluated initially and upon final removal from storage. Duplicate measurements were performed at each stage.

Before use the TBA reagent was recrystallized twice in distilled, deionized water.

5.6.2 Color change in pepperoni

The amount of nitric oxide heme pigments (cured pigments) in the pepperoni was determined using the extraction technique developed by Hornsey (1956). The procedure was modified slightly as described in the following way. Pepperoni from each pizza was cut into pieces and weighed to 5 gm to 5.05 gm, then transferred into a 50 ml plastic tube which was then capped prior to being chilled in a refrigerator for at least one hour. The tube was capped to prevent moisture loss from the sample. The chilling helped suppress heat buildup during blending with solvent. After chilling, the sample was homogenized with a portion of 22 ml of acetone:water (20:2, v/v) for 15 sec or until a smooth paste was obtained. The remaining solvent was then added and remixed for 5 sec. The mixture in the capped tube was allowed to stand for an additional 5 min., and was then centrifuged using an International centrifuge (model V, International Equipment Co.) at a speed of 2000 rpm for 10 min. A portion of the extracted red supernatant was then carefully transferred into a 1 cm cell and % transmission measured against a blank (80% acetone in water, v/v) at a wavelength of 540 nm using the Beckman DU Spectrophotometer. The absorbance was calculated. Three measurements (one from each pizza) were obtained from each

treatment.

The amount of solvent used (22 ml) was about one half of that used in the Hornsey method (43 ml, acetone/water = 40/3) because 5 gm of sample were used instead of 10 gm.

The total heme pigments (uncombined and oxidized) were estimated using the adapted technique of Hornsey (1956) where 0.5 ml of water in the solvent (22 ml) is replaced by 0.5 ml of concentrated hydrochloric acid. The mixture was then allowed to stand for 1 hour prior to centrifugation. Absorbance of the brown colored supernatant was measured at 640 nm. Two samples (from a mixture of pepperoni from three pizzas) were tested for each treatment.

In the Hornsey technique, factors of 290 and 680 were used to multiply the absorbance representing the amount of nitric oxide heme pigments and of total heme pigments, respectively. The resultant values were represented as ppm, concentration of the respective pigments. Percent cured pigment conversion was determined using a concentration ratio of nitric oxide heme pigments to total heme pigments, which then was multiplied by a factor of 100.

The use of the Hunterlab D-25 Colorimeter for measuring surface color of pepperoni was investigated as an alternative technique to permit a simple, fast technique to follow color change in pepperoni. The technique which was developed consisted of measuring the color of three pepperoni slices alternatively while they were stacked and

placed between a pair of microslides. Three slices of pepperoni were removed from three pizzas (one per pizza) for each treatment. They were selected with respect to a round shape and a uniform thickness. The stack height of the three pepperoni slices was between 7 mm and 10 mm. Because the window of the instrument was larger than the diameter of a pepperoni slice, the excess space in the instrumental window was covered with black paper. The instrument was standardized using the standard red tile (L = 24.1, a = 27.4, b = 12.5) as a reference for comparison to the sample color. Values for L, a and b were recorded but only "value a " is used to represent the " redness" of the pepperoni. It was found that tomato sauce sometimes adhered to the pepperoni. Therefore, it was removed prior to measurement of the pepperoni color.

5.6.3 Color change of tomato sauce

To determine the color of tomato sauce on the pizzas, the Hunterlab D-25 was used. Because only a small amount of tomato sauce is on the surface of each pizza, a dilution technique was employed to make up an enough volume for testing. Tomato sauce was carefully scraped from each pizza using two small spatulas to avoid contamination with small pieces of other ingredients. From three pizzas for each treatment, two samples were prepared. Each sample consisted of four gm of scraped tomato sauce which was diluted with 6 ml of distilled, deionized water and then mixed by

homogenization for 5-10 sec or until a homogeneous mixture was obtained. Air bubbles which sometimes occurred during blending were removed before measuring. The mixture was transferred into a Polystyrene dish, 2 inches in diameter. The values of L, a and b were read against the reference red tile, the same one used for measuring pepperoni color.

5.6.4 Water vapor permeation

The water vapor transmission of the test package was determined using a gravimetric method as described in ASTM E-96 (1980). A test specimen was sealed to an open mouth of a test dish containing a desiccant, and the assembly was placed in a controlled atmosphere. Two test conditions ($22.2^{o}$ C, 50%RH and $37.7^{o}$ C, 85%RH) were used. Two samples and two blanks (no desiccant) were prepared and placed under each test condition. Each test dish was weighed initially and reweighed at daily intervals to determine the weight gain, resulting from the absorption by the desiccant of moisture vapor permeating through the film material. The test ended when a constant rate of moisture gain was obtained or a plot of moisture gain against elapsed time approached linearity (equilibrium permeation rate).

5.6.5 Oxygen permeation

The oxygen permeation rate was determined according to ASTM D-3585 (1981) applied to plastic film and sheet using a coulometric sensor. The oxygen gas transmission rate is determined after the film specimen has equilibriated in a

dry test environment (less than 1% RH). The specimen is mounted as a sealed semi-barrier between two chambers at ambient atmospheric pressure. One chamber contains oxygen and the other is slowly purged by a stream of nitrogen gas which serves as carrier of oxygen permeating through the film. The mixed gas is transported to the coulometric detector where it produces an electrical current the magnitude of which is proportional to the amount of oxygen flowing into the detector per unit time. In this determination, the Mocon Oxtran 100 (Modern Controls,Inc., Minneapolis, MN) was employed. Film samples were maintained in a desiccator prior to testing. A standard polyester film was used to calibrate the chart scale prior to testing the film sample. The measurement was duplicated.

5.6.6 Light transmission

The light transmission profile of the test package material was determined by a Spectrophotometric method using a Perkin-Elmer Lambda 3B Double beam UV/VIS Spectrophotometer, which combines an optomechanical system with microcomputer electronics. The optical system, mainly, consists of a light source which generates UV or VIS light, a reflectance grating which disperses the light beam into a spectrum and selects a desired monochromatic light beam to pass through a slit, an optical chopper which splits the monochromatic light into a reference beam and a sample beam, and a photomultiplier detector which measures the amount of

light transmitting through the sample in comparison to the reference beam.

In this test, a test specimen was mounted on the sample holder of the instrument and placed into the sample light beam while nothing was placed into the reference beam. %Transmittance was determined from 250 nm to 800 nm. Using a strip chart recorder, a light transmission profile was obtained. Light transmission of the original packaging material was also determined in this manner.

5.6.7 Sensory evaluation

Initially and at monthly intervals through the fifth month of storage, pizzas from the first six treatments were evaluated sensorially using a scoring scale hedonic test. The scale ranged from 1 to 7 where 1 equaled extremely dislike and 7 equaled extremely like. Both appearance of frozen pizzas (color of pepperoni, pork sausage and tomato sauce before cooking) and flavor of cooked pizzas (pepperoni and pork sausage, and overall taste) were evaluated. The scoring sheets for both tests are shown in Appendix B. Approximately twenty panelists, students and MSU employees, participated in the test which was usually performed between 2:00 pm and 3:00 pm.

The test consisted of presenting each participant with two sets of samples, uncooked and cooked, one at a time. Either one could be served first. Random numbers were assigned to samples representing different treatments. The

number of samples was varied according to the number of treatments to be tested. A control sample representing a fresh pizza was not available for the subsequent months of storage, therefore only the treated samples were evaluated.

During the day of the test, four pizzas from each treatment were removed from storage. For each treatment one of the frozen pizzas was cut into four pieces with each piece placed onto a paper plate marked with its respective number. Two sets (containing a piece from each treatment) of frozen samples were prepared. For the other three pizzas, each was cut into eight pieces and heated in a conventional oven at 400 °C for 5 min. or until they became brown and then served while hot. The cooked samples were served with a glass of water. The untrained test panelist was encouraged to rinse his/her mouth with water between each sample. Each panelist evaluated the samples independently without discussion with the other panelists. The test was performed in a tasted panel room where noise and light were controlled. Normal fluorescent light was used during testing. The results were expressed as mean scores from all panelists for each category.

5.6.8 Statistical analysis

The BMDP statistical software program (Dixon and Brown, 1981) for use on the CDC 6000 Computer operated by the Michigan State University Computer Laboratory was employed to assist in statistical analysis. Analysis of

variance and covariance for fixed effects factorial design, included repeated measures were determined using subprogram P2V. Therefore, the effect of each treatment on pizza quality was statistically evaluated.

A specific test was used to compare mean values from the analytical methods. The Bonferroni test as described by Gill (1978) was employed. Correlation between sensory and analytical methods was determined, based on their mean values, using a normal correlation model. The statistical analyses are described in Appendix C.

# RESULTS AND DISCUSSIONS

## 6.1 Rancidity of pork sausage

Flavor change in pork sausage resulting from lipid oxidation (LO) and influenced by environmental factors of temperature, freeze-thaw cycling, display light, and packaging type and method was determined. In this study, the influence of these factors on LO were investigated using fourteen treatments (see Table 3). The results represented by the mean TBA values for each treatment taken at monthly intervals are shown in Table 5. It was observed that the mean TBA values increased with increased storage time, at different rates for each treatment. To determine if the treatment had a significant effect on LO, a statistical analysis was performed.

Grouping some factors together and reducing them to three main factors (P, H and T) allowed an easier statistical analysis. The P factor refers to the combination of three primary factors, packaging (type and method), temperature, and freeze-thaw cycling. The factor H refers to display light and T refers to storage time. Each main factor had several sub levels which are denoted by numerical numbers as shown in Table 6. Therefore, in the statistical analysis a treatment referred to a combination of the three

Table 5 The mean TBA values (O.D. unit) in pork sausage during storage

| Treatment no. | Time (month) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 |
| 1 | .115 | .144 | .150 | .154 | .157 | .176 |
| 2 | | .150 | .150 | .154 | .175 | .177 |
| 3 | | .141 | .146 | .149 | .164 | .182 |
| 4 | | .139 | .150 | .154 | .177 | .197 |
| 5 | | .181 | .200 | .231 | .280 | .302 |
| 6 | | .177 | .195 | .219 | .269 | .316 |
| 7 | .079 | .085 | .088 | .107 | .103 | .102 |
| 8 | | .088 | .086 | .102 | .102 | .102 |
| 9 | | .088 | .083 | .110 | .106 | .106 |
| 10 | | .084 | .094 | .107 | .111 | .104 |
| 11 | | .098 | .104 | .118 | .121 | .155 |
| 12 | | .094 | .109 | .130 | .128 | .145 |
| 13 | | .091 | .106 | .127 | .112 | .146 |
| 14 | | .090 | .108 | .127 | .108 | .153 |

Table 6 Notations for factors used in statistical analysis

| Main factor | Level | Description |
|---|---|---|
| P | 1 | Poor barrier, -18 °C, w/o F/T |
| | 2 | poor barrier, -18 °C, w/ F/T |
| | 3 | poor barrier, -7 °C |
| | 4 | Vacuum, -18 °C, w/o F/T |
| | 5 | Vacuum, -18 °C, w/ F/T |
| | 6 | Atmosphere, -18 °C, w/o F/T |
| | 7 | Atmosphere, -18 °C, w/ F/T |
| H | 1 | Without light exposure |
| | 2 | With light exposure |
| T | 0 | Initial |
| | 1 | One month |
| | 2 | Two months |
| | 3 | Three months |
| | 4 | Four months |
| | 5 | Five months |

Treatment combination

| Treatment no. | P | H | T |
|---|---|---|---|
| 1 | 1 | 1 | 0-5 |
| 2 | 1 | 2 | 0-5 |
| 3 | 2 | 1 | 0-5 |

Table 6 (cont'd.)

| | | | |
|---|---|---|---|
| 4 | 2 | 2 | 0-5 |
| 5 | 3 | 1 | 0-5 |
| 6 | 3 | 2 | 0-5 |
| 7 | 4 | 1 | 0-5 |
| 8 | 4 | 2 | 0-5 |
| 9 | 5 | 1 | 0-5 |
| 10 | 5 | 2 | 0-5 |
| 11 | 6 | 1 | 0-5 |
| 12 | 6 | 2 | 0-5 |
| 13 | 7 | 1 | 0-5 |
| 14 | 7 | 2 | 0-5 |

main factors instead of only primary factors as described previously in Table 3. A BMDP statistical package ( Dixon and Brown, 1981) was used to obtain analysis of variances on the mean TBA values for pork sausage and the results are shown in Table 7. It was found that exposure to display light (H) did not result in significantly increased TBA values ($P<0.05$). P and T factors had a significant effect ($P<0.01$) on TBA values. Also, the interaction of P and T factors was significant.

The insignificant effect of display lighting on the rate of oxidation could have been because of the low temperatures ($-18^{o}C$ and $-7^{o}C$) under the product was held. At these temperatures the lipid oxidation would proceed

Table 7 Analysis of variances of TBA values in pork sausage

| Source | Degree of freedom | Sum of Square | Mean square | F-ratio | P-value |
|---|---|---|---|---|---|
| P | 6 | .41812 | .06969 | 671.46 | <.01 |
| H | 1 | .00013 | .00013 | 1.25 | >.05 |
| T | 5 | .14480 | .02896 | 279.04 | <.01 |
| PH | 6 | .00047 | .00008 | .76 | >.05 |
| PT | 30 | .07908 | .00264 | 25.40 | <.01 |
| HT | 5 | .00025 | .00005 | .47 | >.05 |
| PHT | 30 | .00206 | .00007 | .66 | >.05 |
| ERROR | 180 | .01868 | .00010 | | |

slowly. The test was performed one day after exposure to light, therefore not allowing much time for to take place.

The insignificant effect of light on TBA values in pork sausage was in agreement with that reported by Simard et al. (1983) who found that rancidity in frankfurters was not affected by 100 ft-c of fluorescent light during 49 days of storage.

Although P and T factors did have a significant effect on the rate of oxidation in pork sausage, it was unclear whether one or all three primary factors had contributed to the increased rate of oxidation. To clarify this, a specific test based on comparison of mean TBA values for the treatments was performed using the Bonferroni method (Gill, 1978). Ignoring the H factor, the mean TBA values in each P sub level for a specified month were pooled and analysed. In the Bonferroni test, the first treatment served as control, therefore the difference between the control and other treatments or between treatments was determined. Initially it was noted that the mean TBA values for pork sausage in the first six treatments (the first shipment) were higher than those in the second eight treatments (the second shipment). Therefore, the mean TBA values were adjusted prior to testing.

In Table 8 are shown the selective pairwise mean comparisons and their significance. It was found that freeze-thaw cycling had no significant effect on oxidation.

The increased storage temperature up to -7 °C contributed to the greatest increase in TBA values and was highly significant ( $P<0.01$ ). In general, the higher the temperature the more lipid oxidation is likely to occur. The use of the high barrier structure and vacuum packing significantly slowed down the rate of lipid oxidation. In contrast, the high barrier structure and atmospheric packing did not reduce the rate of oxidation as compared to the control.

Each pairwise comparison was analysed at monthly intervals and inference was made according to the results which show change in trends (denoted by the same arithmetic sign) for every month. This reflects the difference between two treatments. It was difficult to interpret the results when fluctuation was observed.

For the freeze-thaw cycle, the temperature profile during thawing at 5 °C for 7 hours was determined and is shown in Table 9. It was found that when the pizzas were removed from storage (-18 °C) the temperature of pizzas was between -16.1 °C and -16.7 °C and that the temperature increased in a rate which varied depending upon the position of the pizza in the box. The temperatures of pizza in the middle increased less rapidly in comparison with that at the top and in the bottom, which increased at the same rate. The final temperature was -10 °C for pizza in the middle and about -6.7 °C at the top and in the bottom positions of the boxes. All pizzas remained solidly frozen. The increase in

Table 8 The selective pairwise comparisons of mean TBA values in pork sausage

| Pairwises | Time (month) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| P1-P2 | NS | NS | NS | -NS | -NS |
| P1-P3 | -S** | -S** | -S** | -S** | -S** |
| P1-P4 | S** | S** | NS | S** | S** |
| P1-P5 | S** | S** | NS | S** | S** |
| P1-P6 | NS | NS | -NS | NS | -NS |
| P1-P7 | S** | NS | -NS | S** | -NS |
| P4-P5 | NS | -NS | -NS | -NS | -NS |
| P4-P6 | -NS | -S** | -S** | -S** | -S** |
| P6-P7 | NS | -NS | -NS | NS | NS |

** Significant level at P<.01

NS = Not significant difference

S = Significant difference

temperature was between 6°C and 10°C which should have had an effect on the increased rate of oxidation. However, statistical analysis showed that the freeze-thaw cycling had no significant effect on the rate of oxidation. It was possible that because the short period of time at -6.7°C, oxidation was not accelerated. They were always frozen. A Longer period of time during thawing may be required to increase the temperature of pizzas up to the point where they are no longer solidly frozen. More than one freeze-thaw cycle may also be required.

Table 9 The change in temperature of pizzas during thawing

| Time (hrs.) | Temperature (°C)* | | |
|---|---|---|---|
| | Top | Middle | Bottom |
| 0 | -16.1 | -16.1 | -16.7 |
| 1 | -11.7 | -14.4 | -13.3 |
| 2 | -10.5 | -13.9 | -11.7 |
| 3 | -8.9 | -12.2 | -10.0 |
| 4 | -8.3 | -11.7 | -8.9 |
| 5.5 | -7.2 | -11.1 | -6.7 |
| 7 | -6.7 | -10.0 | -6.1 |

* Temperature in the top, middle and bottom of the box

The lack of apparent effect due to freeze-thaw cycling on rancidity in pork sausage was supported by the work of Winter et al. (1952) who found that temperature fluctuating between -17.7 °C and -12.2 °C did not affect the flavor change in ground beef during 10 months of storage.

Storage of pizzas at -7 °C resulted in an increased rate of oxidation in pork sausage. The influence of temperature was highly significant and contributed the greatest effect to lipid oxidation in pork sausage. Choice of packaging material and method also effected the lipid oxidation rate. Vacuum packing using the high barrier material retarded the rate of oxidation, thereby, increasing the shelf life of pork sausage.

The amount of available oxygen had a major influence on lipid oxidation in pork sausage. The insignificant difference between oxidation of pizza packed in poor barrier (control) and good barrier packages packed under atmosphere may be due to the fact that the rate of oxygen uptake by pork sausage is so slow at -18 °C that the entrapped oxygen in the package was in excess during the storage period. Therefore, the oxidation rate was not slowed down as compared with the control. A significant difference was observed between the two treatments after five months probably because the oxygen partial pressure in the headspace of the good barrier is lowered, which results in a reduced rate of autoxidation. In Table 10 is shown the oxygen permeation rate of the good barrier material. The

amount of oxygen permeating through the package would probably not affect to a significant level the existing partial pressure of oxygen in the headspace of the atmospheric pack. The oxidation rate of pork sausage in the test package packed under atmosphere could be lower after five months of storage. The exclusion of oxygen by vacuum packing decreased lipid oxidation in pork sausage and was in agreement with results reported by Fox (1966). The development of rancidity in pork sausage was proceeded by heme-catalyzed lipid oxidation where ferric iron in uncured pork sausage acted as an active catalyst in the reaction (Younathan and Watts, 1959).

The water vapor transmission rate (WVTR) of the test package material was determined and is shown in Table 10. The WVTR at 22.2 °C, 50%RH and 37.7 °C, 85%RH were relatively low. In freezer conditions with low temperature, the laminate structure should maintain its moisture and oxygen barrier. The use of the laminate structure under vacuum provided protection to frozen pizzas from desiccation and freezer burn due to reduction of headspace in the package. There was no visual indication in the desiccation in the other packages.

As a whole, the factors most affecting lipid oxidation in pork sausage were storage temperature, and packaging material and method. For these treatments, the mean TBA values were pooled and are shown in Table 11. Also, those values were plotted and are shown in Figure 9. It was

Table 10 The oxygen permeation and WVTR of the barrier test package material*

| WVTR ( gm. per m2.day.mmHg) | | Oxygen permeation rate (ml per m2.day.mmHg) |
|---|---|---|
| 22.7 °C, 50%RH | 37.7 °C, 85%RH | |
| 0.124 | 0.146 | 0.041 |

* Nylon/PE laminate structure

apparent that an increased rate in TBA values in pork sausage from the control samples and from the atmospheric packed samples were equivalent. This agreed with the results from mean comparison testing. Pork sausage from the vacuum pack showed the lowest levels of TBA values while samples stored at -7 °C had the highest amount.

Table 11 The pooled mean TBA values for all treatments except freeze-thaw cycling and lighting

| Treatments | Time (month) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 |
| T1-T4 | .115 | .144 | .149 | .153 | .168 | .183 |
| T5-T6 | | .179 | .198 | .225 | .274 | .309 |
| T7-T10 | .079 | .086 | .088 | .106 | .105 | .103 |
| T11-T14 | | .093 | .107 | .126 | .117 | .150 |

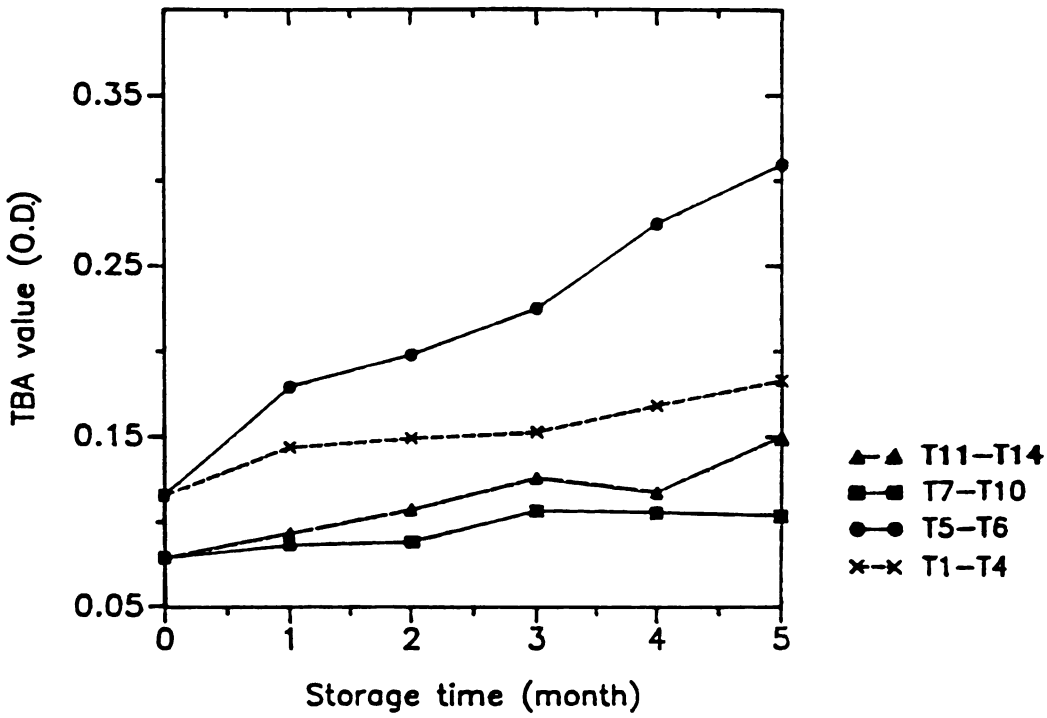



Figure 9 Change of TBA values in pork sausage at different storage times

## 6.2 Rancidity in pepperoni

Pepperoni can undergo lipid oxidation which results in rancidity. The mean TBA values for pepperoni were determined initially and at final removal from storage, and are presented in Table 12. Higher TBA values were observed during the last month of storage and were about two to three times as much as that for fresh samples. At -18 °C, lipid oxidation in pepperoni occurred.

Table 12 The mean TBA values in pepperoni initially and at final storage

| Shipment no. | Time (month) | |
|---|---|---|
| | 0 | 5 |
| 1 | .022 | .058 |
| 2 | .033 | .057 |

The TBA values in pepperoni were less than that of pork sausage because of the preservative action of sodium nitrite, antioxidants (BHA, BHT, and citric acid) which are components in the pepperoni. Despite its higher fat content (Table 1), the pepperoni had much lower TBA values than pork sausage. In addition to the preservatives, the influence of salt and spices in the pepperoni had an interfering effect on the perception of rancid flavor by taste panelists.

Heme-catalyzed lipid oxidation in muscle tissues has been widely accepted (Greene and Price, 1975; Tarladgis, 1971; and Younathan and Watts, 1959), which could be the mechanism by which pepperoni oxidizes. Younathan and Watts (1959) found that change in color in cured ham pigments from bright red to brown upon storage at refrigerator temperatures related to the increased rancidity. They suggested that the ferric form of the oxidized pigment was the catalyst in tissue rancidity. Therefore, it is possible that color may be used to follow oxidation in tissue lipids. The greater the loss of color the greater rancidity. However, Simard et al. (1983) found that color and odor change in frankfurters packed under vacuum and nitrogen were not correlated to TBA values.

## 6.3 Color of pepperoni

### 6.3.1 Nitric oxide myoglobin

The amount of nitric oxide myoglobin (NO-Mb) retained in pepperoni during pizza storage was estimated using the Hornsey technique (1956). The NO-Mb pigments were solvent extracted and measured spectrophotometrically at 540 nm. The mean NO-Mb values (in O.D. unit) in pepperoni at different storage times are shown in Table 13. Some fluctuation in the values obtained from the same treatment was observed and may be because of variability of pigment intensity in samples. Display light, storage temperature, freeze-thaw cycling and packaging could influence the change

Table 13 The mean NO-Mb values for pepperoni during storage

| Treatment no. | Time (month) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 |
| 1 | .563 | .571 | .559 | .538 | .533 | .549 |
| 2 | | .557 | .534 | .488 | .475 | .499 |
| 3 | | .528 | .544 | .543 | .543 | .558 |
| 4 | | .473 | .503 | .506 | .444 | .488 |
| 5 | | .441 | .418 | .404 | .386 | .358 |
| 6 | | .361 | .367 | .324 | .316 | .310 |
| 7 | .742 | .726 | .678 | .762 | .729 | .730 |
| 8 | | .753 | .700 | .718 | .693 | .726 |
| 9 | | .678 | .672 | .765 | .703 | .774 |
| 10 | | .668 | .696 | .725 | .685 | .761 |
| 11 | | .727 | .730 | .721 | .690 | .696 |
| 12 | | .688 | .734 | .702 | .654 | .665 |
| 13 | | .728 | .743 | .792 | .754 | .736 |
| 14 | | .659 | .692 | .692 | .650 | .665 |

in color. Statistical analysis was made and the results are shown in Table 14. It was found that all three main factors (P,H and T) had a significant impact on pepperoni color at $P<0.01$. The interaction of PH, PT, and HT was significant but that of all three was not. To clarify the effect of each factor, the Bonferroni test for mean comparisons was performed. The results of selective pairwise comparison of mean NO-Mb amounts are shown in Table 15.

It was found that the effect of freeze-thaw cycling was not significant at $P<0.01$ (T1-T3, T7-T9 in Table 15) under dark conditions. A slight effect ($P<0.05$) due to freeze-thaw cycling was observed in pepperoni packed under atmosphere in the third and the fourth months (T11-T13) but a decrease was observed in the fifth month. Results reported by Townsend and Bratzler (1958) also found that discoloration in steak was not increased by freezing (24 hr $-28.9^{o}$C) and thawing (24 hr $2.2^{o}$C) alternately.

The effect of increased storage temperature on color fading in pepperoni was highly significant ($P<0.01$). Greater discoloration was observed at $-7^{o}$C than at $-18^{o}$C. The increase in temperature accelerated the rate of oxidation in cured meat pigments in the presence of oxygen. Exposure to light tended to enhance color fading by dissociation of nitric oxide in cured meats (Watts, 1954). The effect of lighting on discoloration in pepperoni was significant though its effect was somewhat inconsistent from

Table 14 Analysis of variances of NO-Mb values in pepperoni

| Source | Degree of freedom | Sum of square | Mean square | F-ratio | P-value |
|---|---|---|---|---|---|
| P | 6 | 3.72906 | .62151 | 695.28 | <.01 |
| H | 1 | .07515 | .07515 | 116.72 | <.01 |
| T | 5 | .13545 | .02709 | 42.07 | <.01 |
| PH | 6 | .02963 | .00494 | 7.67 | <.01 |
| PT | 30 | .20786 | .00963 | 10.76 | <.01 |
| HT | 5 | .02689 | .00538 | 8.35 | <.01 |
| PHT | 30 | .01584 | .00053 | .82 | >.05 |
| ERROR | 180 | .11590 | .00064 | | |

month to month (Table 15). It may have been because of the fluctuation in light intensity from the lamps during storage. It is recognized that the light intensity affects discoloration in cured meats and that the effective intensity required to cause the change is variable depending upon the sensitivity of the product. Simard et al. (1983) reported on the effect of 100 ft-c fluorescent light on color change in frankfurters while the influence of 60 ft-c on the color of bologna was reported by Kraft and Ayres (1954). In this study, color change in pepperoni resulted from co-effects between light and temperature and/or packaging method.

Table 15 The selective pairwise comparisons of mean NO-Mb values in pepperoni

| Pairwise | Time (month) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| T1-T3 | NS | NS | -NS | -NS | -NS |
| T1-T5 | S** | S** | S** | S** | S** |
| T1-T7 | NS | S* | -NS | -NS | -NS |
| T1-T9 | S* | S** | -NS | NS | -NS |
| T1-T11 | NS | NS | -NS | NS | NS |
| T1-T13 | NS | -NS | -S** | -NS | -NS |
| T1-T2 | NS | NS | NS | NS | NS |
| T3-T4 | NS | NS | NS | S** | S* |
| T5-T6 | S** | NS | S** | S* | NS |
| T7-T8 | -NS | -NS | NS | NS | NS |
| T9-T10 | NS | -NS | NS | NS | NS |
| T11-T12 | NS | -NS | NS | NS | NS |
| T13-T14 | S* | NS | S** | S** | S* |
| T7-T9 | NS | NS | -NS | NS | NS |
| T7-T11 | -NS | -NS | NS | NS | NS |
| T11-T13 | -NS | -NS | -S* | -S* | -NS |

S = Significant difference

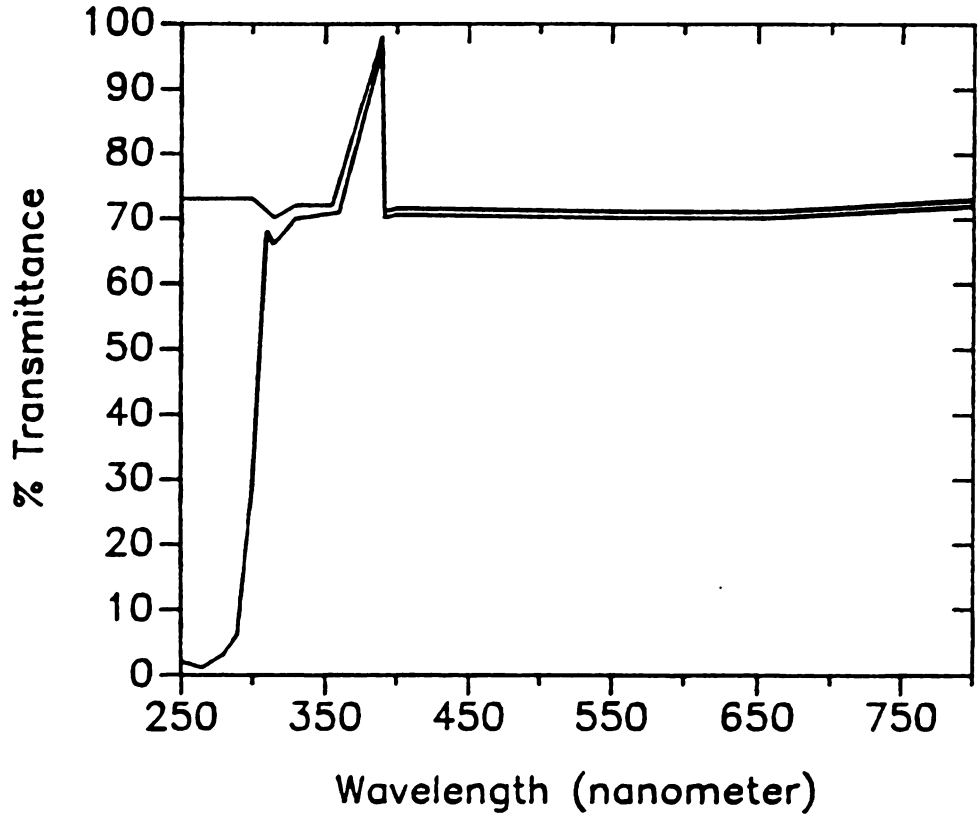
NS = Not significant difference

* Significant at P<0.05

** Significant at P<0.01

In pepperoni without freeze-thaw cycling, light had no effect in discoloration at -18°C (T1-T2, T7-T8, T11-T12 in Table 15). In samples which were subjected to freeze-thaw cycling, light had a slight effect on discoloration in the original package (T3-T4), a greater effect in atmospheric pack (T13-T14) and no effect for samples in the vacuum pack (T9-T10). For the pepperoni, the use of the vacuum pack tended to retain better color than the atmospheric pack and than the original pack. However, during five month storage the vacuum pack did not show a significant difference from the others (T1-T7, T1-T9, T1-T11, T1-T13 and T7-T11).

The insignificant difference due to light exposure between both types of package materials could result from the similarity in light transmission particularly in the range of 560 nm to 630 nm where the greatest loss of meat color occurs (Townsend and Bratzler, 1958). In Figure 10 are presented the light transmission profiles of both package materials from 250 nm to 800 nm. The barrier structure absorbed UV light in the 250 nm to 300 nm range but beyond 300 nm both materials permitted visible light to pass through at approximate 70% transmission. In addition, the small amount of residual oxygen in the vacuum pack could still promote the oxidation of cured meat pigments therefore, resulting in no difference between the vacuum and the atmospheric packs. The use of vacuum at 610 mm Hg in this study may not have been high enough to eliminate oxygen in the package headspace to the level required to completely




Top profile : Original package material

Bottom profile : Test package material

Figure 10 Light transmission profiles for the test package and original package materials

prohibit discoloration. Ledward (1970) stated that 1-2 % oxygen inside the package can cause surface discoloration in beef packed under inert gases.

Table 16 shows the pooled mean values of NO-Mb for pepperoni in treatments excluding freeze-thaw cycling. The plot of change in NO-Mb values at different storage times is presented in Figure 11.

Table 16 The pooled mean NO-Mb values of pepperoni for all treatments except freeze-thaw cycling

| Treatment no. | Time (month) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 |
| T1/T3 | .563 | .550 | .552 | .540 | .538 | .553 |
| T2/T4 | | .515 | .519 | .497 | .460 | .494 |
| T5 | | .441 | .418 | .404 | .386 | .358 |
| T6 | | .361 | .367 | .324 | .316 | .310 |
| T7/T9 | .742 | .702 | .675 | .764 | .716 | .752 |
| T8/T10 | | .711 | .698 | .722 | .689 | .743 |
| T11/T13 | | .728 | .737 | .757 | .722 | .716 |
| T12/T14 | | .674 | .713 | .697 | .652 | .665 |

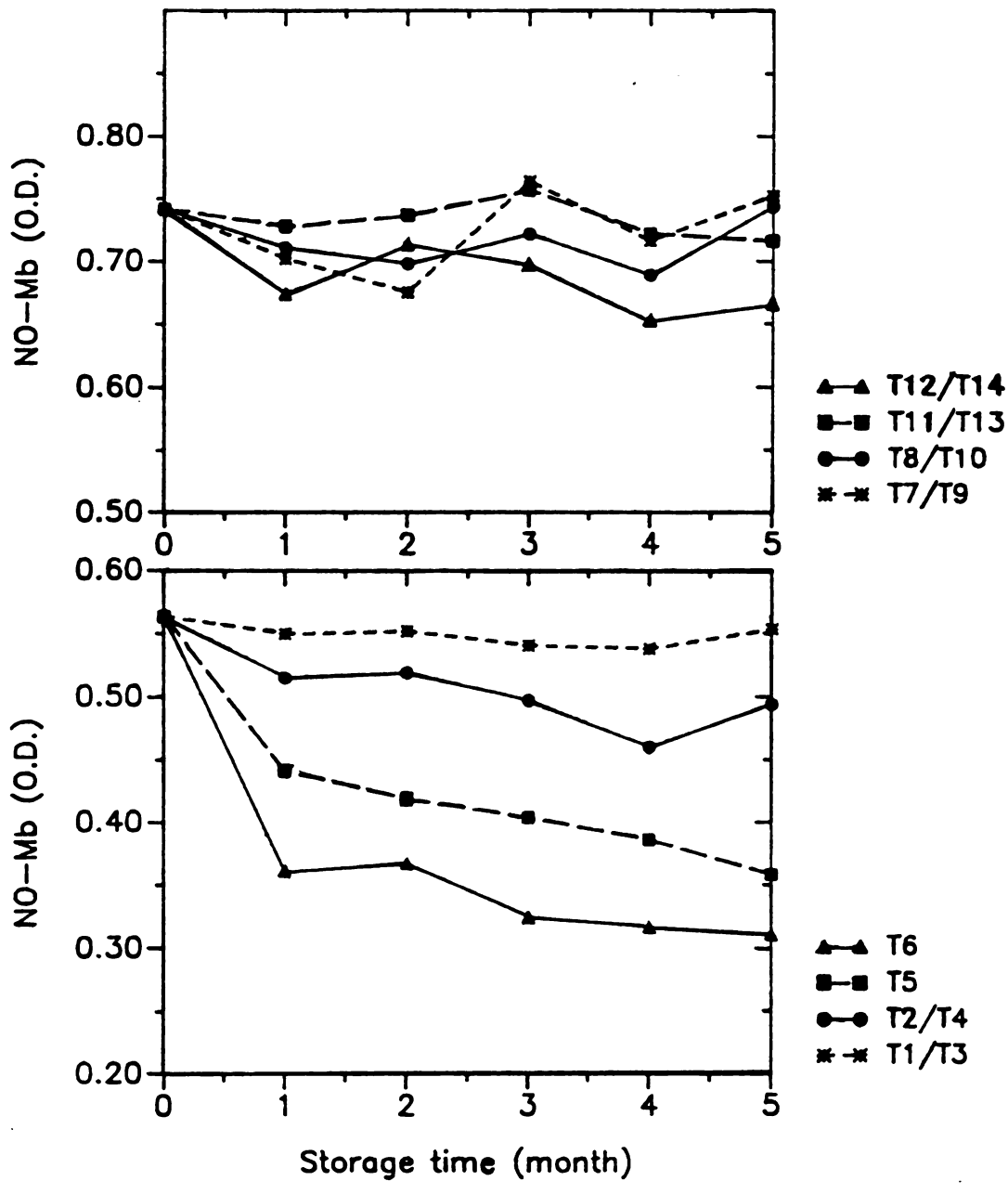



Figure 11 Change in NO-Mb values in pepperoni at different storage times

6.3.2 % Pigment conversion

By considering the amount of NO-Mb in a comparison with the amount of total haem pigments (uncombined and oxidized), a pigment conversion ratio was determined in percent. The higher the % conversion the greater the red color. In Table 17 are expressed the mean values of % NO-Mb conversion in pepperoni at different storage times. There was some fluctuation in the values which may have resulted from variation within samples or the effect of treatments. Analysis of variance of the data representing % NO-Mb conversion in pepperoni is presented in Table 18. Mean comparisons of selected pairs were analysed and the results are shown in Table 19.

There was no apparent effect of freeze-thaw cycling on promotion of discoloration in pepperoni using this method. Increasing the storage temperature to -7 $^{o}$C reduced % NO-Mb conversion significantly at $P<0.05$. As temperature increased the rate of oxidation of the cured meat pigments accelerated. The amount of NO-Mb decreased while the amount of oxidized pigments increased. This resulted in the lower % conversion. The use of the barrier package under vacuum maintained color under exposure to display light, though there was no significant improvement with the barrier package sealed under atmosphere as compared with the control package. The oxygen present in the package may have caused color fading which is in agreement with results reported by Karel (1976). The effect of display light on color

reduction was highly significant at -7°C but not at -18°C.

Greater color retention was observed in the vacuum pack which reflects the protection the barrier package provides from oxygen and which results in retardation of cured pigment oxidation. Rikert et al. (1956a) also noted that use of high vacuum packaging may accelerate the recovery of red color after long storage. Table 20 shows the mean values of % No-Mb conversion as influenced by display light, temperature and packaging. These values were plotted and are shown in Figure 12.

It was shown that either NO-Mb or % NO-Mb conversion can be used to measure the color of pepperoni during storage.

Table 17 The mean % NO-Mb conversion for pepperoni during storage

| Treatment no. | Time (month) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 |
| 1 | 85.0 | 76.5 | 80.4 | 75.1 | 76.0 | 79.3 |
| 2 | | 73.4 | 75.2 | 71.2 | 68.8 | 70.7 |
| 3 | | 70.6 | 76.6 | 79.2 | 78.6 | 80.7 |
| 4 | | 67.0 | 71.5 | 72.9 | 68.9 | 73.3 |
| 5 | | 79.5 | 73.1 | 69.4 | 68.4 | 59.3 |
| 6 | | 63.5 | 61.2 | 57.6 | 54.8 | 54.2 |
| 7 | 87.5 | 84.4 | 88.2 | 94.0 | 89.6 | 91.1 |
| 8 | | 89.5 | 82.6 | 85.9 | 90.9 | 89.2 |
| 9 | | 85.8 | 90.5 | 89.0 | 89.5 | 91.9 |
| 10 | | 85.7 | 83.8 | 90.8 | 90.3 | 87.4 |
| 11 | | 87.8 | 84.9 | 83.3 | 89.1 | 85.6 |
| 12 | | 77.9 | 86.6 | 77.0 | 81.3 | 79.6 |
| 13 | | 88.3 | 86.4 | 82.6 | 85.3 | 85.0 |
| 14 | | 76.2 | 78.6 | 77.3 | 82.6 | 79.2 |

Table 18 Analysis of variances of % No-Mb conversion in pepperoni

| Source | Degree of freedom | Sum of square | Mean square | F-ratio | P-value |
|---|---|---|---|---|---|
| P | 6 | 12728.58026 | 2121.43004 | 192.60 | <.01 |
| H | 1 | 1443.33763 | 1443.33763 | 131.60 | <.01 |
| T | 5 | 1839.89093 | 367.97819 | 33.41 | <.01 |
| PH | 6 | 432.15265 | 72.02544 | 6.54 | <.01 |
| PT | 30 | 3304.84125 | 110.16137 | 10.00 | <.01 |
| HT | 5 | 289.12185 | 57.82437 | 5.25 | <.01 |
| PHT | 30 | 757.47425 | 25.24914 | 2.29 | <.01 |
| ERROR | 180 | 1982.67500 | 11.01476 | | |

Table 19 The selective pairwise comparisons of mean % NO-Mb conversion in pepperoni

| Pairwises | Time (month) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| T1-T3 | NS | NS | -NS | NS | -NS |
| T1-T5 | -NS | S* | NS | NS | S* |
| T1-T7 | -NS | -NS | -S* | -S* | -S* |
| T1-T9 | -NS | -S* | -S* | -S* | -S* |
| T1-T11 | -NS | -NS | -NS | -S* | -NS |
| T1-T13 | -S* | -NS | -NS | -NS | -NS |
| T1-T2 | NS | NS | NS | NS | S* |
| T3-T4 | NS | NS | NS | S* | NS |
| T5-T6 | S* | S* | S* | S* | NS |
| T7-T8 | -NS | NS | S* | -NS | NS |
| T9-T10 | NS | NS | -NS | -NS | NS |
| T11-T12 | NS | -NS | NS | NS | NS |
| T13-T14 | S* | NS | NS | NS | NS |
| T7-T9 | -NS | -NS | NS | NS | -NS |
| T7-T11 | -NS | NS | S* | NS | NS |

S = Significant difference

NS = Not significant difference

* Significant at $P<0.05$

Table 20 The pooled mean % NO-Mb conversion of pepperoni for all treatments except freeze-thaw cycle

| Treatment no. | Time (month) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 |
| T1/T3 | 85.0 | 73.6 | 78.5 | 77.1 | 77.3 | 80.0 |
| T2/T4 | | 70.2 | 75.5 | 72.4 | 68.7 | 72.0 |
| T5 | | 79.5 | 73.1 | 69.4 | 68.4 | 59.3 |
| T6 | | 63.5 | 61.2 | 57.6 | 54.8 | 54.2 |
| T7/T9 | 87.5 | 85.1 | 89.4 | 91.5 | 89.6 | 91.5 |
| T8/T10 | | 87.6 | 83.2 | 88.3 | 90.8 | 88.3 |
| T11/T13 | | 87.0 | 85.7 | 83.0 | 87.2 | 85.3 |
| T12/T14 | | 77.5 | 82.7 | 77.1 | 81.9 | 79.3 |

6.3.3 Redness (a) in pepperoni

Although it is claimed that the Hornsey technique is a fast method for measuring color in cured meats and has been widely used, for a certain work such as measuring a surface color, use of the Hunter Colorimeter may be more appropriate and in fact simpler. In this study, this method was also used to measure the color of pepperoni. The color was measured using the standard red tile as a reference. Only the a value (redness) was considered. The average values are shown in Table 21. Analysis of variance of data for redness in pepperoni is shown in Table 22. The results show the significant effect of all three main factors. Mean

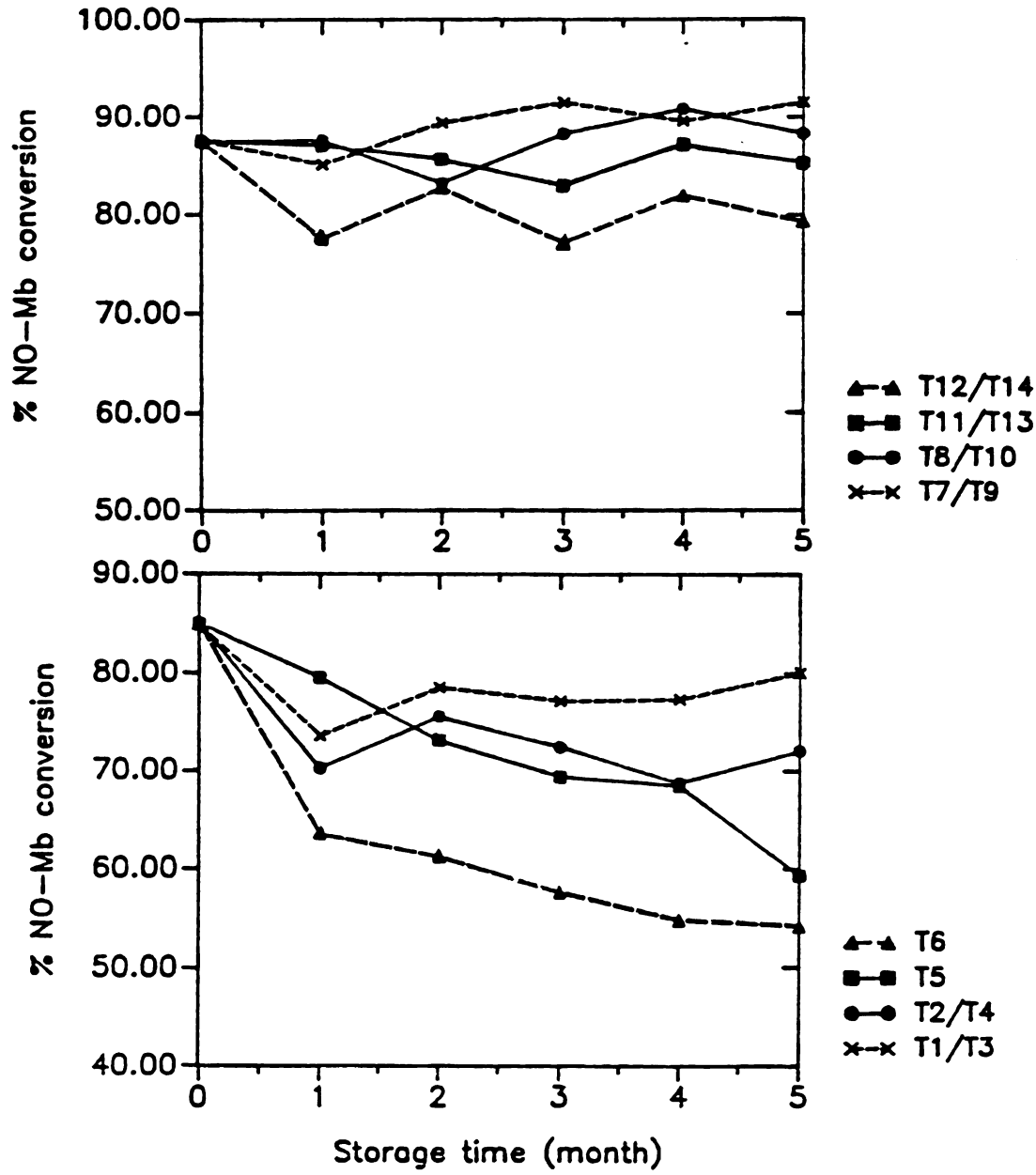



Figure 12 Change in % NO-Mb conversion in pepperoni at different storage times

Table 21 Mean redness (a) values of pepperoni at different storage times

| Treatment no. | Time (month) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 |
| 1 | 23.17 | 20.53 | 21.90 | 21.90 | 18.03 | 20.83 |
| 2 | | 15.63 | 20.48 | 18.60 | 15.33 | 16.27 |
| 3 | | 19.70 | 22.65 | 21.60 | 18.63 | 19.13 |
| 4 | | 18.07 | 20.13 | 19.33 | 13.63 | 19.17 |
| 5 | | 20.70 | 21.07 | 17.57 | 16.17 | 18.33 |
| 6 | | 16.97 | 19.07 | 15.30 | 14.60 | 16.30 |
| 7 | | 20.23 | 19.80 | 20.30 | 19.20 | 18.07 |
| 8 | | 19.37 | 20.97 | 19.27 | 17.97 | 19.10 |
| 9 | | 20.73 | 21.00 | 20.37 | 20.23 | 18.30 |
| 10 | | 19.67 | 19.50 | 19.67 | 19.50 | 18.77 |
| 11 | | 20.57 | 19.63 | 20.70 | 18.63 | 19.03 |
| 12 | | 18.20 | 17.73 | 15.97 | 16.40 | 15.90 |
| 13 | | 20.60 | 18.73 | 20.83 | 19.17 | 20.07 |
| 14 | | 16.53 | 18.87 | 17.07 | 15.93 | 16.90 |

Table 22 Analysis of variances of redness (a) for pepperoni

| Source | Degree of freedom | Sum of square | Mean square | F-ratio | P-value |
|---|---|---|---|---|---|
| P | 6 | 83.68375 | 13.94729 | 17.33 | <.01 |
| H | 1 | 193.89659 | 193.89659 | 240.89 | <.01 |
| T | 5 | 840.98400 | 168.19680 | 208.96 | <.01 |
| PH | 6 | 53.61972 | 8.93662 | 11.10 | <.01 |
| PT | 30 | 154.86670 | 5.16222 | 6.41 | <.01 |
| HT | 5 | 56.92508 | 11.38502 | 14.14 | <.01 |
| PHT | 30 | 67.27527 | 2.24251 | 2.79 | <.01 |
| ERROR | 180 | 144.88333 | .80491 | | |

comparisons for selected pairs were tested and the results are shown in Table 23. Higher storage temperature was significant at $P<0.05$ in the third and the fifth months of storage. Effect of freeze-thaw cycling on color was not significant. Display light reduced the redness of pepperoni significantly except for the vacuum pack.

For storage at -18 °C, it was found that the difference between the control and the vacuum pack was uncertain (T1-T7 and T1-T9 in Table 23). In fact the control retained better red color than the vacuum pack did. Also, the control retained better color than the atmospheric pack did in some months (T1-T11 and T1-T13). It was highly possible that samples from the two shipments were different.

Table 23 The selective pairwise comparisons of mean redness (a) values in pepperoni

| Pairwises | Time (month) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| T1-T3 | NS | -NS | NS | -NS | NS |
| T1-T5 | -NS | NS | S** | NS | S** |
| T1-T7 | NS | S** | NS | -NS | S** |
| T1-T9 | -NS | NS | NS | -S** | S** |
| T1-T11 | -NS | S** | NS | -NS | NS |
| T1-T13 | -NS | S** | NS | -NS | NS |
| T1-T2 | S** | NS | S** | S** | S** |
| T3-T4 | NS | S** | S** | S** | -NS |
| T5-T6 | S** | NS | S** | NS | NS |
| T7-T8 | NS | -NS | NS | NS | -NS |
| T9-T10 | NS | NS | NS | NS | -NS |
| T11-T12 | S** | -NS | S** | S** | S** |
| T13-T14 | S** | -NS | S** | S** | S** |
| T7-T11 | -NS | NS | -NS | NS | -NS |
| T7-T9 | -NS | -NS | -NS | -NS | -NS |
| T11-T13 | -NS | NS | -NS | -NS | -NS |

S = Significant difference

NS = Not significant difference

** Significant at P<0.01

Neglecting the effect of freeze-thaw cycling, the mean values (a) were pooled and are presented in Table 24 and are plotted in Figure 13. It was observed that less fluctuation in color occurred in the high barrier package than in the bad barrier package.

Table 24 The pooled mean redness* (a) of pepperoni for all treatments except freeze-thaw cycling

| Treatments | Time (month) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| T1/T3 | -3.10 | -0.90 | -1.45 | -4.90 | -3.25 |
| T2/T4 | -6.35 | -2.90 | -4.25 | -8.75 | -5.45 |
| T5 | -2.50 | -2.10 | -5.60 | -7.00 | -4.90 |
| T6 | -6.20 | -4.10 | -7.90 | -8.60 | -6.30 |
| T7/T9 | -2.75 | -2.80 | -2.85 | -3.50 | -5.00 |
| T8/T10 | -3.65 | -2.95 | -3.70 | -4.45 | -4.25 |
| T11/T13 | -2.60 | -4.05 | -2.45 | -4.30 | -3.65 |
| T12/T14 | -5.85 | -4.90 | -6.65 | -7.25 | -6.55 |

* calculated from the difference between the subsequent months and the initial values

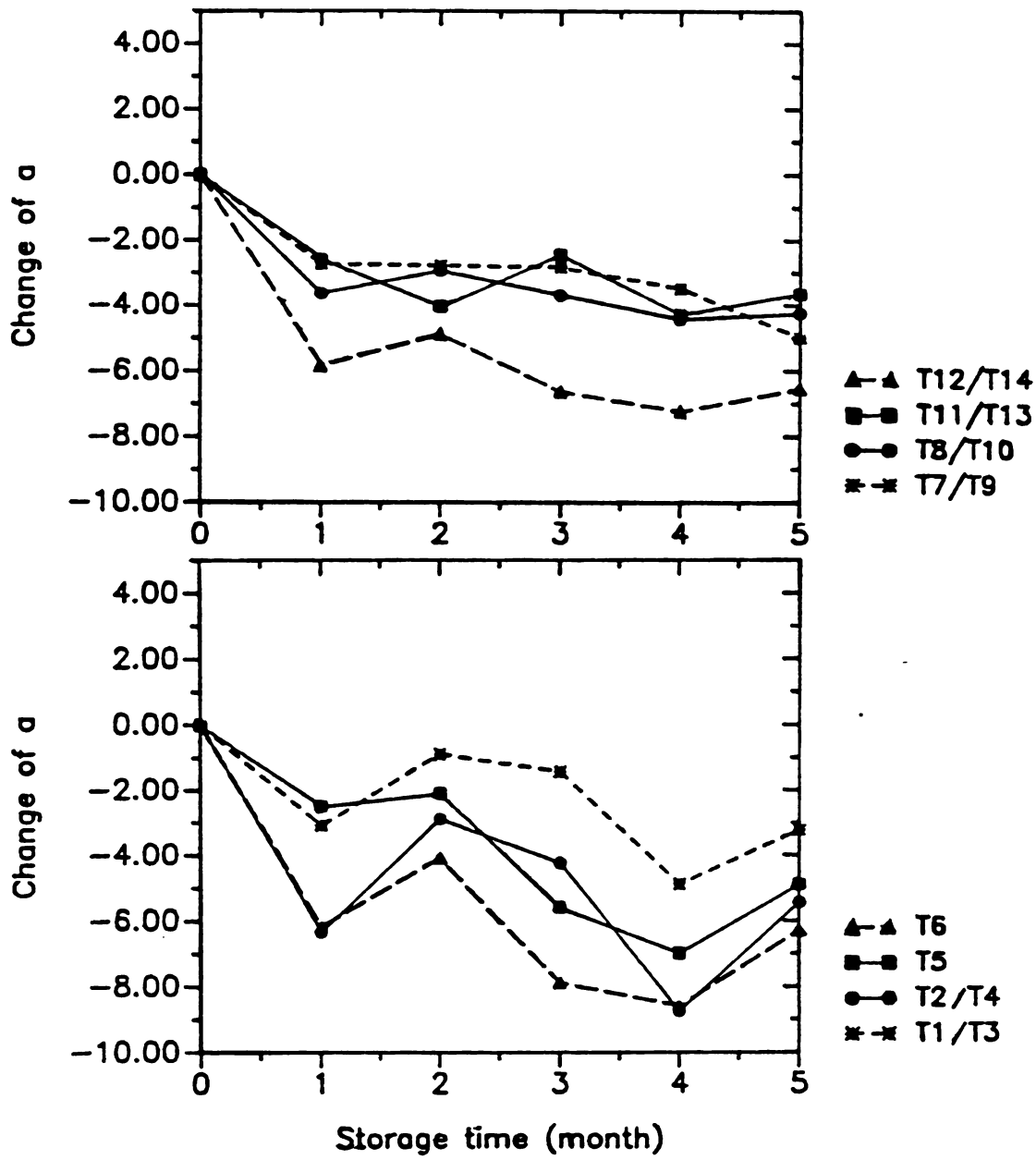



Figure 13 Change in redness (a) for pepperoni at different storage times

## 6.4 Color of tomato sauce

The color of tomato sauce removed from frozen pizzas was determined using a dilution method and the Hunter Colorimeter. Tomato sauce was removed from the pizza and diluted with distilled water, blended and measured on a scale of L, a and b against the standard red tile. The mean values of L, a, b and a/b ratio for treatments are presented in Tables 25 and 26. Analysis of variance of the values are shown in Tables 27 and 28. It was found that a and a/b representing redness gave the same results. Display light (H) did not affect tomato sauce color while P and T factors did at $P<0.01$. The interaction of the two factors (PT) also was significant at $P<0.01$. For L (lightness) and b (yellowness), all three factors affected L and b values significantly ($P<0.01$) but only the interaction of P and T (PT) was significant.

The mean values of L, a, b and a/b were pooled and analysed by mean comparisons. The results of the selective pairwise comparisons of means for a and a/b are shown in Table 29. Both agreed that the tomato sauce color was not affected by freeze-thaw cycling (P1-P2, P4-P5 and P6-P7 in Table 29) but significantly affected by storage temperature at $P<0.01$ (P1-P3 in Table 29). An increase in storage temperature to $-7^{o}C$ resulted in a greater loss of redness in tomato sauce than at $-18^{o}C$. The higher barrier package retained the red color of tomato sauce better than the bad

Table 25 The mean values of a and a/b ratio for tomato sauce during storage

| Value | Treatment no. | Time (month) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 |
| a | 1 | 25.33 | 23.60 | 23.78 | 24.90 | 23.55 | 26.00 |
| | 2 | | 23.90 | 23.78 | 25.00 | 23.45 | 26.15 |
| | 3 | | 23.00 | 24.13 | 25.45 | 23.35 | 25.60 |
| | 4 | | 23.30 | 24.16 | 25.25 | 23.55 | 26.10 |
| | 5 | | 23.35 | 23.05 | 22.80 | 20.60 | 22.55 |
| | 6 | | 24.35 | 23.95 | 22.35 | 20.95 | 22.90 |
| | 7 | | 24.80 | 24.70 | 27.30 | 26.80 | 24.80 |
| | 8 | | 25.40 | 24.55 | 27.40 | 27.05 | 25.05 |
| | 9 | | 25.50 | 24.85 | 26.85 | 27.20 | 24.95 |
| | 10 | | 25.30 | 24.75 | 26.80 | 27.25 | 24.90 |
| | 11 | | 25.15 | 24.55 | 26.80 | 26.65 | 27.90 |
| | 12 | | 24.70 | 24.50 | 27.25 | 27.20 | 26.85 |
| | 13 | | 24.50 | 24.65 | 27.15 | 24.45 | 27.20 |
| | 14 | | 24.90 | 24.55 | 26.90 | 27.35 | 27.10 |
| a/b | 1 | 1.47 | 1.41 | 1.43 | 1.37 | 1.29 | 1.34 |
| | 2 | | 1.42 | 1.44 | 1.33 | 1.24 | 1.33 |
| | 3 | | 1.42 | 1.44 | 1.39 | 1.27 | 1.27 |
| | 4 | | 1.42 | 1.41 | 1.32 | 1.22 | 1.29 |
| | 5 | | 1.28 | 1.15 | 1.08 | 0.97 | 1.00 |
| | 6 | | 1.30 | 1.19 | 1.05 | 0.94 | 1.01 |

Table 25 (cont'd.)

| | | | | | |
|---|---|---|---|---|---|
| 7 | 1.45 | 1.42 | 1.51 | 1.47 | 1.55 |
| 8 | 1.44 | 1.40 | 1.52 | 1.48 | 1.52 |
| 9 | 1.42 | 1.44 | 1.47 | 1.45 | 1.54 |
| 10 | 1.43 | 1.40 | 1.51 | 1.48 | 1.53 |
| 11 | 1.42 | 1.44 | 1.46 | 1.48 | 1.42 |
| 12 | 1.39 | 1.40 | 1.48 | 1.46 | 1.43 |
| 13 | 1.38 | 1.41 | 1.44 | 1.46 | 1.42 |
| 14 | 1.40 | 1.39 | 1.47 | 1.48 | 1.43 |

Table 26 The mean values of L and b for tomato sauce during storage

| Value | Treatment no. | Time (month) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 |
| L | 1 | 30.50 | 30.00 | 29.74 | 31.60 | 31.70 | 33.35 |
| | 2 | | 29.70 | 29.38 | 32.70 | 32.85 | 33.55 |
| | 3 | | 28.95 | 29.73 | 31.70 | 31.90 | 34.22 |
| | 4 | | 29.15 | 30.50 | 33.30 | 33.40 | 34.35 |
| | 5 | | 31.90 | 33.85 | 34.95 | 35.70 | 38.10 |
| | 6 | | 32.80 | 33.95 | 35.60 | 37.35 | 38.85 |
| | 7 | | 30.30 | 30.15 | 31.55 | 32.05 | 29.25 |
| | 8 | | 31.25 | 30.30 | 31.45 | 32.05 | 29.95 |
| | 9 | | 32.05 | 29.55 | 31.70 | 32.70 | 29.15 |
| | 10 | | 31.65 | 30.45 | 31.00 | 32.05 | 29.65 |
| | 11 | | 31.25 | 29.15 | 31.80 | 31.40 | 33.65 |
| | 12 | | 31.40 | 30.00 | 31.95 | 32.30 | 32.25 |
| | 13 | | 31.40 | 29.80 | 32.45 | 32.55 | 32.85 |
| | 14 | | 31.35 | 30.20 | 31.60 | 32.10 | 32.70 |
| b | 1 | 17.30 | 16.80 | 16.68 | 18.10 | 18.20 | 19.40 |
| | 2 | | 16.80 | 16.50 | 18.80 | 18.85 | 19.70 |
| | 3 | | 16.20 | 16.75 | 18.30 | 18.40 | 20.20 |
| | 4 | | 16.40 | 17.16 | 19.20 | 19.25 | 20.25 |
| | 5 | | 18.30 | 20.00 | 21.20 | 21.35 | 22.45 |
| | 6 | | 18.80 | 20.15 | 21.30 | 22.35 | 22.75 |

Table 26 (cont'd.)

| | | | | | |
|---|---|---|---|---|---|
| 7 | 17.05 | 17.40 | 18.10 | 18.30 | 16.05 |
| 8 | 17.65 | 17.55 | 18.05 | 18.25 | 16.60 |
| 9 | 17.95 | 17.25 | 18.25 | 18.70 | 16.05 |
| 10 | 17.70 | 17.65 | 17.80 | 18.40 | 16.25 |
| 11 | 17.75 | 17.10 | 18.30 | 18.05 | 19.60 |
| 12 | 17.80 | 17.50 | 18.45 | 18.60 | 18.75 |
| 13 | 17.70 | 17.55 | 18.80 | 18.80 | 19.15 |
| 14 | 17.75 | 17.70 | 18.30 | 18.50 | 19.00 |

Table 27 Analysis of variances of a and a/b values for tomato sauce

| Value | Source | Degree of freedom | Sum of square | Mean square | F-ratio | P-value |
|---|---|---|---|---|---|---|
| | P | 6 | 179.70286 | 29.95048 | 186.03 | <.01 |
| | H | 1 | .33679 | .33679 | 2.09 | >.05 |
| | T | 5 | 60.93050 | 12.18610 | 85.69 | <.01 |
| a | PH | 6 | .96452 | .16075 | 1.00 | >.05 |
| | PT | 30 | 160.80159 | 5.36005 | 33.29 | <.01 |
| | HT | 5 | .50646 | .10129 | .63 | >.05 |
| | PHT | 30 | 3.85603 | .12853 | .80 | >.05 |
| | ERROR | 109 | 17.54883 | .16100 | | |
| | P | 6 | 1.85394 | .30899 | 571.90 | <.01 |
| | H | 1 | .00137 | .00137 | 2.54 | >.05 |
| | T | 5 | .34153 | .06831 | 126.42 | <.01 |
| a/b | PH | 6 | .00414 | .00069 | 1.28 | >.05 |
| | PT | 30 | 1.02496 | .03417 | 63.24 | <.01 |
| | HT | 5 | .00179 | .00036 | .66 | >.05 |
| | PHT | 30 | .02259 | .00075 | 1.39 | >.05 |
| | ERROR | 109 | .058889 | .00054 | | |

Table 28 Analysis of variances of L and b values for tomato sauce

| Value | Source | Degree of freedom | Sum of square | Mean square | F-ratio | P-value |
|---|---|---|---|---|---|---|
| L | P | 6 | 247.42327 | 41.23721 | 102.66 | <.01 |
| | H | 1 | 3.00870 | 3.00870 | 7.49 | <.01 |
| | T | 5 | 219.54314 | 43.90863 | 109.31 | <.01 |
| | PH | 6 | 4.52394 | .75399 | 1.88 | >.05 |
| | PT | 30 | 205.87129 | 6.86238 | 17.08 | <.01 |
| | HT | 5 | 1.85564 | .37113 | .92 | >.05 |
| | PHT | 30 | 13.39867 | .44662 | 1.11 | >.05 |
| | ERROR | 109 | 43.78250 | .40167 | | |
| b | P | 6 | 138.00781 | 23.00130 | 158.38 | <.01 |
| | H | 1 | 1.00769 | 1.00769 | 6.94 | <.01 |
| | T | 5 | 103.53479 | 20.70696 | 142.58 | <.01 |
| | PH | 6 | 1.58162 | .26360 | 1.82 | >.05 |
| | PT | 30 | 117.05539 | 3.90185 | 26.87 | <.01 |
| | HT | 5 | .61814 | .12363 | .85 | >.05 |
| | PHT | 30 | 4.39071 | .14636 | 1.01 | >.05 |
| | ERROR | 109 | 15.83000 | .14523 | | |

Table 29 The selective pairwise comparisons of mean a and a/b values for tomato sauce

| Value | Pairwises | Time (month) | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| a | P1-P2 | NS | NS | -NS | NS | NS |
| | P1-P3 | -NS | NS | NS | S** | S** |
| | P1-P4 | -S** | -S** | -S** | -S** | -S** |
| | P1-P5 | -S** | -S** | -S** | -S** | -S** |
| | P1-P6 | -S** | -S** | -S** | -S** | -S** |
| | P1-P7 | -S** | -S** | -S** | -S** | -S** |
| | P4-P5 | -NS | -NS | NS | -NS | NS |
| | P4-P6 | NS | NS | NS | NS | S** |
| | P6-P7 | NS | -NS | NS | NS | NS |
| a/b | P1-P2 | -NS | NS | NS | NS | S** |
| | P1-P3 | S** | S** | S** | S** | S** |
| | P1-P4 | -NS | NS | -S** | -S** | -S** |
| | P1-P5 | -NS | NS | -S** | -S** | -S** |
| | P1-P6 | NS | NS | -S** | -S** | -S** |
| | P1-P7 | NS | NS | -S** | -S** | -S** |
| | P4-P5 | NS | -NS | NS | NS | -NS |
| | P4-P6 | NS | -NS | NS | NS | S** |
| | P6-P7 | NS | NS | NS | NS | NS |

** Significant level at P<0.01

barrier at -18°C (P1-P4, P1-P5, P1-P6 and P1-P7 in Table 29). There was no significant difference between the vacuum and atmospheric pack in maintaining the redness of tomato sauce (P4-P6).

The results of selective pairwise comparisons of mean L and b values are shown in Table 30. L and b were not affected by freeze-thaw cycling (P1-P2, P4-P5, and P6-P7 in Table 30) but affected by storage temperature ($P<0.01$). The higher values of L and b indicate the greater color fading. Use of the high barrier package did not affect a significant change in L and b values as compared with the bad barrier package during the five month storage (P1-P4, P1-P5, P1-P6 and P1-P7 in Table 30). Also, there was no significant difference between vacuum and atmospheric packing (P4-P6 in Table 30).

Light can catalyze the oxidation of lycopene, the major pigment in tomato products, in the presence of oxygen (Gould, 1983; and Mackinney and Little, 1962). However, in this study no significant light effect was observed possibly because other ingredients especially cheese covered some of the sauce and could have interfered with the light fading process. Freeze-thaw cycling did not affect the color of the tomato sauce.

The average changes in L, a, b and a/b values in tomato sauce compared to the fresh samples are shown in Table 31 and the plots of these values are shown in Figures 14 and 15.

Table 30 The selective pairwise comparisons of mean L and b values for tomato sauce

| Value | Pairwises | Time (month) | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| L | P1-P2 | NS | -NS | -NS | -NS | -NS |
| | P1-P3 | -S** | -S** | -S** | -S** | -S** |
| | P1-P4 | -NS | -NS | NS | NS | S** |
| | P1-P5 | -S** | -NS | NS | -NS | S** |
| | P1-P6 | -S** | -NS | NS | NS | -NS |
| | P1-P7 | -S** | -NS | NS | -NS | -NS |
| | P4-P5 | -NS | NS | NS | -NS | NS |
| | P4-P6 | -NS | NS | -NS | NS | -S** |
| | P6-P7 | -NS | -NS | -NS | -NS | NS |
| b | P1-P2 | NS | -NS | -NS | -NS | NS |
| | P1-P3 | -S** | -S** | -S** | -S** | -S** |
| | P1-P4 | -NS | -S** | NS | NS | S** |
| | P1-P5 | -S** | -S** | NS | NS | S** |
| | P1-P6 | -S** | -S** | NS | NS | NS |
| | P1-P7 | -S** | -S** | -NS | -NS | NS |
| | P4-P5 | -NS | NS | NS | -NS | NS |
| | P4-P6 | -NS | NS | -NS | -NS | -S** |
| | P6-P7 | NS | -NS | -NS | -NS | NS |

** Significant level at P<0.01

Table 31 The pooled mean changes* of L, a, b and a/b of tomato sauce for all treatments except freeze-thaw cycling and lighting

| Value | Treatments | Time (month) | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| L | T1-T4 | -1.08 | -0.68 | 1.83 | 1.98 | 3.33 |
| | T5-T6 | 1.85 | 3.35 | 4.75 | 6.00 | 7.95 |
| | T7-T10 | 0.78 | -0.43 | 0.90 | 1.68 | -1.05 |
| | T11-T14 | 0.83 | -0.73 | 1.43 | 1.58 | 2.33 |
| a | T1-T4 | -1.87 | -1.37 | -0.50 | -1.87 | 0.65 |
| | T5-T6 | -1.50 | -1.85 | -2.75 | -4.55 | -2.60 |
| | T7-T10 | -0.05 | -0.65 | 1.78 | 1.75 | -0.40 |
| | T11-T14 | -0.50 | -0.78 | 1.70 | 1.58 | 1.95 |
| b | T1-T4 | -0.75 | -0.53 | 1.30 | 1.40 | 2.59 |
| | T5-T6 | 1.25 | 2.75 | 3.95 | 4.50 | 5.30 |
| | T7-T10 | 0.29 | 0.16 | 0.75 | 1.11 | -1.06 |
| | T11-T14 | 0.45 | 0.16 | 1.16 | 1.19 | 1.83 |
| a/b | T1-T4 | 1.42 | 1.43 | 1.35 | 1.26 | 1.31 |
| | T5-T6 | 1.29 | 1.17 | 1.06 | 0.95 | 1.01 |
| | T7-T10 | 1.44 | 1.42 | 1.50 | 1.47 | 1.54 |
| | T11-T14 | 1.40 | 1.41 | 1.46 | 1.47 | 1.43 |

* L, a and b values presented as changes in a comparison to the initial values; a/b are means which initially is 1.47

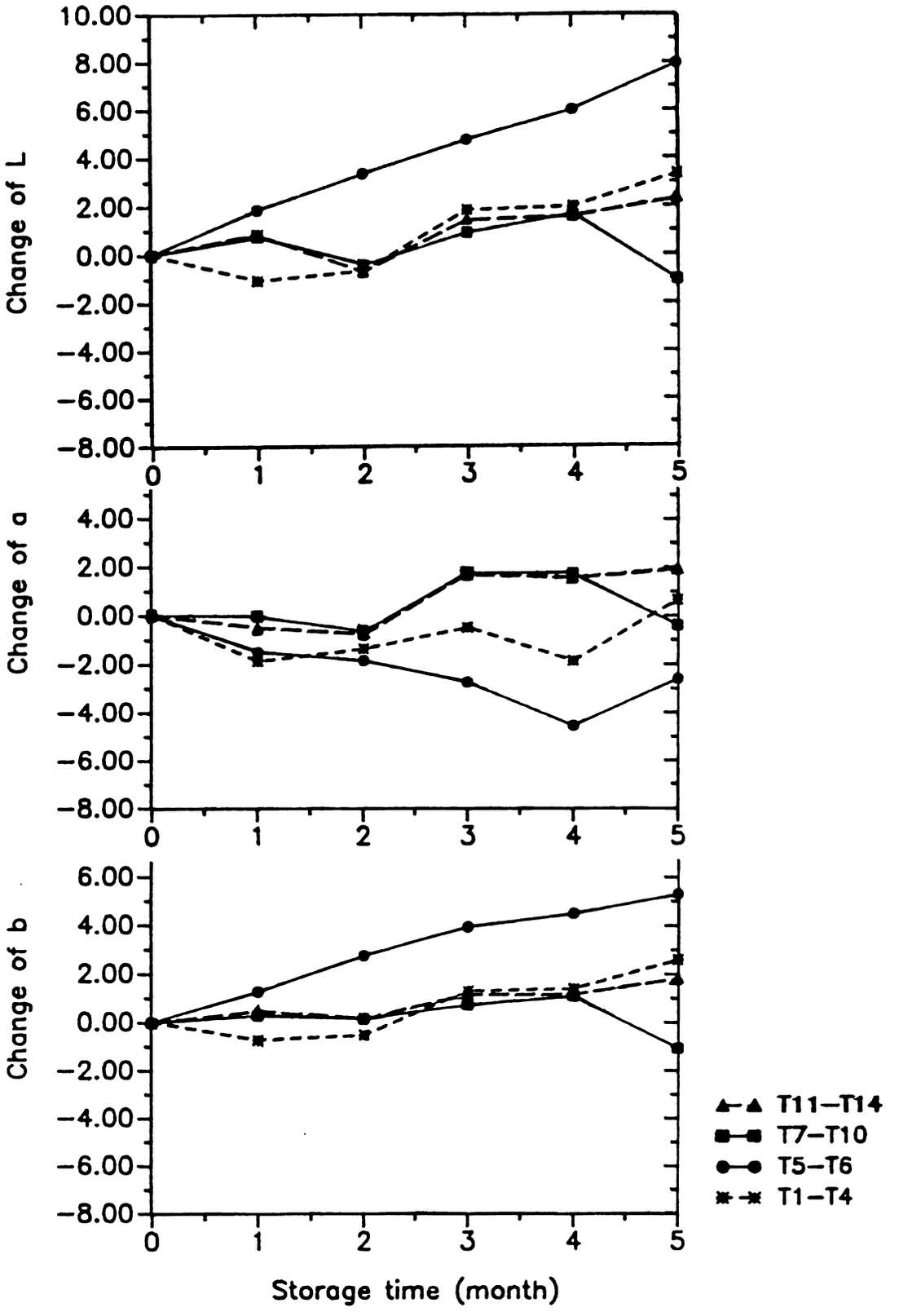



Figure 14 Change of L, a and b in tomato sauce at different storage times

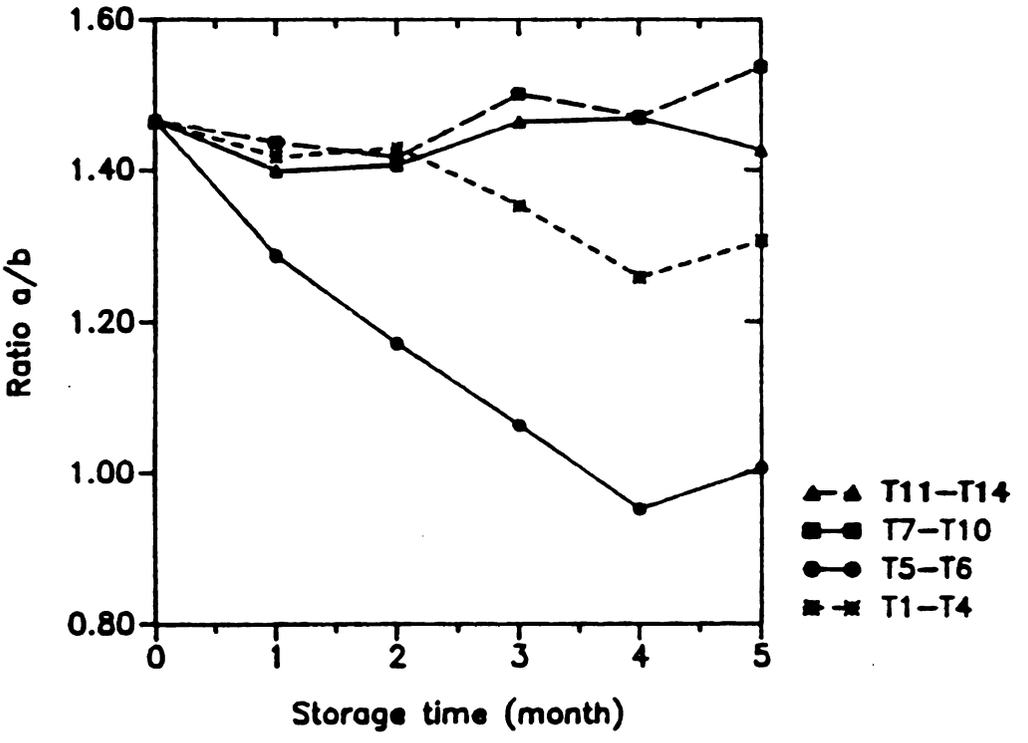



Figure 15 Change of a/b ratio in tomato sauce at different storage times

## 6.5 Sensory evaluation

Color and flavor change in the frozen pizzas from the first six treatments were evaluated sensorially using a group of untrained test panelists. Both appearance of uncooked and flavor of cooked pizzas were assessed initially and at approximately monthly intervals through the fifth month.

### 6.5.1 Appearance of frozen pizzas

The appearance characteristic of frozen pizzas was considered relative to color of pepperoni, pork sausage and tomato sauce. Appearance was evaluated using a scoring scale ranging from 1 to 7 where 1 equaled extremely dislike and 7 equaled extremely like. The scoring sheet is shown in Appendix B. About twenty untrained panelists evaluated the samples.

The mean color scores for pepperoni, pork sausage and tomato sauce are shown in Table 32. It was found that discoloration of pepperoni resulting from exposure to light was detected by the panelists (treatment 2, 4 and 6 in Table 32) though display light did not affect color of tomato sauce. Freeze-thaw cycling did not have any effect on discoloration of the ingredients. As a whole, appearance of pepperoni did not change much at -18 $^{o}$C storage. It was assumed that a score of four (midpoint) was the minimal acceptable value. Therefore, all pizzas stored at -18 $^{o}$C (treatment one to four) still were acceptable up to five

months. A large decrease in the mean color scores was observed in pizzas stored at -7°C (treatment five and six). This indicated that discoloration in pepperoni and tomato sauce was accelerated as storage temperature increased. The color of pork sausage was seemingly unchanged and received low color scores from the beginning, thus making the results difficult to interpret.

6.5.2 Flavor of cooked pizzas

The flavor pepperoni, pork sausage and overall pizza quality was evaluated using the same scoring scale as used for appearance. The scoring sheet is shown in Appendix B. The results represented by mean flavor scores for each category are shown in Table 33. Some fluctuation in flavor scores occurred but generally they decreased with increased storage time. Pizzas stored at -7°C had much lower flavor scores than those at -18°C.

Display light and freeze-thaw cycling did not seem to affect the mean flavor scores for pepperoni, pork sausage and overall quality according to their mean flavor scores which were in a range of four to six. Therefore, they were acceptable during the five month storage. In contrast, the mean flavor scores of those for treatment five and six were lower than four after two months which resulted in unacceptability. In the fifth month, pizzas stored at -7°C had low scores for appearances resulting from marked discoloration of pepperoni and tomato sauce.

The frozen pizzas packed under vacuum and atmosphere were not evaluated sensorially. The use of the high barrier package should have extended their shelf life, thus the acceptability during the five month storage was anticipated.

Table 32 The mean color scores* for pepperoni, pork sausage and tomato sauce in frozen pizzas (treatment 1-6)

| Treatment | | Time (month) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 |
| Pepperoni | 1 | 5.2 | 5.0 | 5.8 | 5.2 | 5.2 | 5.2 |
| | 2 | | 3.5 | 3.8 | 3.7 | 4.1 | 4.4 |
| | 3 | | 4.7 | 4.9 | 4.8 | 5.6 | 5.2 |
| | 4 | | 4.3 | 3.9 | 3.8 | 4.2 | 4.5 |
| | 5 | 5.5 | 3.8 | - | 4.6 | - | 3.4 |
| | 6 | | 3.0 | - | 2.4 | - | 2.7 |
| Pork sausage | 1 | 3.7 | 3.9 | 4.3 | 4.1 | 4.3 | 4.0 |
| | 2 | | 3.6 | 4.0 | 3.7 | 3.6 | 4.2 |
| | 3 | | 3.7 | 4.4 | 4.3 | 4.4 | 4.3 |
| | 4 | | 3.4 | 4.1 | 3.7 | 4.2 | 3.8 |
| | 5 | 4.2 | 4.2 | - | 4.1 | - | 3.8 |
| | 6 | | 3.8 | - | 2.8 | - | 3.3 |
| Tomato sauce | 1 | 6.2 | 5.5 | 5.5 | 5.4 | 6.0 | 5.1 |
| | 2 | | 5.2 | 4.8 | 5.2 | 6.1 | 5.4 |
| | 3 | | 5.4 | 4.8 | 5.3 | 5.7 | 5.3 |
| | 4 | | 5.2 | 5.0 | 5.0 | 5.0 | 4.3 |
| | 5 | 5.8 | 4.7 | - | 3.5 | - | 2.7 |
| | 6 | | 4.0 | - | 3.5 | - | 2.5 |

* 1-7 scale where 1 = extremely dislike; 7 = extremely like

Table 33 The mean flavor scores* of pepperoni, pork sausage and overall quality for cooked pizzas (treatment 1-6)

| | Treatment | Time (month) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 |
| Pepperoni | 1 | 5.7 | 5.1 | 5.0 | 4.5 | 5.0 | 4.3 |
| | 2 | | 4.8 | 4.6 | 4.5 | 5.1 | 4.7 |
| | 3 | | 4.9 | 5.1 | 4.8 | 4.7 | 4.4 |
| | 4 | | 4.6 | 5.2 | 4.5 | 5.4 | 4.9 |
| | 5 | 4.8 | 4.3 | - | 3.4 | - | 3.2 |
| | 6 | | 4.1 | - | 3.3 | - | 2.7 |
| Pork sausage | 1 | 5.5 | 5.4 | 5.0 | 4.8 | 4.8 | 4.3 |
| | 2 | | 4.8 | 4.7 | 4.4 | 4.7 | 4.4 |
| | 3 | | 4.9 | 4.5 | 4.9 | 4.2 | 4.6 |
| | 4 | | 4.7 | 5.2 | 5.1 | 4.1 | 4.7 |
| | 5 | 4.4 | 3.8 | - | 2.4 | - | 2.2 |
| | 6 | | 3.6 | - | 2.6 | - | 2.5 |
| Overall | 1 | 5.7 | 4.9 | 5.3 | 4.8 | 4.7 | 4.1 |
| | 2 | | 4.7 | 4.7 | 4.4 | 4.7 | 4.3 |
| | 3 | | 5.0 | 4.9 | 4.8 | 4.9 | 4.8 |
| | 4 | | 4.5 | 5.0 | 5.0 | 4.7 | 4.8 |
| | 5 | 4.4 | 4.5 | - | 3.2 | - | 2.6 |
| | 6 | | 4.1 | - | 2.9 | - | 2.3 |

* 1-7 scale where 1 = extremely dislike; 7 = extremely like

## 6.6 Correlation between analytical and sensory tests

Correlation between sensory and analytical tests was performed using a normal correlation model. Generally, the results are represented by correlation coefficients which theoretically are between +1 and -1. The higher the correlation coefficient (r), regarding to its sign, the stronger the evident of a high correlation should be. However, it does not always mean that the high correlation is significant unless a large number of measurements is used. When the number of measurements is large, the power of the test statistic increases which makes the analysis more efficient.

In pork sausage, the correlation between the mean TBA values and the mean flavor scores was determined and the results are shown in Table 34. It was found that r values for treatments one and two were relatively high (-0.935 and -0.864) and highly significant at $P<0.01$. The r values for treatments five and six were somewhat high (-0.941 and -0.919) but slightly significant ($P<0.10$). The r value for treatment four was low and not significant at $P<0.01$.

The insignificant or low significant correlation may result from the wide variability in individual untrained panelist scoring and inconsistencies in the TBA test (Greene and Cumuze, 1983). Gokalp et al. (1983); Tarladgis et al., (1960); Turner et al. (1954); and Zipser et al. (1964) reported that a high correlation between sensory test and TBA test for cooked meat was obtained when using trained

Table 34 Correlation coefficients between the mean flavor scores and the mean TBA values for pork sausage

| Treatment | Correlation coefficient (r) |
|---|---|
| 1 | -0.935** |
| 2 | -0.864** |
| 3 | -0.767† |
| 4 | -0.676 |
| 5 | -0.941† |
| 6 | -0.919† |
| 1-4 | -0.628** |
| 1-6 | -0.883** |

† Significant at P<0.10

** Significant at P<0.01

panelists.

The r value of combined data from the first four treatments was low (-0.628) but still significant at $P<0.01$. When six treatments were combined the r value increased to -0.883 and was significant at $P<0.01$. This indicated that the results from treatment five and six contributed to an increase in correlation between sensory and analytical methods. It was possible that the strong rancid flavor in pork sausage from these treatments was easily detected by the panelists and thus resulted in a lower variability in flavor scores.

Although some individual treatments did not show highly significant correlation, they all showed the same direction of negative correlation. Also, the combined treatments did show the negative correlation. This implied that there was a strong evident of real correlation between The sensory and the TBA tests. Therefore, the TBA test may be used to measure degrees of rancidity in pork sausage.

Table 35 shows correlation coefficients for pepperoni color with regard to three analytical measurements including NO-Mb, %No-Mb conversion and redness (from the Hunter cell). It was found that the mean values from those measurements did not correlate with the mean color scores. Each treatment had low r value and was not significantly correlated. Only treatment six showed a high r value and it was significant at $P<0.05$.

Table 35 Correlation coefficients between the mean color scores and NO-Mb, % NO-Mb conversion and redness values for pepperoni

| Treatment | Correlation coefficients (r) | | |
|---|---|---|---|
| | NO-Mb | % NO-Mb conversion | Redness (a) |
| 1 | 0.065 | 0.478 | 0.406 |
| 2 | 0.358 | 0.751 | 0.601 |
| 3 | 0.531 | 0.624 | -0.245 |
| 4 | 0.626 | 0.789 | 0.540 |
| 5 | 0.848 | 0.710 | 0.591 |
| 6 | 0.989** | 0.974* | 0.996** |
| 1-4 | 0.587** | 0.715** | 0.515** |
| 1-6 | 0.798** | 0.815** | 0.567** |

* Significant at P<0.05

** Significant at P<0.01

The insignificant correlation may result from either inconsistencies in the test methods or variability in individual panelist scoring or both. The results could not be compared with those from previous works due to lack of available published data.

The r values of combined data from the first four treatments were relatively low but significant at $P<0.05$. %NO-Mb conversion values showed better correlation ($r = 0.715$) with the mean color scores than the others. When all treatment data were combined, the r values increased and were highly significant at $P<0.01$. %NO-Mb conversion gave the highest r value, follow by that from NO-Mb and that from redness ($r = 0.815$, 0.798 and 0.567, respectively). Only the redness values did not show the same direction of positive correlation for all individual treatments, thus it may not be an appropriate value to represent pepperoni color.

Table 36 shows correlation coefficients for tomato sauce with respect to redness a and ratio a/b from the Hunter cell. An individual treatment had low r and was not significant at $P<0.05$. Although some treatments showed high r values but they were insignificant. The r values of combined data from the first four treatments were very low and insignificant. When all treatment data were used, a higher r value was observed. Ratio a/b gave better correlation ($r = 0.765$) with the color scores than value a singly ($r = 0.385$) and r from ratio a/b was significant at

$P<0.01$ but r from value a was insignificant. The addition of results from treatment five and six contributed to the increased r value because tomato sauce in these treatments had marked color fading which was easily detected by the panelists, thus variability in the scoring decreased.

However, there was not a strong evident to suggest that a/b value could represent color of tomato sauce because the correlation of individual treatments did not follow in the same direction.

Table 36 Correlation coefficients between the mean color scores and the mean redness (a) and a/b values for tomato sauce

| Treatment | Correlation coefficients (r) | |
|---|---|---|
| | Redness (a) | Ratio a/b |
| 1 | -0.329 | 0.254 |
| 2 | 0.148 | -0.262 |
| 3 | 0.098 | 0.091 |
| 4 | -0.172 | 0.676 |
| 5 | 0.929† | 0.993** |
| 6 | 0.848 | 0.941† |
| 1-4 | -0.290 | 0.026 |
| 1-6 | 0.385 | 0.765** |

† Significant at P<0.10

** Significant at P<0.01

## SUMMARY

Of all the factors included in this study, storage temperature impacted the most on quality deterioration of frozen pizzas. Freeze-thaw cycling did not have a significant effect on the quality. This may imply that using one continuous freeze-thaw cycle such as in this experiment was not the right approach and did not simulate actual conditions.

During storage, quality loss was observed in all treatments with varied rates of deterioration. Packaging technique played an important role in the reduction of quality loss. The use of a barrier package under vacuum at $-18\ ^{o}C$ provided the best quality maintenance. The same package sealed under atmosphere did not provide as good protection, with little difference observed in comparison to the existing poor barrier package. This indicates that level of oxygen is an important factor governing deterioration. Both lipid oxidation in pork sausage and discoloration in pepperoni were reduced with vacuum packing. The display light condition used in this study did not increase lipid oxidation of pork sausage or color fading of tomato sauce and had only little effect on promotion of color fading in pepperoni.

Based on the results from analytical and sensory tests, the pizzas packed in the original packages were still acceptable up to five months of storage, thus vacuum packing can prolong shelf life of frozen pizzas perhaps up to one year. The initial quality of fresh pizzas is also critical. In the same type of package, pizzas with a lower degree of rancidity and discoloration will have longer shelf life.

The use of vacuum packaging is of some concern. The production cost relating to the increase in material cost, line slow down, equipment cost and inspection for seal integrity need to be examined. Also, the product itself must not be crushed under high vacuum which could result in loss of appearance and crispy texture. Other than vacuum packaging, the exclusion of oxygen may be achieved by alternative techniques such as headspace control, nitrogen flush and the use of oxygen scavenger. Further study should be done to compare these options, thus the most feasible one could be selected in regard to quality and cost.

# APPENDICES

# APPENDIX A

## Layer thickness measurements

The thickness of the respective layer of the test package material was determined by means of viewing edge on through a microscope fitted with an ocular micrometer. A test specimen was placed into a freezer (-80 $^{o}$F) for about 15 hours and then cut into 20 mm x 30 mm and mounted securely in a frame (clamping device) made from two stainless steel blocks with dimensions of 3 in.x 1/4 in.x 1/4 in. in length, width and thickness respectively. The mounted film/frame assembly then was placed in the freezer for 10-15 min. prior to cutting to ensure a clean cut edge for viewing. The sample was swiftly cut with a razor while the assembly was in the freezer.

An American Optical Company Model 60 microscope fitted with an ocular micrometer (American Optical Company) was used with 43x10 magnification. The ocular micrometer was calibrated to give one division = 0.725 mil. It was found that the test package material was fabricated from two materials where the inner ply was 1.96 mil, 0.87 mil for the outer ply and 0.22 mil for the adhesive ply.

To determine type of material for each layer, attenuated total reflectance procedure was employed.

Attenuated total reflectance (ATR)

ATR procedure is a technique used to examine the surface of a film material. The phenomenon of ATR involves the internal reflectance of light within a high refractive index material (analyzing crystal such as KRS-5) along the interface between the crystal and a film sample (the reflecting surface). The light entering the crystal is reflected within the crystal and at each reflection the light beam penetrates the sample a small amount and is absorbed at the characteristic absorption frequencies. The variation in intensity of the exit beam with wavenumber in the Infrared region is recorded as the spectrum of the sample. The IR spectrum is identified in the same manner as an IR transmission spectrum where absorption bands are analysed and compared to the known samples.

In practice, a multiple internal reflection accessory (MIR) was used with a Perkin-Elmer 180 IR spectrophotometer. The MIR accessory consists of a base with three angle positions, one flat mirror, two spherical mirrors, a KRS-5 crystal and a sample holder assembly. A test specimen was cut and placed on the parallel faces of the crystal which was placed in the sample holder. The sample holder assembly then was positioned at $45^{o}$ interface angle. A reference beam attenuateor was used to correct for losses in energy transmittance through the sample beam resulting from use of the MIR accessory. The reference beam attenuator removed

comparable amounts of energy from the surface.

It was found that the inner layer was Polyethylene and the outer layer was Nylon.

## APPENDIX B

### EVALUATION OF FROZEN PIZZA APPEARANCE

Name _____

| Pepperoni Color | Sausage Color | Sauce Color |
|---|---|---|
| 7 - Most desirable | 7 - Most desirable | 7 - Most desirable |
| 6 | 6 | 6 |
| 5 | 5 | 5 |
| 4 | 4 | 4 |
| 3 | 3 | 3 |
| 2 | 2 | 2 |
| 1 - Least desirable | 1 - Least desirable | 1 - Least desirable |

Sample No.

| | | |
|---|---|---|
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |

Comments : _____

EVALUATION OF COOKED PIZZA FLAVOR

Name _____

Please evaluate the samples for any off flavors. Spiciness, texture or temperature should not be considered for the rating but do feel to comment on these. Determine where on a scale of 1 to 7 each sample best fits then transfer that number down corresponding to each sample number. Thank you.

| Pepperoni Flavor | Sausage Flavor | Overall Flavor Quality |
|---|---|---|
| 7 - Fresh - No off flavor | 7 - Fresh - No off flavor | 7 - Like very much |
| 6 | 6 | 6 |
| 5 | 5 | 5 |
| 4 - Moderately fresh moderate off flavor | 4 - Moderately fresh moderate off flavor | 4 Neither like nor dislike |
| 3 | 3 | 3 |
| 2 | 2 | 2 |
| 1 - Severe off flavor | 1 - Severe off flavor | 1 - Dislike very much |

Sample No.

| | | |
|---|---|---|
| | | |
| | | |
| | | |
| | | |

Comments : _____

_____

_____

# APPENDIX C

## Analysis of Variance

BMDP statistical software version MSU, was used and the results are shown in Tables 7, 14, 18, 22, 27 and 28.

## Bonferroni t-test

$$t_b = \text{diff.} / [\, MS_E (1/n1 + 1/n2)\,]^{1/2}$$

Critical values : $t_b$ (tabulated)

| Sample | Values | $MS_E$ | D.F.(v) | m | | d | $t_b$ (tabulated) |
|---|---|---|---|---|---|---|---|
| Pork sausage | TBA | 0.00010 | 180 | 9 | | 0.05 | 2.814 |
| | | | | | | 0.01 | 3.327 |
| Pepperoni | NO-Mb | 0.00064 | 180 | 16 | \| | 0.10 | 2.774 |
| | % NO-Mb | 11.01476 | 180 | 16 | > | 0.05 | 3.005 |
| | a | 0.80491 | 180 | 16 | \| | 0.01 | 3.496 |
| Tomato sauce | L | 0.40167 | 109 | 9 | \| | 0.05 | 2.832 |
| | a | 0.16100 | 109 | 9 | \| | 0.01 | 3.354 |
| | b | 0.14523 | 109 | 9 | > | | |
| | a/b | 0.00054 | 109 | 9 | \| | | |

where m = number of pairwise mean comparisons

Sample of calculation

Mean TBA value of P1 (1st month) = (0.144 + 0.150)/2

= 0.147

Mean TBA value of P2 (1st month) = (0.141 + 0.139)/2

= 0.141

$$t_b = (0.147 - 0.141)/[0.00010(1/6 + 1/6)]^{1/2}$$

$$= 1.039$$

This number is smaller than $t_b$, thus there is insignificant difference between P1 and P2 at $P<0.05$.

Normal correlation model

Coefficient of correlation between variable 1 and 2 is expressed as :

$$r_{12} = S_{12} / S_1 S_2$$

and t-test statistic : $t = r_{12} (n-2)^{1/2} / (1-r_{12}^2)^{1/2}$

where n = number of mean values involved in the analysis.

The correlation coefficients are shown in Tables 34, 35 and 36.

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# BIBLIOGRAPHY

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