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THE EFFECT OF FLUORESCENT LIGHT ON RIBOFLAVIN AND FLAVOR QUALITY OF 2% MILK PACKAGED IN HIGH DENSITY POLYETHYLENE CONTAINERS

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# THE EFFECT OF FLUORESCENT LIGHT ON RIBOFLAVIN AND FLAVOR QUALITY OF 2% MILK PACKAGED IN HIGH DENSITY POLYETHYLENE CONTAINERS

By

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ABSTRACT

THE EFFECT OF FLUORESCENT LIGHT ON RIBOFLAVIN AND FLAVOR QUALITY OF 2% MILK PACKAGED IN HIGH DENSITY POLYETHYLENE CONTAINERS

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The effect of 100 foot-candles of unshielded fluorescent light and 90 foot-candles of shielded light on 2% milk packaged in High Density Polyethylene quarts, half-gallons, gallons and yellow pigmented gallon bottles were studied.

The containers were subjected to these lighting conditions over a 24 hour period. Samples were removed at 0, 5, 10 and 24 hours and analyzed for riboflavin content by High Pressure Liquid Chromatography.

Riboflavin losses were greater in the quarts and half gallons than in the gallon bottles. Degradation also tended to be slower under shielded light than unshielded light. The riboflavin content of the yellow pigmented containers did not change over the testing period. Overall, riboflavin losses from fluorescent light exposure did not vary significantly during the experimental study.

A taste panel was also assembled to determine the degree of light activated flavor in 2% milk subjected to the same conditions as previously described. Light activated flavor developed more rapidly under the unshielded light. The protection provided by the yellow-colored shields shows a notable reduction in activated flavor for all container types.

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# INTRODUCTION

Milk is a nutritionally dense food with a rich history. The earliest written record appears in the Sanskrit of ancient India, nearly 6000 years ago. Since this ancient time to about 1850, milk production and processing experienced very little change. Due to a lack of refrigeration and transportation, most milk had to be consumed within a few miles of where it was produced because of its extreme perishability. However, with a shift in population from a rural environment to an urban one came many changes: sanitation regulations, pasteurization and bottling plants for fluid milk, the shifting of the processing and delivery functions from the farmer producers to milk dealers, modern milking equipment, the cream separator, mechanical refrigeration, special milk trains, tank cars and tank trucks (8).

The packaging and delivery of milk has also changed through the years. Milk was first home delivered in glass bottles and placed into metal boxes for protection from dawns early light. When buying habits changed and people began purchasing milk from supermarkets, glass bottles became difficult to return. Gallon size glass containers were also very heavy to carry. In the late 1920's dairies began packaging milk in paperboard cartons. These became popular with consumers in the mid-1940's because they weighed much less than glass. After introduction in 1964, plastic bottles became widely accepted by the 1970's (9,11). Thus, what paperboard did to glass, plastic is currently doing to

paperboard.

With this change in packaging, many controversial statements have been made by the Paperboard Packaging Council among others. They claim that, unlike paperboard, plastic bottles do not protect milk from light and thus nutritional losses occur and flavor changes result when exposed to fluorescent lighting in dairy cases. However, the plastic industry believes the issue is an economic one and not nutritional (14,76,115).

95% of all milk sold today is packaged in one gallon plastic containers (69). Until recently, paperboard companies have enjoyed a dominance in the half gallon segment of this market. However, improvement in plastic container fabrication techniques have now made it economical to produce half gallon bottles at a price competitive to that of paperboard (11,14,67). Many consumers prefer plastic bottles, thus paperboard companies may lose their dominance in this market to the plastic bottle industry.

Nevertheless, loss of nutritional value and flavor changes in milk as a result of exposure to fluorescent light are important factors.

In this study, the effect of 100 foot-candles of unshielded fluorescent light and 90 foot-candles of shielded light on 2% milk packaged in High Density Polyethylene (HDPE) quarts, half-gallons, gallons and yellow pigmented gallon bottles is examined. The objective is to determine changes in riboflavin content and flavor quality over 24 hours. Results will then determine if these variations are significant to warrant changes in milk packaging and dairy case lighting.

# REVIEW OF THE LITERATURE

## Nutritional Value of Milk

"Every person, young and old, should drink milk. Milk contains a large variety of nutritional constituents and, considering its cost per pound, more food for the money than any other food material available" - Charles H. Mayo, M.D. (36).

Milk is considered one of man's most important foods. It is nutritionally dense, meaning the major nutrients are in high concentration in relation to it's caloric value. Milk is also very complex with over 100 compounds identified. Milk consists of approximately 87% water and 13% solids. The percent total solids portion is comparable to that of many solid foods. For example, lettuce and tomatoes have solid contents of only 5 and 6 percent respectively (36). The solids portion contains fat, fat soluble vitamins and solids not fat. The solids non-fat include protein, carbohydrate, water soluble vitamins and minerals.

The National Dairy Council ( 6 ) estimated for 1977 fluid milk contributed only 6.1 percent of the caloric intake. However, milk provided 44.1 percent of the calcium, 24.2 percent of the riboflavin, 20.8 percent of the phosphorus, 14.1 percent of vitamin B-12, 13.9 percent of the magnesium, 12.0 percent of the protein, 6.3 percent of vitamin B-6, 6.0 percent of the fat, 5.8 percent of the thiamin, 4.8 percent of the vitamin A, 4.5 percent of the carbohydrate and 3.3 percent

of the ascorbic acid that was consumed. In addition, milk provides significant amounts of vitamin D, iron and niacin.

These nutritional qualities were emphasized by Campbell and Marshall (36), who indicated that daily consumption of a quart of cows' milk furnishes an average man approximately all the fat, calcium, phosphorous, and riboflavin; one-half the protein; one-third of the vitamin A, ascorbic acid, and thiamin; one-fourth the calories; and with the exception of iron, copper, manganese, and magnesium, all the minerals needed daily.

To summarize, Hippocrates, the father of medicine, emphasized the nutritional importance of milk in his statement that "milk is the most nearly perfect food" (36).

Lowfat milk has a fat content of ≤2% and contains about 8.25% nonfat solids (6). Milk must be pasteurized or ultra pasteurized at 71.5$^{o}$C and 138$^{o}$C respectively (6,87). Since much of the natural vitamin A is lost during removal of the milkfat, 2000 international units (IU) of vitamin A per quart must be added in accordance with federal law (6). Homogenization and vitamin D fortification are optional. However, when vitamin D is added, levels must be 400 IU per quart (6). Other optional ingredients include; carriers for vitamins, characterizing flavorings, fruit and fruit juices, natural and artificial flavorings (8). Emulsifiers and stabilizers may also be used as optional low level ingredients to keep added milk ingredients dissolved. An eight-ounce glass of 2% milk contains about 120 calories (5,6). When nonfat solids are added to lowfat milk to reach the 10% level, the product must be labeled either protein fortified or fortified with

protein. With the increase in nonfat solids the calorie count also increases.

Table 1 shows the composition of 2% and whole milk (6). The most notable differences are with the water and fat content.

Food producers and processors often use the United States Recommended Daily Allowance (U.S. RDA) to relate the nutrient content of their products. These nutrient amounts are expressed as percentages of the U.S. RDA on food packaging. The U.S. RDA's are the amounts of nutrients when consumed, provide a margin of nutritional well-being for practically all healthy people in this country. The mean intake of riboflavin by consumers from all food types exceeds the U.S. RDA of 1.7 mg (5). This is especially important when considering the effect of fluorescent light on riboflavin in milk.

Many different milk and milk products are commercially available. A few of these are presented below (11,16):

Fluid Milk

Whole, lowfat, nonfat and chocolate

Milk Types

Evaporated, condensed and dry

Specialty Milks

Certified, low sodium, imitation and filled

Other Products

Buttermilk, half-and-half, yogurt, eggnog, whipping creams, sour creams, light and heavy creams, ice cream, ice milk, sherbet, butter, cheese and cottage cheese.

Table 1

Composition of 2% Milk vs Whole Milk - Expressed as a Percentage of Each Component

| Component | 2% Milk | Whole Milk |
|---|---|---|
| Water | 89.21% | 87.99% |
| Fat | 1.92% | 3.34% |
| Protein | 3.33% | 3.29% |
| Carbohydrates | 4.80% | 4.66% |
| Vitamins and Minerals | .7-1% | .7-1% |

## Regulations

The Grade A Pasteurized Milk Ordinance (PMO) includes a set of recommendations developed by the United States Public Health Service (USPHS) and the Food and Drug Administration (FDA) for voluntary adoption by states and other local jurisdictions (6,14). The PMO is designed to assure the quality of Grade A milk. Even though the ordinance is voluntary, many states and local jurisdictions follow more rigid provisions than those laid out by the PMO. The PMO is periodically updated as new advances in processing, equipment and research are made. Practices adopted by the PMO include: maintaining healthy herds, inspecting farm and dairy plants for sanitary conditions, instructing personnel engaged in production, processing and distribution of milk on sanitary practices, conducting laboratory examinations on milk, insuring proper pasteurization and monitoring milk supplies for unintentional adulterations.

## Sales and Trends

Over the past ten years sales of whole milk have declined while those of the low fat variety have increased. The Milk Industry Foundation (16) noted that in 1984 low fat milk continued its upward climb with a 5% increase, while plain whole milk declined by 2.4% over the same period. On a per capita basis, sales totaled 38 quarts of low fat milk and 57 quarts of whole milk. In regard to percent of fluid milk sales by product, whole, 2%, 1% and skim milk represented 53.2%, 28.8%, 6.2% and 5.2% respectively.

Recently Dairy Field (117) surveyed 350 consumers in seven cities to determine if they had a preference for milk in paperboard or plastic containers. According to Trudeau (117) of the 350 consumers, 41% preferred plastic and 36% paperboard, the remaining 23% did not have a preference. The survey specifically avoided the vitamin loss/ nutritional value issue and found some interesting results to why consumers liked one package over the other. Reasons cited included "only package available in the size we buy" and "our favorite dairy uses it". This suggests a lax attitude among consumers toward their favorite dairy's packaging choice (117).

As another part of the survey "taste better" was not listed as a possible choice for preference. Despite this omission, some consumers commented on the flavor quality in both container types.

Consumers were also asked the type of milk they preferred. Over half said fat content was the influencing factor in their choice. Overall, Trudeau (116) notes 42% of the total respondents listed whole milk, 43% 2% lowfat, 6% 1% lowfat and 8% skim milk.

74 milk bottlers also responded to a similar survey. In regard to container type, 45.9% preferred plastic, 33.8% chose paperboard as their primary container and 20.3% gave no preference (69). Of the reasons given for choosing plastic, "customers prefer them" was the number one response.

Recently, a war of words has occurred between the producers of plastic and paperboard milk containers. Each claim their container is a suitable package for milk.

The Paperboard Packaging Council claimed the following (14):

1) Milk packaged in plastic suffers significant vitamin losses, particularly losses of riboflavin and vitamin A when exposed to light under normal conditions;
2) Milk is a primary source of riboflavin and vitamin A in the diets of the general public;
3) Over 50 independent studies conducted by scientists have now been published to show the damage light does to milk;
4) Milk packaged in plastic loses sufficient nutrients through exposure to fluorescent light to pose a nutritional threat to the American consumers; and
5) Consumers, especially children, may dislike the taste of milk packaged in plastic.

However, Hoover Universal, a leader in the plastic industry has come to a number of different conclusions concerning the effects of fluorescent light on milk (12,14):

1) The United States Food and Drug Administration determined there was no significant nutritional problems with milk packaged in plastic bottles;
2) Scientists disagree among themselves concerning the effects of fluorescent light on milk in dairy cases;
3) Even though riboflavin and vitamin A are effected by exposure to fluorescent light, milk is not the only source for these nutrients. Many of the foods we consume contain these vitamins. In fact only 12% of our Recommended Daily Allowance comes from dairy products;

4) Hoover Universal believes good nutrition or health is not seriously effected by the influence of fluorescent light on milk;
5) Many of the studies referenced by the paperboard industry have been conducted under laboratory conditions, which have little relevance to actual dairy case conditions;
6) To alleviate concerns, Hoover Universal developed the gold shield to protect milk from fluorescent light.

An issue other than nutrition may be at the root of the current controversy. Blair (27) found in recent years that paperboard sales have declined while those of plastic have increased. Today, over 60% of the milk sold is packaged in plastic (16,67,71). Over 95% of the milk sold in the gallon size is contained in plastic (69,117). With the advent of the plastic half-gallon and loss of their once dominant gallon market, paperboard companies foresee further declines. Until recently it has not been economical to produce a plastic half gallon milk bottle. However, this situation is rapidly changing. New developments in bottle designs, dairy equipment and lower prices for High Density Polyethylene resin all point toward the plastic bottle in the half gallon market (11,71,115,118).

Many dairies seem to prefer plastic whether in a gallon or half gallon. The reasons given include (14,66,68,90): the most sanitary container available, has excellent handling features, high consumer acceptance, opens new markets, removes production problems, lowers inventory costs, reduces packaging inventory, eliminates leaks in the dairy store, allows a high degree of product visibility, extends product shelf-life and permits reuse of scrap material.

Consumers also seem to prefer plastic bottles. They like the built-in handle for ease of carrying and pouring, the resealable cap to preserve freshness, the high product visability and the fact that plastic containers are sanitary and leakproof (14,90).
Changing demographics have and will continue to play a role in growth of the plastic half gallon market. With the trend toward smaller households, an older population, more single people and delays in young people having children, a larger number of smaller product sizes are becoming available (14). Thus, the potential market for the plastic half gallon is enormous.

Country Fresh Dairy (15) recently decided that the plastic half-gallon would probably be the container of the future. Thus, to remain competitive, Country Fresh decided to switch from paperboard cartons to plastic bottles. Reasons for the switch include consumer preference, elimination of leakers and preservation of the half gallon segment of the market from takeover by the plastic gallon.

In an attempt to counteract the emergence of the blow molded half gallon and weaken consumer preference for plastic bottles, the paper-board industry has introduced the paper gallon using several advertising campaigns.

The 2-Pak consists of two half gallon containers joined together with a paperboard or polystyrene handle. Dairy Field (7) reports that this package is directed toward consumers which have been educated to buy a gallon of milk, because traditionally that is where the savings have been. In addition, the Twin-Pak is convenient, allowing for easy carrying and handling once individual cartons are separated. Also,

one carton can be kept sealed while the other is in use. The Ex-Cell-O Corporation (10) reports the paperboard 2-Pak permits 12 to 13% more product space in the dairy case and saves one U.S. dairy 2¢ per gallon in comparison to the plastic gallon. Finally, the package is made from a renewable resource, trees.

In addition to the introduction of the twin-pak, a number of advertising claims have been endorsed by the Paperboard Packaging Council (PPC). These campaigns are designed to inform consumers of the advantages of paperboard in protecting milk quality and to bolster sales. Johnson (71) reported the ads by the PPC claim that riboflavin and vitamin A are being lost when milk in plastic containers is exposed to fluorescent light in dairy cases and that when informed, some consumers make the switch to paperboard. Once the switch has been made, consumers are reluctant to go back to plastic. For example, in Sioux Falls, South Dakota, pre-advertising sales showed paperboard with a 41% market share. After the campaign, paperboard captured a 64% share of the market. Fifteen months later, paperboard still had a 65% share. In Boston, paperboard's market share increased from 26 to 47% and in Seattle, it jumped from 28 to 37% (71).

In a recent court hearing Densford (41) reported that federal Judge Thomas A. Flannery refused to halt the controversial advertising campaign being used for the Paperboard Packaging Council. The judge ruled the PPC "can accurately claim that certain laboratory studies do, in fact, indicate that milk in plastic may be subject to greater vitamin losses than milk packaged in paperboard." He also ruled that two claims appearing in the ads had to be withdrawn: The first made

reference to the amount of riboflavin being lost after 24 hours. The ads made claims of 14 percent. However, this referred to skim milk which accounts for only 5.2 percent of total fluid milk sales. A better representation would have been whole milk which showed an 8 percent loss of riboflavin over 24 hours according to the study supporting this claim (41,102). The judge also made it clear that most of the 62 university studies cited in PPC literature did not replicate actual retail conditions as they had implied. Only three studies tried to duplicate an actual dairy case simulation. And of these studies, no difference was indicated between the two container types after exposure to light for a given period.

These campaigns have made the Society of the Plastic Industry Inc. (SPI) angry. They claim that the PPC is blowing the problem way out of proportion (27,41,71). Thus, SPI has forced the PPC to admit in their ads that fluorescent light shields can be utilized to minimize vitamin losses by harmful rays. SPI also points out that vitamin losses in milk occur only after it has been exposed to fluorescent light for long periods of time and that most milk stays on the shelf for only a few hours, therefore avoiding any significant nutritional loss.

SPI also argues that consumers are being mislead into believing PPC claims that losses of vitamin A and riboflavin from milk packaged in plastic containers are nutritionally significant. Judge Flannery, however, did not agree. He stated (41) that "Clearly, a 10 percent loss of vitamin A could be of 'nutritional importance' to a person whose diet already fails to meet the Recommended Daily Allowance (for these vitamins) and who relies on milk to meet his dietary needs."

Despite this and the current disagreement among scientists about the whole situation, the PPC is going to continue their ad campaigns in markets they deem appropriate.

Hoover Universal (67) points out that if consumers were dissatisfied with the nutritional aspects of milk packaged in plastic bottles, they would discontinue to buy milk in these containers. Current sales figures show a rise nationally in plastic rather than a decline (14). Also, if the PPC continues with its ads against plastic, this could hurt already sagging milk sales.

## Energy Distribution of Fluorescent Light and Effect on Milk

Several forms of energy are emitted by fluorescent light. Satter and deMan (97) report near ultraviolet radiation accounts for only a small part (0.5%) of the lamp output, while about 1/3 of the output (36%) is emitted as infrared energy. The rest of the energy 42% and 22% is dissipated as heat and light respectively (Figure 1).

Dimick (44) observed that white fluorescent lights have a spectral output ranging from 300-750 nanometers (nm) with maximum radiant emissions peaking in the visible region of the electromagnetic spectrum at 470 nm and 600 nm (Figure 2). Fanelli et al. (48) reported a similar spectral emission for a 40 watt cool white fluorescent lamp (Figure 3). The Soltex Polymer Corporation (109) observed energy output for a typical supermarket white fluorescent lamp to have maximum absorption at approximately 410 and 430 nm (Figure 4).

Lamp energy and emission spectra are very important parameters affecting light induced flavor change in milk. Dimick (44) found that

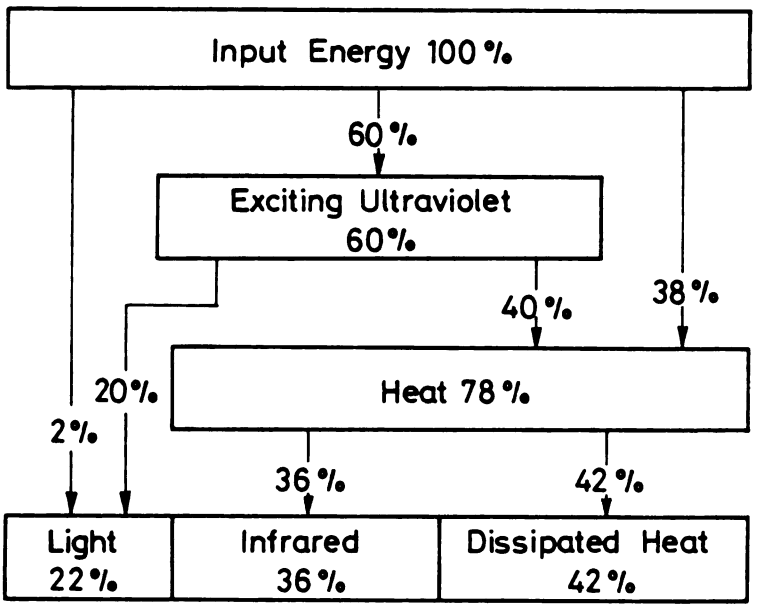



Figure 1. The Energy Distribution of a Typical Cool White Fluorescent Lamp, Satter and deMan (97).
(Courtesy of General Electric Company, USA)

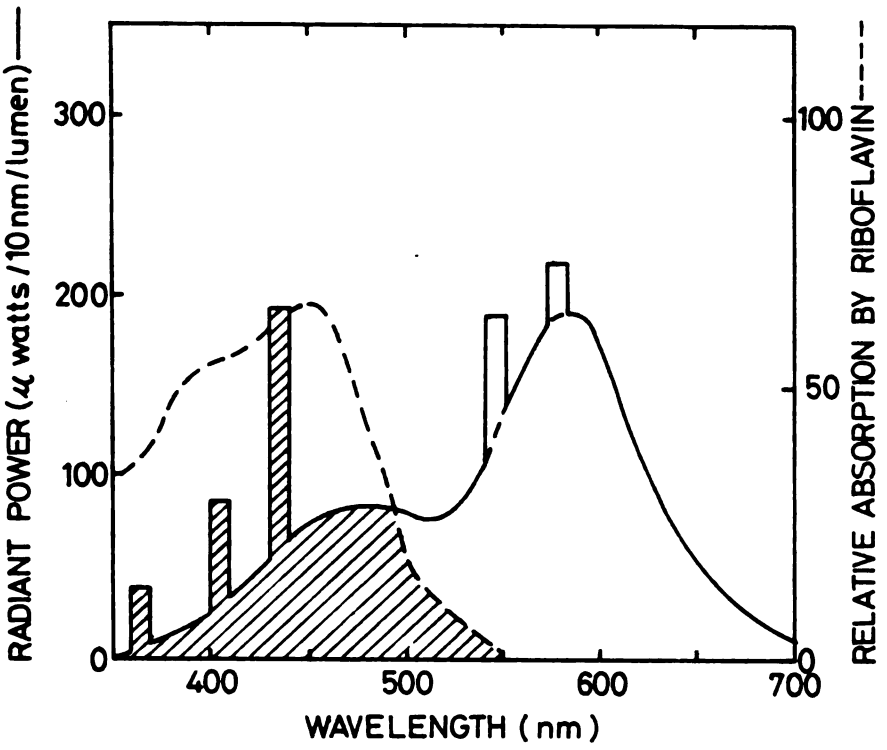



Figure 2. Emission Spectra of a Cool White Fluorescent Lamp Compared with the Absorption Spectrum of Riboflavin, Dimick (44). (Published with permission of The National Office of the Canadian Institute of Food Science and Technology)

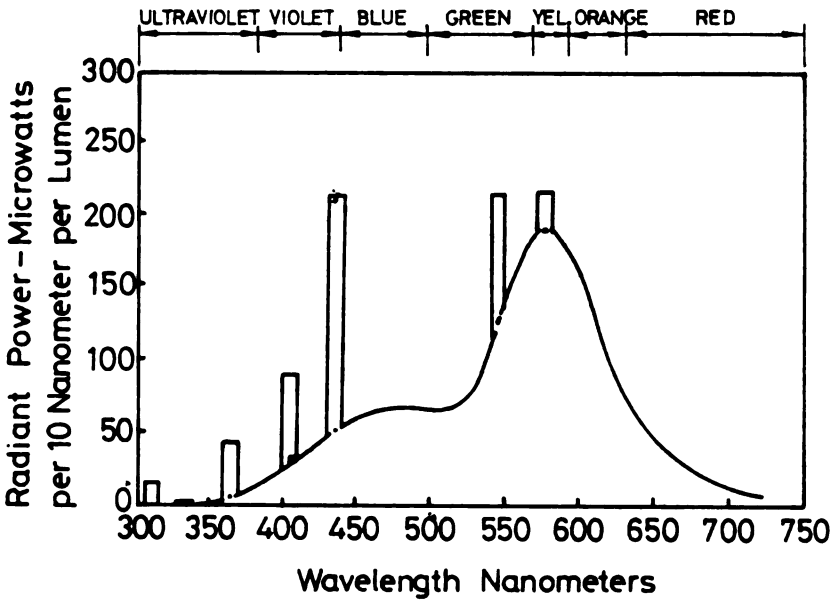



Figure 3. Spectral Energy Distribution of a 40 watt Cool White Fluorescent Lamp, Fanelli et al. (48).
(Courtesy of General Electric Company, USA)

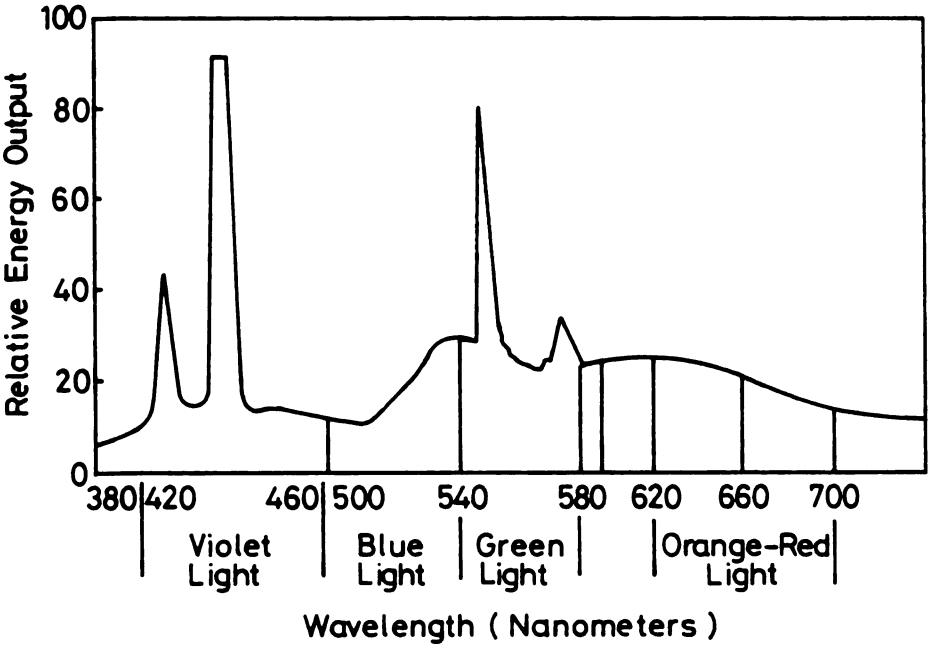



Figure 4. Energy Output Curve for a Typical Supermarket White Lamp. (Courtesy Soltex Polymer Corporation)

chemical reactions which occur in milk may be initiated by the absorbed radiant energy (Figure 2).

When an applied voltage accelerates electrons in a fluorescent light bulb, gas atoms inside the tube are struck and become excited. The excited atoms emit UV photons. The photons strike a fluorescent coating on the inside of the tube resulting in light emission. These emissions are spread through the entire visible spectrum. Light in the blue-violet area is considered to be the most destructive for riboflavin and light activated flavor development in milk (56,109), while those in the red and yellow are considered less destructive (47).

Fanelli et al. (48) have noted through the work of Dimick (44) that the 450 nm band is implicated as the principal source of electromagnetic irradiation leading to degradation. Satter and deMan's (97) survey of over 200 articles found light ranging from 325-460 nm most critical. White (121) considered 300-500 nm most destructive, while Nelson and Cathcart (82) reported 400-500 nm most harmful. Farrer (49) noted a reduction in riboflavin losses when light having wavelengths less than 500 nm were eliminated by protective packaging. Hansen et al. (60) observed similar results.

The Soltex Polymer Corporation (109) found ultraviolet light below 380 nm and visible light above 500 nm having little effect on vitamin and nutrient losses in milk. However, Shipe et al. (105) noted light having wavelengths greater than 500 nm did aid in development of light induced flavor.

## Influence of the Package and Light Shields

The amount of light able to penetrate the wall of a milk container has a very important effect upon riboflavin losses and off-flavor development. Fat content of milk also plays a role. Senyk and Shipe (102) noted that a low fat content allows light to pass more deeply into milk, thus increasing the chance of a reduction in riboflavin and flavor quality. For a 63 gram unpigmented High Density Polyethylene (HDPE) container about 55% light transmission occurred in the blue-violet region of 400-500 nm (109). Incorporation of titanium dioxide into the same container reduced light transmission in the blue-violet region to about 9%.

Nelson and Cathcart (81) reported that light transmission through polyethylene containers varied from 50-70% depending on wall thickness. They also found (82) pigmentation of bottles with titanium dioxide ($TiO_2$) substantially reduced light transmission over unpigmented ones, with the amount of reduction depending on the level of $TiO_2$. However, light between 400-550 nm is not totally blocked through pigmentation. Barnard et al. (25) also concluded that incorporation of titanium dioxide into blow molded containers readily improved its protective ability.

In earlier studies, Bradfield and Duthie (28,29) show light damage to milk in blown polyethylene bottles occurred even with the incorporation of a titanium dioxide blocking agent. deMan (40) also noted incorporation of 1/2 to 2% titanium dioxide into plastic jugs was not effective in reducing light transmission.

Other researchers have described the light transmission character of blow molded containers; Coleman et al. (38) and Levey (76) reported that plastic bottles have a light transmission 35 times greater than paperboard containers which block out 98% of the harmful light.

Senyk and Shipe (102) as do Bradfield and Duthie (28) suggest use of packaging materials to limit light induced problems that are potentially harmful to milk. Poulsen and Blaauw (88) go one step further in recommending that the maximum permissible light transmission of a milk container material should be 8% at 500 nm and 2% at 400 nm.

The volume/area ratio is a given volume of product divided by the face area of its container. For example, 1000 milliliters (ml) of milk with a container face area of 136 centimeters ($cm^2$) has a volume to area ratio of 7.4 $ml/cm^2$. This ratio plays a key role in light-induced flavor defects in milk. Farrer (49) indicates small volume to area ratios are apt to cause more degradation because less milk is present to "dilute" a given amount of light. Fluckiger (52) reports that a smaller ratio between the surface of the packaging material and the product is more disadvantageous. Bradley (31), Mottar (80) and Allen and Parks (3) also point out the importance of volume to area ratio in protecting milk quality.

During the blow molding operation wall thickness can vary widely from nonuniformity of resin distribution. Container wall thickness can therefore be an important aspect in the development of light induced flavor in milk.

Nelson and Cathcart (82) reported on percent transmission versus wall thickness for two pigmented containers. Thicknesses for white

tinted specimens ranged from 13.0 to 33.1 millimeters (mils). Light transmission ranged from 29 to 10% at 800 nm to 20 to 8% at 420 nm. Below this wavelength, light transmission decreased rapidly (a sigmoidal curve) to zero. This occurred at about 400 nm. Sample specimens were opaque to the ultraviolet light.

Yellow tinted containers had wall thicknesses from 14.3 to 28.2 mils. Light transmission ranged from 30 to 21% at 800 nm and decreased to 27 to 16% at 600 nm. Below this, transmission decreased in sigmoidal fashion until it reached 7 to 1% at 490 nm. The amount of light able to penetrate the container was recorded as zero at 370 nm. Sample specimens proved to be opaque below this wavelength. Nelson and Cathcart also reported on wall thickness vs light transmission for unpigmented bottles. Thicknesses ranged from 21.9 to 13.0 mils. Light transmission ranged from 50 to 78% at 800 nm, and 40 to 62% at 350 nm. Mottar (80) and Farrer (49) also commented on the importance of wall thickness in relation to light transmission and off-flavor development. Thicker walls, therefore, provide better protection from the damaging effects of light.

As early as the 1920's experiments were conducted to change the coloration of packaging to protect milk from light. Clear flint glass was recognized as providing little protection from light. Therefore, red and amber bottles were introduced. Red colored bottles provided milk with the most protection; however, it was expensive to produce. Amber bottles also provided excellent protection but met with poor consumer acceptance.

In the 1940's, paperboard containers became widely available. These containers provided milk with much more light protection than did glass.

Today, with the popularity of plastic milk bottles, pigmented milk jugs have been introduced to prevent transmission of light into milk. Natural High Density Polyethylene is a milky to clear colorless product. However, when the crystalline compound titanium dioxide ($TiO_2$) is added to the resin, this results in a white opaque container with reduced light penetration properties (81,82). Care must be taken when mixing $TiO_2$ pigment with resin to ensure maximum dispersion, otherwise poor container appearance and a non-uniform color may result. Tests indicate that the use of pigments to tint containers seems to weaken to the point where leakage or breakage could occur (14). To eliminate this problem, dairies have to compensate by producing a heavier weight bottle. This results in a bottle containing more resin, thus increasing material costs.

Titanium based pigment also causes extra wear on blow molding equipment, because it is abrasive (14). The end result may be earlier replacement of equipment than might otherwise be planned. Pinholing and bottle blowouts also increase significantly with $TiO_2$ pigmented containers (109).

The change to a colored pigment would likewise add an additional three to four cents to bottle costs, which would ultimately be passed on to the consumer (14). White (120) conducted a survey of nearly 400 consumers. Close to 74% said they would buy milk in a colored container if it was the same price as the currently utilized translucent bottle. Only 35% indicated they would pay a three to five cent increase for the same container. The same consumers also chose 2% white $TiO_2$ containers as the popular choice over cream-colored, translucent and yellow bottles.

Lee and Harper (75) reported 3% $TiO_2$ pigmented containers afforded the same light protection as paperboard cartons, whereas 1% $TiO_2$ provided one-half the protection.

Shipe and Senyk (104) observed a reduction in the losses of riboflavin and flavor scores of 80 and 50% respectively when 5% $TiO_2$ was added to half gallon blow molded containers. The protective effect of titanium dioxides was reported as being nearly proportional to the concentration over a 1 to 10% range. Shipe et al. (105) also noted a reduction in losses of riboflavin through the addition of $TiO_2$. White (121) observed a similar protective effect and pointed out that pigmented containers afforded more protection from light activated flavor as well.

The incorporation of yellow pigments into HDPE bottles have proven very effective in allowing virtually no light transmission below 500 nanometers (nm) which eliminates the harmful blue-violet rays. These containers therefore can maintain milk quality despite prolonged exposure to fluorescent light.

Shipe and Senyk (104) examined two yellow pigments produced by the CIBA-GEIGY Corporation. The pigments blocked most of the light in the 400 to 500 nm region. Incorporation of .2% of these yellow pigments into plastic containers effectively reduced riboflavin losses and flavor scores by about 75 and 25% respectively. When combined with .2% $TiO_2$ the pigments further increased the protective ability of the containers.

Shipe et al. (105) reported on the protective effectiveness of FDA yellow No. 5 pigment. In combination with $TiO_2$ it was again shown to be an effective barrier against light induced flavor changes. When 2% $TiO_2$

was added with .2% FDA yellow No. 5, polyethylene containers displayed protective capabilities similar to those of paperboard cartons. Fanelli et al. (48) observed that the pigment FD&C yellow #5 provided excellent protection against light by absorbing critical visible and ultra-violet wavelengths, in particular those at 450 nm.

Nelson and Cathcart (82) also examined the pigmentation of polyethylene milk bottles with a yellow colorant. The yellow pigment along with $TiO_2$ reduced light transmission through the containers. For wavelengths below 500 nm, the effectiveness was almost equal to that of paperboard. However, some light in the wavelength range, 400-500 nm, was not totally blocked through pigmentation.

However, despite the good blocking power of yellow pigments, yellow containers are selected last by consumers in preference over other colored bottles, according to White (120).

In 1983, Hazelton Laboratories (13,14) conducted a study for Hoover Universal to determine the effect of fluorescent light on milk packaged in plastic containers for a period of 48 hours. Riboflavin content was reduced by 13%. To reduce possible vitamin degradation due to dairy case lighting (and to reduce consumers anxiety), Hoover Universal developed a gold-colored shield. The shield was designed to slip over the fluorescent tube and to match a 40 watt gold bug light which is the ultimate in light filtering efficiency. In several tests, the gold shield effectively protected milk from riboflavin losses and light induced flavor for 96 hours. According to the Soltex Polymer Corporation (109), "a properly selected shield is the most cost effective means of screening out harmful light rays", namely those in the

blue-violet region of the visible spectrum.

The protective ability of the shields are so effective, the Society of the Plastics Industry (SPI) has forced the Paperboard Packaging Council (PPC) to admit in their controversial advertisements that fluorescent light shields can be utilized to minimize vitamin losses due to light.

Shipe et al. (105) also examined the use of gold shields. The shields blocked most of the light below 500 nm, providing effective riboflavin protection during 48 hours of exposure. The shields also reduced flavor degradation during the first 8 hours of exposure. However, after 24 hours, flavor deterioration climbed to 40% for the shielded samples and 50% for the unshielded samples. Bradley (32) also reported that shields did not stop all radiation from penetrating milk containers.

Hansen et al. (61) observed that yellow filters protected milk from light activated flavor for up to 30-40 hours. White (121) and others (27,102,120) also suggest the use of gold shields in protecting milk from the harmful effects of fluorescent light.

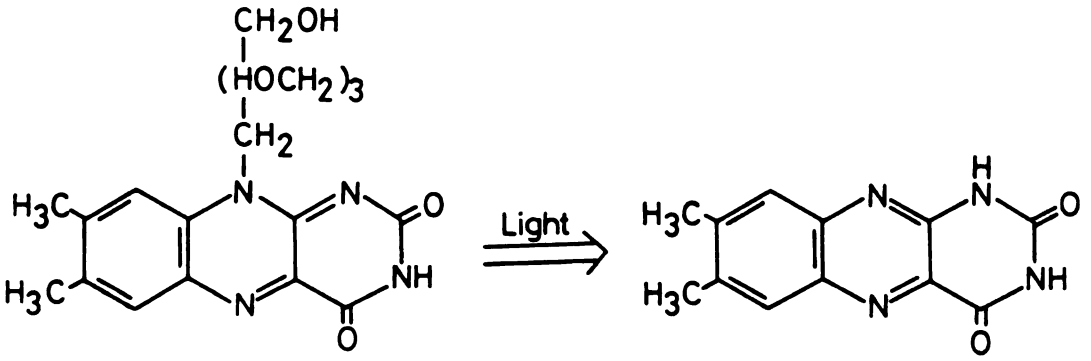
## Riboflavin

Riboflavin ($C_{17}H_{20}N_4O_6$) is a water soluble vitamin which plays a key role in energy metabolism and helps keep the skin, tongue, mouth and lips healthy. Riboflavin is also needed for growth and reproduction. Other sources of riboflavin besides milk and milk products include eggs, pork, liver, cereals, cottage cheese, poultry, noodles, pasta, green and leafy vegetables, dried beans, peas and lentils.

Riboflavin is a very sensitive vitamin and will degrade when exposed to light. According to Dimick (43) any treatment that alters or destroys its structure is detrimental. Light exposure tends to make riboflavin unstable. A gradual disappearance of nutritional quality is noted when such exposure occurs. Senyk and Shipe (102) observed that in low fat milk, light can pass more deeply into the product increasing the likelihood of riboflavin degradation.

Maniere and Dimick (78) studied the effect of fluorescent light on riboflavin in homogenized milk and in the fat, casein and acid whey isolated therefrom. 82% of the riboflavin was associated with the acid whey fraction, while 15% was connected to the casein and 3% with the fat phase. Results indicate riboflavin loss was greatest in the whey fraction. 95% of this riboflavin is in a free form and not associated with whey proteins. It is the free riboflavin that is most susceptable to fluorescent light.

Lumichrome has been indicated as one product of riboflavin breakdown. Thin-layer chromatography and exposure of skim milk to sunlight enabled Parks and Allen (84) to determine lumichrome as a photodegradation product of riboflavin. Lumichrome formation was found to be dependent on the wavelength of light, amount of exposure time, presence or absence of both oxygen and electron donors and the pH of the medium. The breakdown of riboflavin to lumichrome is shown by Cairns and Metzler (35) in the following reaction:




Riboflavin Lumichrome

Light severs the ribityl side chain ($HO\text{-}CH_2(CHOH)_3CH_2$) which then serves as an electron donor resulting in the formation of lumichrome.

A number of analytical methods are currently available for analysis of riboflavin. These include chemical, physical, microbiological and animal assays. According to Williams et al. (124) the choice of methodology usually depends upon the accuracy and sensitivity required in addition to interferences encountered in the sample.

Freed (54) and Gyorgy and Pearson (57) indicate both microbiological and animal assays are very sensitive to low levels of vitamin in food products. In addition, complex samples can be analyzed without sample clean-up. Despite these advantages, analysis times can range up to 20 plus hours. This can present a problem for many experiments. Therefore, chemical and physical techniques are often preferred methods. When applicable, they are faster and simpler than microbiological and animal assays. A variety of methods are available including colorimetric, fluorometric and spectrophotometric techniques. For these techniques, problems such as sample impurity can lead to

inaccurate measurement and laborious sample clean-up procedures.

Williams et al. (124) found that liquid chromatography has advantages over each of the other analytical approaches. These advantages include accurate or reproducible behavior, quick analysis and a minimum of sample clean-up. The authors also reported high speed ion exchange liquid chromatography has been used to separate and quantitatively analyze riboflavin. Mobile phase pH plays a key role in the amount of riboflavin retained on a strong cation exchange column. Vitamin B-2 is weakly retained in a neutral or slightly acidic pH whereas retention becomes stronger at a pH of 2 or less. Elution from the column takes less than five minutes.

Wittmer and Haney (126) utilized high speed liquid chromatography for analysis of riboflavin in multivitamin preparations. Once prepared, samples were injected into a stainless steel column packed with silicic acid. The retention time was eight minutes.

Kamman et al. (72) described a technique utilizing high performance liquid chromatography to analyze riboflavin in enriched and fortified foods. The extracted vitamin was assayed using a Waters Associates reverse phase $\mu$Bondapak $C_{18}$ column. Absorbance was monitored at 254 nm. The retention time was approximately five minutes.

Ashoor et al. (18) also described a method of riboflavin separation. Milk proteins were separated from the whey portion through acidification and centrifuging. Extracts from the whey were analyzed by High Pressure Liquid Chromatography (HPLC). The column was a Waters Associates 3.9 mm x 39 cm $\mu$Bondapak $C_{18}$ fitted with a $C_{18}$ Porasil $\beta$ guard column. The mobile phase consisted of a water-methanol-acetic acid mixture

(68:32:.1). Ultraviolet detection was at 270 nm. Retention time was 12.4 minutes. Ashoor et al. (19) also described an improved procedure used to determine the amount of riboflavin in milk and dairy products. A Waters Associates $C_{18}$ μBondapak reverse phase stainless steel column was utilized. The mobile phase consisted of a water-methanol-acetic acid mixture (65-35-0.1). The eluting riboflavin was detected with an ultraviolet absorbance detector set at 270 nm with .02 sensitivity. The μBondapak $C_{18}$ reverse phase column packing consisted of 10 μ irregular silica particles bonded with a 10% carbon load (17). The packing is also end capped or bonded to minimize the unwanted effect of Si-OH groups upon separation. This is to ensure good peak symmetry. In reverse phase columns, the packing material is non-polar while the mobile phase is a polar liquid. Here the greater the non-polarity of the sample the longer it will adhere to the column, Yost et al. (127). Highly polar (water soluble) samples like riboflavin can therefore be analyzed quite well by reverse phase chromatography.

## Light Activated Flavor

Exposing milk to light will also cause a chemical process called oxidation to occur. Oxidation has an effect on milk flavor which varies according to the type and strength of light, length of exposure time and temperature, Mottar (80). The oxidation of milk is often referred to by scientists as light activated flavor (LAF). LAF in milk occurs through the oxidation of the amino acid, methionine to methional. When milk is exposed to light for prolonged periods, LAF can occur.

The effect of light on milk products has a rich history (110). Reports in Europe as early as 1890 by Hanus (62) and later in 1907 by Burr (34) did not elucidate the exact nature of the flavor defect observed. References to this subject, in the United States, are made by Hammer and Cordes (58) in 1920 and Frazier (53) in 1928. Light induced off-flavor was brought into focus in the late 1920's and early 1930's with the advent of vitamin D fortification by ultraviolet irradiation. Drummond (46) however, sounded an ominous note in 1927 when he conjected that the benefits of irradiating milk might be outweighed by the possibilities of off-flavor development and destruction of photosensitive vitamins. The problem became more noticeable during the period when dairy products were distributed in glass containers and left on consumers doorsteps (106).

Several authors (50,56,65,100) have reported that light can induce two oxygen dependent flavors in milk; activated and oxidized. The former develops rapidly from degradation of milk proteins, while the latter develops more slowly and is attributed to lipid oxidation. Therefore, activated flavor will predominate initially while two to three days later oxidized flavor becomes more pronounced.

The use of High Density Polyethylene (HDPE) milk containers by the dairy industry has increased the incidence of light-induced flavor in milk. The translucent containers coupled with exposure to fluorescent lights in dairy cases have significantly increased the occurrence of light induced flavor in milk (22,64,77,112).

Richmond (90) and other authors (13,14,22,31,32,80,109) have observed that both light and container play important roles in the

amount of light induced flavor. Light intensity, wavelengths, distance between source and container, duration of exposure, packaging material, container thickness, amount of surface area exposed, surface to volume ratio and storage temperature all influence light activated flavor.

Several investigators (14,23,80,90,121) have suggested a variety of protective measures which include; checking the sale date, packaging the milk in brown paper bags for protection from sunlight, proper stock rotation, covering crates, filling dairy cases in the refrigerated zone only and keeping air circulation ducts unblocked.

Reduction of light induced effects can also occur through utilization of opaque or colored bottles, yellow and pink fluorescent lights, turning out lights in the dairy case and through the application of light shields.

Despite the protective measures taken by many stores, Richmond (90) reports consumers generally cannot detect a difference if comparisons are not made between exposed and protected milk.

Tracy (113) first indicated that light reacting with milk proteins caused the development of activated flavor. Doan and Meyers (45) showed that this flavor originated in casein. Keeny and Josephson (73) later confirmed these results. Flake et al. (51) reported that the protein fraction of milk was considered an active ingredient in the development of light activated flavor. Weckel and Jackson (119) indicated the same from the results of their own work as well as that of other investigators. In their review, the authors report the work of Rohr and Schultz (92,93) who found abnormal flavor associated with the effects of radiation on protein.

Patton and Josephson (86) were the first to implicate that the breakdown of the amino acid methionine to methional (3-methylthio-propanal) could cause an off-flavor to develop in milk. Patton (85) considered methional to be a product of a light induced reaction between methionine and riboflavin. Proteins in milk are considered the primary source of sunlight flavor. Of these, casein is the most important in the origin of off-flavor, because it is present in the greatest concentration, contains the highest level of methionine and is the principal factor limiting light absorption. Samuelsson and Harper (95) later confirmed the work of Patton and Josephson by demonstrating the importance of the Strecker degradation in converting methione into methional, ammonia and carbon dioxide. In addition, riboflavin and oxygen were found to play important parts in this reaction. The authors also noted the importance of methional in the development of off-flavor in milk. Hicks and Draper (65) observed light activated flavor resulting from the oxidation of the amino acid, methionine. Allen and Parks (2) reported that methional is the dominant flavor compound which develops in skim milk after exposure to direct sunlight for about ten minutes. Cohen and Ojanpera (37) reported that methional is produced from the photoreduction of methionine at pH 7.

Tada et al. (111) proposed a mechanism for the breakdown of methionine to methional. Methionine can be broken either one of two ways by the interaction of oxygen, light and riboflavin (Figure 5). Samuelsson and Harper (95) proposed that this reaction mechanism (Figure 5) follows the Strecker degradation (Figure 6). This results in the conversion of an amino acid to an aldehyde of one less carbon

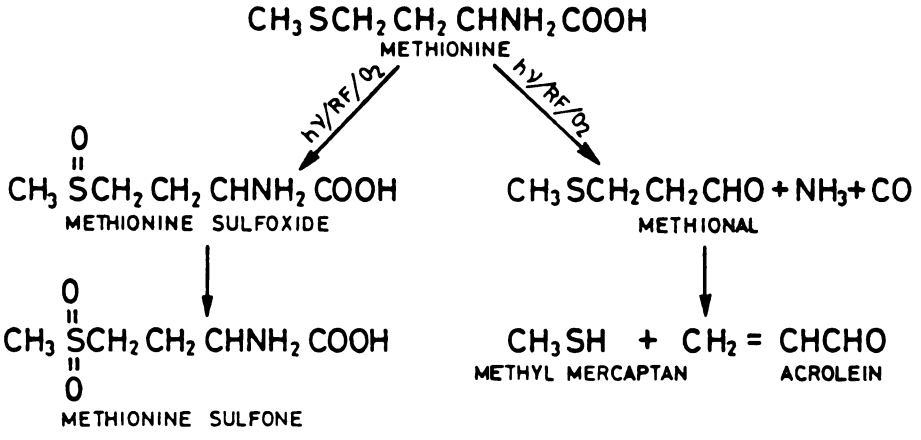



Figure 5. Photochemical Degradation of Methionine by Riboflavin. Published with permission of Dimick (43).

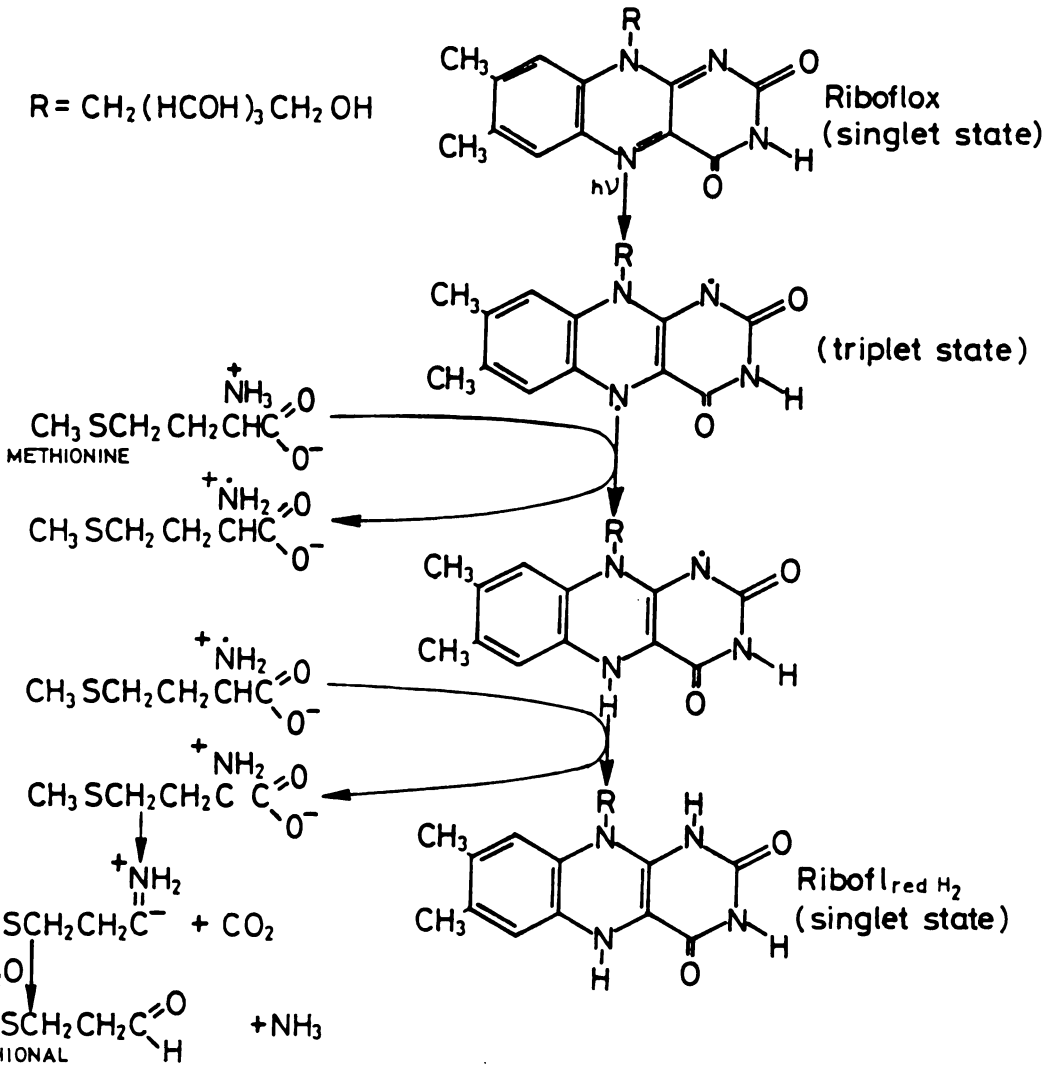



Figure 6. The Strecker Degradation Mechanism, Dimick (44). (Published with permission of The National Office of the Canadian Institute of Food Science and Technology.)

atom, carbon dioxide and ammonia. Dimick (44) stated that when riboflavin absorbs light, it becomes excited to first a singlet state and then a triplet state (Figure 6). The triplet state attracts methionine through redox reactions and is thus reduced. At this point, methionine is oxidized to methional. Patton (85) noted methional is capable of producing light-induced flavor at concentrations as low as 50 ppb. Dimick (43,44) pointed out that this compound would not develop in the absence of riboflavin.

Methional has been shown to be very important in the development of light-induced flavor in milk. However, several authors have indicated that off-flavors in milk can develop by other mechanisms. In a review of milk quality, Allen and Joseph (1) noted that riboflavin is incriminated as the primary factor responsible for light induced lipid oxidized flavor. The oxidized flavor is suggested to develop through the oxidation of unsaturated fatty acids to yield peroxides which then degrade to form carbonyl compounds. These carbonyl compounds are organoleptically detectable at levels as low as parts per billion. Thus, flavor problems can develop only after a small amount of lipid oxidation has occurred.

Dimick (43) also described the importance of carbonyl compounds in light induced flavor in milk. Formaldehyde, acetaldehyde, propanal, $C_6$-$C_{10}$ alk-2-enals, butanone, pentanone, acrolein and glyoxial are all carbonyl compounds which have been isolated from milk exposed to light.

Bassette (26) pointed out that a compound of the nonfat fraction is a precursor to acetaldehyde, while other carbonyl compounds are associated with the fat portion. The author also gave evidence which

indicated that an increase in the volatile compounds; n-pentanal and n-hexanal occurred with exposure to light.

Samuelsson (94) reported that upon irradiation, mercaptans, sulfides and disulfides are likely to contribute to the activated flavor component. Finely and Shipe (50) reported that a low density lipid-protein is a principal source of light-induced flavor in milk. The investigators found that the protein portion of the fraction appeared to undergo a partial degradation resulting in loss of tryptophan, tyrosine, lysine, cysteine and methionine. The partially-oxidized lipid portion was characterized by a decrease in oleic and linoleic acid and the production of a series of 2,4 dinitrophenylhydrazine reaction products.

Mottar (80) reviewed available literature and found several factors influence off-flavor development in milk. Two different flavors were described: a light activated flavor which develops quickly and an oxidized flavor which develops more slowly. Light activated flavor originates in the whey protein fraction of milk. Methional, formed from the amino acid methionine is the main component involved, however thiols, sulfides and disulfides also contribute to this phenomenon. Oxidation in milk begins in the phospholipid fraction. Under the influence of light and oxygen, hydroperoxides are formed from unsaturated fatty acids. The unstable condition of the peroxides give rise to secondary oxidation products such as aldehydes, ketones and shorter chain fatty acids. These products are responsible for oxidized off-flavors in milk. Fat homogenization, riboflavin and ascorbic acid were also pointed out as playing key roles in off-flavor development.

Schroder (99) concludes that light induced oxidized flavor in stored milk could be prevented by restricting access of oxygen. Lipid content of milk was found to be the source for light induced oxidized flavor. This off-flavor developed only after ascorbic acid oxidation was complete. Protection of milk from losses of nutritional value and flavor change caused by oxygen is possible if milk is packaged in an oxygen impermeable container with no headspace and protected from light. Schroder et al. (100) also noted the important role that oxygen plays in the photoreduction of riboflavin. Generally, milk is saturated with oxygen at filling. Therefore, if no additional oxygen is available, the rate of photoreduction slows or stops. However, if container permeability and headspace allow additional oxygen to enter the bottle, oxidative reactions will continue.

Aurand et al. (20,21) proposed a systematic scheme for light induced flavor in milk. From spectral absorption studies, it was found that the proteins in milk form a loosely-bound complex with riboflavin. This complex was dependent on the tryptophan found in the protein. The reaction was observed to be both oxygen and riboflavin dependent with riboflavin being the main component responsible for light induced flavor development. A summary of the reactions follow:

(1) $A + O_2 \rightarrow A - O_2$

(2) $A - O_2 + D \rightarrow (A - O_2, D)$

From 1 $\xrightarrow{hv}$ $(AO_2^-, D+)^*$

(3) $(A\ O_2^-, D+)^* \rightarrow$ "S" + $DO_2$

where A = acceptor riboflavin; D = donor (protein containing tryptophan); hv = sunlight; * = excited state; "S" = photoproduct of riboflavin; $DO_2$ = oxidized protein (sunlight flavor).

Heath (63) described several factors which can add to off-flavor development in milk. These include forage, pesticides in forage, prolonged storage and increases in storage temperature. Sunlight, processing temperatures and lipid autoxidation were also linked to off-flavor development. Thomas (112) also detailed several off-flavor problems including heat-induced, light-induced, microbially-induced, lipolyzed, oxidized and transmitted flavors. Thus, many factors can influence the flavor of milk.

## Riboflavin and Flavor Quality

Riboflavin has been reported by many researchers as a photosensitizer or catalytic agent involved in the development of light activated flavor in milk. Riboflavin has also been implicated as a photosensitizer for ascorbic acid, proteins and amino acids. Dimick (44) noted that riboflavin is destroyed by the same wavelengths of light as that producing light activated flavor. Allen and Parks (3) indicated that the photodegradation of riboflavin proceeds prior to the appearance of light induced flavor.

However, Wishner (125) indicated that riboflavin by itself was not capable of producing this off flavor upon irradiation. Bradley (31) pointed out in a review of available literature, the rate of destruction of vitamin C is proportional to the amount of light transmitted through the container, the wavelength of that energy and the presence of riboflavin. Aurand et al. (21) observed that light induced flavor is influenced by light, riboflavin, milk protein and oxygen.

Weinstein and Trout (120) reported oxidation of ascorbic acid is accelerated in the presence of riboflavin. Aurand et al. (21) showed that riboflavin was the primary factor responsible for the development of light induced oxidized flavor, whereas ascorbic acid was only a secondary factor. Hansen et al. (60) observed that decreases in riboflavin and ascorbic acid were directly proportional to the amount of light exposure. However, Dimick (43) indicated in the absence of riboflavin that the stability of ascorbic acid is maintained.

Satter and deMan (97) reported in a review that the serum proteins found in milk are the primary source of light flavor with riboflavin acting as a sensitizer. Gilmore and Dimick (55) also observed that riboflavin was necessary to catalyze the photochemical changes in milk proteins.

Singleton et al. (108) suggested a direct relationship between the disappearance of riboflavin and the amino acid tryptophan and the appearance of flavor in light exposed milk samples. Both Patton (85) and Tada (111) observed that riboflavin contributed to the conversion of methionine to methional which is also implicated as a light induced flavor found in milk.

Dimick (42) studied the effect of 100 foot candles of fluorescent light on homogenized milk exposed for 144 hours. The milk was packaged in three half gallon containers; unprinted fiberboard, blow molded plastic and clear flint glass. Results from the study show the fiberboard container protected milk from off-flavor development up to 48 hours, whereas plastic and glass bottles protected milk up to 12 hours. Riboflavin destruction in both the plastic and glass containers amounted

to 10-17% after 72 hours of exposure. No significant riboflavin losses could be detected in the fiberboard container.

Satter and deMan (96) studied the effect of fluorescent light (100 and 200 foot candles) on riboflavin and off-flavor development in homogenized whole milk. The study was conducted at 3, 6, 12 and 24 hour intervals. Four packaging materials were used: a clear and opaque polyethylene pouch, a paperboard carton, and a plastic returnable jug. Results from the study indicate that off-flavor development and a significant loss in riboflavin was detected in all the containers except the opaque pouch.

Hansen et al. (60,61) reported on the effect of 200 foot candles of fluorescent light on homogenized milk packaged in polyethylene containers. Off-flavor development and riboflavin loss occurred after two and twelve hours of exposure respectively.

Singh et al. (107) evaluated riboflavin degradation in milk stored in various container types under different lighting conditions. Four types of one gallon containers were utilized in the evaluation: blow molded polyethylene, gold-pigmented blow molded polyethylene, paperboard and glass. Riboflavin losses after 48 hours exposure to 300 foot candles of fluorescent light was about 11% for the glass and blow molded polyethylene containers and 3% for the paperboard and gold-pigmented polyethylene containers. Some loss occurred at 150 foot candles while no significant losses occurred in the dark.

Henrick and Glass (64) examined milk packaged in paperboard and blow molded plastic containers exposed to 150 foot candles of fluorescent light for (a) 5 hours, (b) 10 hours plus 14 hours in the dark,

(c) 24 hours plus 9 days in the dark and (d) 10 days in the dark only. Riboflavin losses were noted after 10 and 24 hours of exposure to fluorescent light. Loss of riboflavin in milk was substantially less in milk packaged in paperboard than the plastic containers.

Senyk and Shipe (101,102) exposed whole, 2%, 1% and skim milk to 186 foot candles of fluorescent light at various time intervals up to 24 hours. Loss of riboflavin amounted to 8, 10, 11 and 14% after 24 hours. The results also showed that paperboard and gold-tinted containers provided the best protection against fluorescent light.

Lee and Harper (75) exposed homogenized pasteurized whole milk to 200 foot candles of fluorescent light. Riboflavin losses of 12-18% were reported after 24 hours for milk stored in plain plastic and glass. Levey (76) reported that riboflavin losses up to 14% were noted for milk packaged in plastic containers exposed to fluorescent light within 24 hours.

Bradley (31) summarized available literature on light-activated flavor development in milk packaged in glass, polycarbonate, high density polyethylene, blow molded polyethylene, plastic bags and paperboard containers. Paperboard containers offered the most protection while the other containers afforded limited protection at best.

Shield (103) studied light activated flavor development in milk packaged in half gallon blow-molded polyethylene bottles and quart polyethylene coated paperboard cartons. The milk was exposed to fluorescent light ranging from 8 to over 3000 foot candles.

Light activated flavor was detected in the polyethylene bottles after 12 hours of exposure to high intensity lighting and at 36 hours

under low intensity lighting. The paperboard cartons showed only a slight activated flavor development after 96 hours under high intensity lighting. At low intensity lighting the milk did not develop a light activated flavor through 96 hours of exposure.

Satter and deMan (97) in a review of available literature also noted the key role fluorescent light plays in the development of off-flavor and riboflavin destruction in milk. However, Hoover Universal (14) position was that when testing is done under real life dairy case conditions, riboflavin losses are minimal. Gregory et al. (56) and Farrer (49) also observed riboflavin degradation and off-flavor development in milk exposed to fluorescent light.

## Taste Panel Surveys

Hansen, Turner and Aurand (60,61) assembled a four-member expert panel trained to identify light induced flavor in milk which had been exposed to fluorescent light in intervals up to 72 hours. Taste panel detection of the off-flavor after exposure were: 2 to 4 hours - very slight; 4 hours - slight; 7 hours - moderate; and more than 24 hours, strong.

Hoskin and Dimick (70) exposed milk in High Density Polyethylene containers to 100 foot-candles of fluorescent light for intervals up to 72 hours. A light-induced flavor was detected by a trained panel after 12 hours of exposure. For the 12-member taste panel conducted by Coleman, Watrous and Dimick (38), samples were evaluated for light induced flavor for up to 144 hours of exposure time. Results indicate

that milk packaged in blow-molded containers decreased in flavor quality after 12 hours of exposure. Hankin and Dillman (59) examined milk taken from retail outlets. They found 33% of milk packaged in polyethylene containers had a light-induced flavor. Reif, Franke and Bruhn (89) arbitrarily collected samples from dairy cases and found 45% of the samples packaged in plastic had developed a light-induced flavor. Barnard and Foley (24) also reported on the flavor quality of milk. Nearly 50% of the milk purchased in plastic gallon and half gallon containers had objectionable light-induced flavors.

Barnard (22) examined more than 1600 samples of milk for light-induced flavor. A trained three-member judging panel found an average of 51% of the samples tested over a 4-year period ranked good to excellent in flavor quality. However a decrease was noted in the percentage of good to excellent samples as blow-molded containers became more prevalent.

White and Bulthaus (123) conducted a taste panel analysis to determine light activated flavor in whole and 2% milk packaged in plastic jugs. Results from the study indicated that 63% of the consumers preferred milk with no off-flavor, 27% preferred the light activated flavor and 10% had no preference. Consumers 25 years and younger were the most successful in detecting the difference between the milks. An expert panel was also used to determine the frequency and severity of light-activated flavor in 90 milk samples. 59% of the samples were rated as having a moderate to strong off-flavor.

Bray, Duthie and Rogers (33) surveyed 2,000 consumers to determine their taste preference for samples of high quality milk and milk with

light-induced flavor. Homogenized milk was packaged in High Density Polyethylene containers and subjected to 400 foot candles of fluorescent light for 40 hours. Over 73% of the people detected off-flavors in the exposed milk. Also, more females than males could taste a difference between the two samples. From the data, the authors suggest prevention of light-induced flavor in milk is very important to the dairy industry.

## Display Time and Light Exposure

Bradfield and Duthie (30) observed that 10% of the half gallon and 20% of the quart containers remained in dairy cases after 20 hours of display. In a 1974 study conducted by Market Facts - New York (4), Bradley (31) reported that 105 retail milk outlets were examined to determine turnover of milk. The survey found that regardless of container size or type, 71% of the milk remained in the dairy case for approximately 5 hours. After 8 hours, 58% was unsold, and after 24 hours, 37% still remained in the cabinets. The survey examined 58,973 time marked containers in 6 cities. Within each city 15 retail outlets were studied. Light intensity varied widely from one dairy case to another.

Bradfield and Duthie (28) observed fluorescent light intensities of 20-500 foot candles with a major portion in the 300-400 foot candle range. Satter and deMan (96) noted that emissions varied from 25-500 foot candles with intensities of 100-200 foot candles most prevalent. Dimick (44) reported on a survey conducted by Market - Facts, New York (4). In the study, an average of 186 foot-candles for 105 retail milk outlets was observed. deMan (40) also noted that light intensities

varied considerably. In a survey conducted in the Toronto area, light intensities ranged from 50-511 foot candles with many between 93-279 foot candles.

## MATERIALS AND METHODS

### Container Wall Thickness

Two samples were taken from the sidewalls of three bottles for each container type. Sample thickness measurements were made with a Testing Machines Inc., Model 549M Micrometer (Testing Machines, Inc., Amityville, New York 11701). Two measurements were made for each sample.

### Transmission Studies

A Perkin-Elmer Lambda 3B UV/VIS Spectrophotometer (The Perkin-Elmer Corporation, Oak Brook, Illinois 60521) was utilized to determine the percent light transmission of the milk bottles in the visible light region. Two samples approximately 1 inch x 1 inch were taken from the sidewalls of individual containers. A total of twelve containers (24 samples) were analyzed; three from each bottle category. Readings were made in intervals of 50 nanometers. The wavelengths studied ranged from 300 to 800 nm. This UV spectrophotometer is unique in that it has an integrating sphere attachment. The attachment is especially made to permit transmittance measurements on turbid samples like the High Density Polyethylene bottles.

## Store Surveys

A survey was conducted of several food store dairy cases to help determine the parameters in the experimental study. These initial results are summarized in Appendix 1.

An important factor contributing to loss of riboflavin and light activated flavor in milk is the length of time it is exposed to fluorescent light. The survey indicated a two day turnover rate for milk. During this two day period fluorescent lights were illuminated for an average of 26 hours. Milk remained on the shelf for approximately 10 hours. After a review of the data, it was decided to expand the length of light exposure from 10 to 24 hours to make for a more thorough investigation.

In this survey spectral emissions from the fluorescent lights varied considerably as was pointed out in the literature. However, the average intensity was a little lower than indicated by other researchers. Light intensities ranged from a low of 1 foot candle to a high of 480 foot candles. Averages for the dairy cases ranged from 37.8 to 155 foot candles.

From these surveys a light intensity for the experimental study was obtained. An intensity of 100 foot candles was chosen because it represented an approximate average of light intensities divided by the number of readings.

## Statistical Analysis

A split plot analysis of variance design was chosen because the samples were subject to treatment combinations of two or more factors and then measured at several sampling intervals. A split plot also separates experimental random error into variation among and within the samples tested. Without a proper statistical analysis differences in treatment means and trends over time can be greatly misleading. The split plot design utilizing four replicates was chosen so that each main effect and all interactions could be tested. The analysis follows.

### Parameters

Light types: unshielded and shielded fluorescent light

Container types: control, quart, half-gallon, gallon, pigmented gallon

Exposure times: 0, 5, 10 and 24 hours

### Split Plot Design

| Source of Variation | Degrees of Freedom |
|---|---|
| Light types (2) | 1 |
| Container types (5) | 4 |
| Light by container interaction | 4 |
| Error a | 30 |
| Exposure times (4) | 3 |
| Exposure by light types | 3 |
| Exposure by container types | 12 |
| Exposure by container by light | 12 |
| Error b | 90 |
| | 159 |
| | n=160 |

Therefore a total of 160 measurements were necessary to have statistically reliable data. This breaks down to four samples for each exposure time with four containers per cell (Table 2).

## Equipment

The High Pressure Liquid Chromatography (HPLC) apparatus included a Waters Associates Model 730 data module, M-45 solvent delivery system, U6K universal liquid chromatograph injector and a Model 441 absorbance detector (Waters Associates, Milford, Massachusetts, 01757). The column was a Waters Associates 3.9 mm x 30 μBondapak $C_{18}$ stainless steel column. Waters Associates $C_{18}$ Guard-Pak Precolumn Inserts were also utilized to remove sample particulate matter that could cause column damage and reduce its efficiency. A Branson Bransonic 221 Sonicator (Branson Cleaning Equipment Company, Shelten, Connecticut, 06484) was used to de-gas and uniformly mix the mobile phase before connection to the system. This process removes dissolved gases which could affect the solvent delivery system as well as column efficiency and life.

The flow rate of the HPLC system was set at 1 ml/minute. A 50 μl Hamilton syringe (Hamilton Company, Reno, Nevada, 89510) was used to inject 30 μl sample volumes into the system.

## Riboflavin Standard Solution

One milligram of riboflavin standard (anhydrous, Sigma Chemical Co., St. Louis, Missouri, 63178) was accurately weighed with a Mettler Analytical Balance; Model AE 160 (Mettler Instrument Corp., Hightstown, New Jersey, 08520. The standard was then dissolved by stirring into 900

Table 2

Statistical Table for the Split-plot Design Involving the Components from the Experimental Study

| | Control | Quart | 1/2 Gallon | Gallon | Pigmented Gallon |
|---|---|---|---|---|---|
| Unshielded light | 0,5,10,24 | 0,5,10,24 | 0,5,10,24 | 0,5,10,24 | 0,5,10,24 |
| Shielded light | 0,5,10,24 | 0,5,10,24 | 0,5,10,24 | 0,5,10,24 | 0,5,10,24 |

milliliters (ml) acetate buffer solution in a one liter volumetric flask wrapped with aluminum foil (Appendix 2). Once dissolved, an additional 100 ml of acetate buffer was added to dilute the solution to volume. The solution was again mixed well and refrigerated until needed.

A calibration curve was made to determine accuracy and repeatability of the High Pressure Liquid Chromatography (HPLC) system. To obtain this curve for riboflavin a small sample of the standard solution was taken from the refrigerator and placed in a foil wrapped beaker. It was then allowed to warm to room temperature for approximately one half hour.

Different volumes of riboflavin standard were then injected and corresponding peak areas obtained. Duplicate runs were made for each particular volume. The average area was then taken and plotted against this volume. A table of the average areas and a graph of the curve are shown on the following pages (Table 3 and Figure 7). System error is within five percent.

A recovery study was conducted to determine the percentage of riboflavin retained, once it had passed through the HPLC system. Equal volumes of three solutions (extracted milk, standard and extracted milk + standard) were prepared and analyzed in 30 microliter (µl) amounts.

| Solutions | Total Amount |
|---|---|
| Milk - 5 ml extracted milk plus 15 ml water | 20 ml |
| Standard - 15 ml standard plus 5 ml water | 20 ml |
| Milk + standard - 5 ml extracted milk plus 15 ml standard | 20 ml |

Table 3

Volumes and Peak Areas for the Standard Calibration Curve

| Volume | Area | Average |
|---|---|---|
| 30 µL | 2346.80<br>2317.05 | 2331.92 |
| 25 µL | 1917.21<br>1930.70 | 1923.95 |
| 20 µL | 1547.64<br>1533.40 | 1540.52 |
| 10 µL | 795.60<br>773.52 | 784.56 |
| 5 µL | 409.29<br>405.24 | 407.26 |
| 2.5 µL | 190.48<br>200.97 | 195.73 |

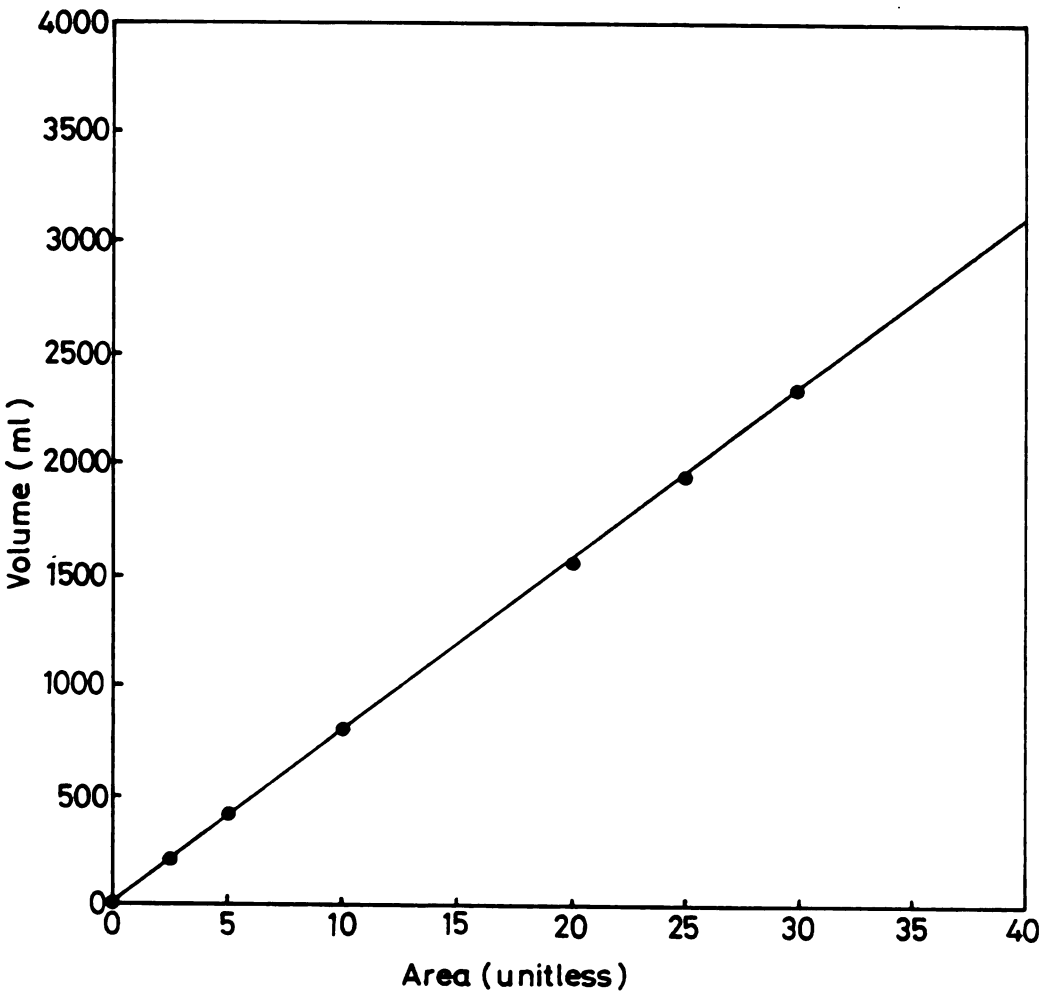



Figure 7. Calibration Curve for Riboflavin Standard.

Recovery Study #1

| | Milk | Standard | Milk & Standard |
|---|---|---|---|
| | 636.96 | 3438.51 | 4123.37 |
| | 632.72 | 3466.84 | 4161.77 |
| Average: | 634.84 | 3452.68 | 4142.57 |

Recovery Study #2

| | Milk | Standard | Milk & Standard |
|---|---|---|---|
| | 635.62 | 3658.59 | 4279.89 |
| | 600.82 | 3709.97 | 4444.22 |
| | 618.22 | 3684.28 | 4362.06 |

(area numbers from riboflavin peaks)

Calculations

Recovery Study #1

3452.68 + 634.84 = 4087.52

4087.52/4142.77 = 98.67

Recovery Study #2

3684.28 + 618.22 = 4302.50

4302.50/4362.06 = 98.63

Average: 98.65

As the above results indicate, recoveries were excellent. If 100% recovery were possible, both the extracted milk and standard samples would equal the two combined. However, due to experimental error and equipment precision, the amount recovered will fall short of that mark. Duplicate runs were made to ensure repeatability.

## Initial Conditions and Set-up

Fresh 2% lowfat milk was transferred from a local Lansing dairy in corrugated containers and immediately placed in a walk-in refrigerator (Chrysler & Koppin Company, Detroit, Michigan, 48238) set at 5.6$^{o}$C ± 1$^{o}$C. Milk was packaged in High Density Polyethylene (HDPE) quarts, half gallons, gallons and yellow pigmented gallons. Both the quarts and yellow pigmented gallons required milk to be transferred from other containers, since production with these two bottles was not available.

The statistical design demonstrated that each container would be examined as a set of four. To simplify the study, only a single set of containers was investigated at any one time. A fifth container served as a control. It was foil-wrapped and placed in a covered corrugated box to prevent degradation from light exposure. The other four bottles were placed in a simulated dairy case (Figure 8). Two 40-watt cool white fluorescent lights were set directly above the milk and adjusted for intensity using a Gossen Panlux Electronic Light Meter (Gossen GMBH, West Germany). The milk was subjected to an intensity of 100 foot-candles under unshielded light and 90 foot-candles under shielded light. This reduction is a result of the yellow light produced by the shield. Samples were taken out of the containers at 0, 5, 10 and 24 hours and placed in foil wrapped beakers. They were then transferred to the laboratory for analysis.

The entire experimental procedure was conducted under yellow lighting to minimize riboflavin loss. The shielded light utilized for the dairy case model was provided by two yellow pigmented tube shields

Figure 8. Simulated Dairy Case Utilized during the Experimental Study.

(McGill Manufacturing Company, Valparaiso, Indiana, 46383). The shields slip over the fluorescent light and reduce light intensity from 100 to 90 foot candles. Laboratory analysis was executed under yellow bug lights. The temperature of the walk-in refrigerator was closely monitored during the testing period. Readings from a centigrade thermometer (Wilkens-Anderson Company, Chicago, Illinois, 60651) were made before samples were removed for analysis. Fluctuations of only $\pm 1^{o}C$ were noted during the study.

An Omega Model 450 ATT Thermocouple thermometer type T (Omega Engineering Inc., Stamford, Connecticut, 06987) was utilized to measure the surface temperature of five one gallon milk containers over a period of 48 hours. Four samples were exposed to 100 foot candles of fluorescent light, while the remaining bottle was kept in the dark and served as a control. Surface temperature readings were taken at 0, 5, 10, 24 and 48 hours.

Data collection indicated that no difference existed between the refrigerator temperature and the surface temperature of the containers exposed to the light. Therefore, heat can be eliminated as a factor influencing riboflavin loss.

The yellow pigmented containers were obtained from Purity Dairy, Nashville, Tennessee, 37210. The yellow pigment is a combination of titanium dioxide and FD and C yellow #5. Formation of gallon containers involves mixing 96% High Density Polyethylene and 4% yellow colorant.

## Extraction of Riboflavin from Milk Samples

Riboflavin was extracted from 2% milk by the method of Ashoor et al. (19). In this procedure, milk samples taken from the walk-in refrigerator were allowed to warm to room temperature for approximately one-half hour. A Waters Associates $C_{18}$ Sep-Pak was then connected to a ten milliliter (ml) glass syringe with a Luer-Lok tip (Beckon-Dickinson and Company, Rutherford, New Jersey, 07070). Five milliliters of methanol, followed by five milliliters water were next passed through the Sep-Pak to activate it. Upon completion, ten milliliters of warmed milk sample was pipetted into the syringe and filtered through the Sep-Pak. The Sep-Pak was then washed twice with ten milliliters of water. Finally, ten milliliters of eluting solution was passed through the Sep-Pak and the eluate was received in a foil-wrapped vial. An advantage of this procedure is that milk proteins are retained on the $C_{18}$ Sep-Pak cartridge. The eluate is therefore practically free of contaminants.

## Reagents Utilized During the Study

Water - Distilled

Methanol - HPLC Grade

Mobile Phase - Water - Methanol - Acetic Acid (65-35-01)

Acetate Buffer - .2 M, pH 4.0

Eluting Solution - A 1:1 mixture of acetic buffer and methanol

## Summary of Experimental Conditions

Container Types: High Density Polyethylene quarts, half gallons, gallons and yellow pigmented gallons

Exposure Times: 0, 5, 10, 24 hours

Light: Two 40 watt cool fluorescent lights, 48 inches long

Light Intensity: 100 foot candles

Milk Type: 2% lowfat

Shield Color: Yellow

Temperature: $5.6^{o}C \pm 1^{o}C$

## Sensory Evaluation

Sensory evaluation was conducted to determine if a semi-trained panel could differentiate between various levels of light activated flavor from milk samples exposed to both shielded and unshielded fluorescent light. A panel of nine subjects was assembled for the analysis. Training of panel members lasted two weeks.

During the first week subjects were given samples with known levels of light activated flavor. The samples consisted of four gallons of 2% milk subjected to 240 foot candles of fluorescent light. One gallon was removed from the light at each one of these time intervals: 0, 5, 10 and 24 hours. The sample at 0 hours was used as the control.

Levels of light activated flavor were next assigned to the samples before being given to panel members. The levels assigned were indicated as follows:

| Hours | Level |
|---|---|
| 0 | None-trace |
| 5 | Moderate |
| 10 | Extreme #1 |
| 24 | Extreme #2 |

Two training sessions were conducted during the initial week. Because of the results obtained, two panel members were dismissed from further testing.

The same conditions were utilized for the second week of training. However, milk samples were given to panel members without classifying the level of off flavor. Two sessions were conducted during the final week of training. All panel members were able to distinguish between levels of activated flavor with some variability. Once training was completed actual testing commenced.

Sampling conditions were similar to those conducted during the training sessions. 2% milk packaged in quarts, half gallons, gallons and pigmented gallons were exposed to 100 foot candles of unshielded fluorescent light and 90 foot candles of shielded light. During each testing session, four bottles of one container type were subjected to the lighting conditions. One container was removed from the light at each of the following time intervals: 0, 5, 10 and 24 hours. Sensory evaluation lasted for four weeks with two sessions conducted per week.

At the end of each testing period, panel members were asked to determine the degree of light activated flavor for each sample at the different time intervals. The Scoring Method for sensory evaluations was utilized to make these determinations. This method involves panel

members recording their judgements on a graduated scale. Responses ranged from "no light activated flavor" to "extreme light activated flavor." An example of the scoring sheet is shown in Appendix 3. Space for additional comments on flavor quality was also provided.

## RESULTS AND DISCUSSION

### Container Wall Thickness

Container wall thickness plays an important role in loss of riboflavin and development of light induced flavor in milk. Generally, thicker walled containers provide better protection from the effects of light (82). Table 4 shows the results for this part of the study.

Test data indicated results similar to those of Nelson and Cathcart (82). Thicknesses for their containers ranged from 14.3 to 28.3 mils for the yellow pigmented bottles and 13.0 to 21.9 mils for the unpigmented ones. The only container tested that was outside this range was the unpigmented half gallon with an average thickness of 23.6 mils. Thicknesses can vary between containers due to the different blow molding processes.

### Transmission Studies

The amount of light able to penetrate a milk container is obviously very important. Loss of riboflavin and off-flavor development depend on how much radiant energy reaches the milk. In particular, those wavelengths in the blue-violet range of the visible spectrum are considered most critical (56,82,109). Table 5 shows the spectrophotometer results for the transmission study.

Readings obtained in this study indicate some variance from the literature. Percent transmission tended to be higher for both the

Table 4

Sidewall Thicknesses for High Density Polyethylene Bottles (in micrometers, mils)

| Container Type | Sample 1 | Sample 2 | Combined Average | Overall Average |
|---|---|---|---|---|
| Quart - 1 | 22.7 | 15.7 | 19.2 | |
| Quart - 2 | 18.0 | 23.5 | 20.8 | 20.5 |
| Quart - 3 | 24.3 | 18.8 | 21.5 | |
| Half Gallon - 1 | 28.0 | 24.3 | 26.1 | |
| Half Gallon - 2 | 24.2 | 22.5 | 23.3 | 23.6 |
| Half Gallon - 3 | 21.8 | 20.8 | 21.9 | |
| Gallon - 1 | 21.4 | 20.5 | 20.9 | |
| Gallon - 2 | 20.6 | 20.3 | 20.5 | 20.0 |
| Gallon - 3 | 20.7 | 16.8 | 18.7 | |
| Pigmented Gallon-1 | 18.8 | 22.3 | 20.5 | |
| Pigmented Gallon-2 | 19.1 | 19.9 | 19.5 | 19.6 |
| Pigmented Gallon-3 | 17.6 | 19.8 | 18.7 | |

Table 5

Percent Transmission of Unpigmented and Pigmented High-Density Polyethylene Bottles

| Container Type | Wavelength Range (nm) | % Transmission |
|---|---|---|
| Quart | 700 - 800 | 94 - 100 |
| | 600 - 700 | 90 - 94 |
| | 500 - 600 | 86 - 90 |
| | 400 - 500 | 80 - 86 |
| | 300 - 400 | 58 - 80 |
| Half-Gallon | 700 - 800 | 93 - 100 |
| | 600 - 700 | 89 - 93 |
| | 500 - 600 | 85 - 89 |
| | 400 - 500 | 78 - 85 |
| | 300 - 400 | 52 - 78 |
| Gallon | 700 - 800 | 94 - 100 |
| | 600 - 700 | 90 - 94 |
| | 500 - 600 | 85 - 90 |
| | 400 - 500 | 79 - 85 |
| | 300 - 400 | 64 - 79 |
| Yellow-Pigmented Container | 700 - 800 | 85 - 100 |
| | 600 - 700 | 72 - 85 |
| | 550 - 600 | 63 - 72 |
| | 500 - 550 | 8 - 63 |
| | 400 - 500 | <1 - 8 |
| | 300 - 400 | 0 - <1 |

unpigmented and pigmented bottles at the upper end of the spectrum. Toward the lower end, the unpigmented samples also tended to be high when compared to the literature. However, percent transmission for the yellow pigmented samples, in particular those below 500 nm were in agreement with Nelson and Cathcart's research. Possible explanations to the differences obtained could range from resin distribution, the blow molding process, container design, material make-up and differences between instruments used to measure light transmission.

## Chromatography Results

To approximate retention time for riboflavin, samples of standard were injected into the HPLC system. An average eluting time of 7.8 minutes was observed (Figure 9).

Samples from extracted milk were then injected. Peak areas at approximately the same time as that from the standard riboflavin solution confirmed the procedure (Figure 10).

Retention times for riboflavin were shorter than those reported by Ashoor et al. (19). The shorter time could have resulted from equipment or column differences in this study.

The unknown peaks at 3.54 and 5.41 minutes (Figure 10) may be products of the metabolic breakdown of riboflavin (39). A number of end products have been suggested which could be responsible for these unknown peaks. Cairns and Metzler (35) observed several photoproducts as a result of riboflavin degradation. These included carboxymethyflavin, formylmethylflavin, lumiflavin and lumichrome. Dimick (44) noted similar findings. Parks and Allen (84) and Treadwell and Metzler

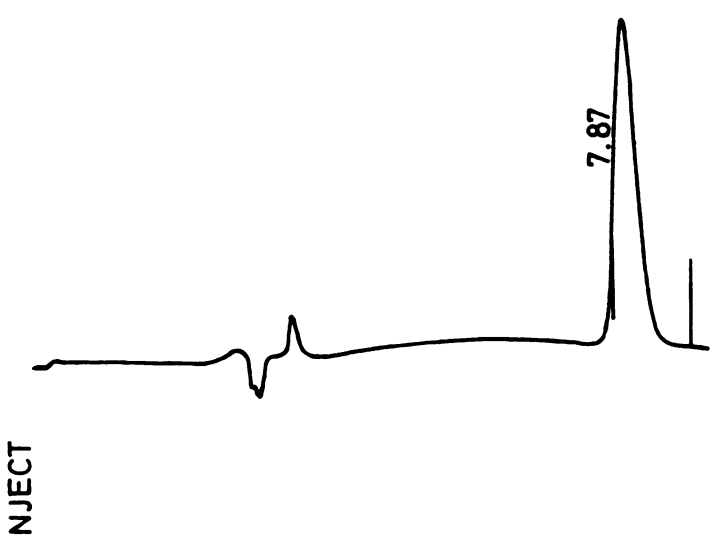



Figure 9. Chromatographic Peak for the Riboflavin Standard Solution.

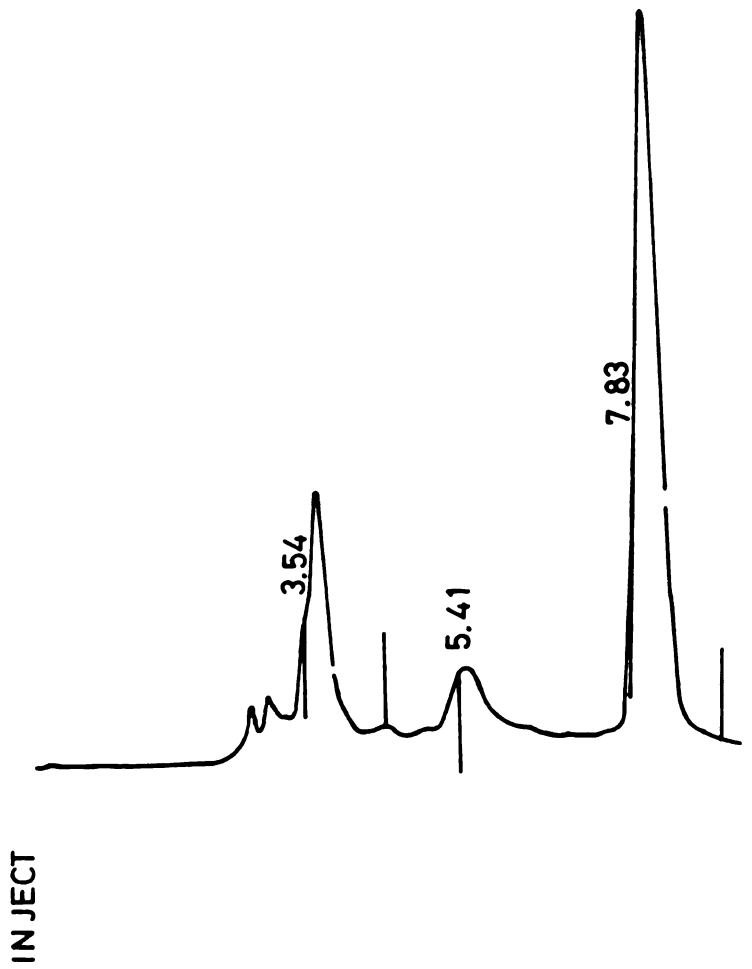



Figure 10. Chromatographic Peak for the Extracted Milk Sample.

(104) demonstrated that lumichrome is produced from light induced degradation of riboflavin. The riboflavin standard (Figure 9) also contained an unknown compound eluting at approximately 3.50 minutes.

## Experimental Results for Riboflavin

The susceptibility of riboflavin to fluorescent light is well documented. Researchers have utilized various techniques to determine the amount of riboflavin loss from milk through such exposure. In this experimental study a model was set up to simulate an "in-store" dairy case (Figure 8). Results should represent vitamin losses expected under actual conditions.

A minimum of two injections were made for each riboflavin sample. Duplicate analysis were made to ensure consistent results. If results varied considerably, a third injection was made. These were averaged and combined with the other three sample averages to get an overall mean for each time period (Table 6). The difference between each mean and the original concentration was then expressed as a percent of riboflavin loss for that time period (Table 7).

Foil wrapped controls were also analyzed for riboflavin degradation under the above conditions. The samples did not change significantly over 24 hours. Comparisons between the controls and the zero hour samples indicate similar results.

Data in Table 6 shows initial riboflavin concentrations for milk of equal freshness to vary widely. Fanelli et al. (48) and Rivlin (91) observed similar findings. Fanelli et al. (48) noted changes in the cows diet as a contributing factor to these fluctuations, while Rivlin (91)

Table 6

Riboflavin Content of Milk Stored in Several Packages
Over a 24 hour Period

| Package | Control | 0 Hrs | 5 Hrs | 10 Hrs | 24 Hrs |
|---|---|---|---|---|---|
| | Unshielded Light-Riboflavin (mg/liter) | | | | |
| Quarts | 2.21 | 2.24 | 2.18 | 2.11 | 2.09 |
| Half Gallons | 2.28 | 2.31 | 2.21 | 2.19 | 2.11 |
| Gallons | 2.15 | 2.13 | 2.15 | 2.12 | 2.06 |
| Pigmented Gallons | 2.09 | 2.05 | 2.08 | 2.03 | 2.05 |
| | Shielded Light-Riboflavin (mg/liter) | | | | |
| Quarts | 2.10 | 2.12 | 2.09 | 2.03 | 2.06 |
| Half Gallons | 2.15 | 2.19 | 2.20 | 2.10 | 2.11 |
| Gallons | 2.10 | 2.10 | 2.11 | 2.11 | 2.03 |
| Pigmented Gallons | 1.99 | 2.02 | 2.02 | 2.03 | 2.06 |

Table 7

Percent Loss of Riboflavin During a 24 hour Period from Milk Stored in Several Packages

| Package | 0 Hours | 5 Hours | 10 Hours | 24 Hours |
|---|---|---|---|---|
| | | Unshielded Light (%) | | |
| Quarts | - | 2.68 | 5.80 | 6.70 |
| Half Gallons | - | 4.33 | 5.19 | 8.66 |
| Gallons | - | 0.00 | 0.47 | 3.29 |
| Pigmented Gallons | - | 0.00 | 0.00 | 0.00 |
| | | Shielded Light (%) | | |
| Quarts | - | 1.42 | 4.25 | 4.25 |
| Half Gallons | - | 0.00 | 4.11 | 4.11 |
| Gallons | - | 0.00 | 0.00 | 3.33 |
| Pigmented Gallons | - | 0.00 | 0.00 | 0.00 |

indicated pasteurization causes slight losses of riboflavin content. Variations in daily production and processing of milk may also contribute to these changes.

Data in Table 7 indicate only a small decline in riboflavin between the shielded and unshielded light. The largest change occurred in the smaller two bottles. This point was brought out by Farrer (49) who indicated that small volume/area ratios are more prone to degradation than the larger ones. The reduced ratios indicate a smaller amount of milk is present to "dilute" the light. Senyk and Shipe (102) also noted light can pass more deeply into milk with a low fat content. Riboflavin degradation is therefore more likely with 2% milk vs. whole fat milk when packaged in smaller size containers. Table 7 also shows that similar percentages were obtained for the gallon containers under both lighting conditions. A large volume/area ratio could explain these results.

When milk is subjected to fluorescent light exposure, the riboflavin content degrades over time. Table 7 shows losses of 6.70%, 8.66% and 3.29% for the quarts, half gallons and gallons respectively. These losses occurred after a 24 hour period. Hedrick and Glass (64) observed riboflavin degradation ranging from 3.78 to 10% for several experiments involving blow molded bottles. Dimick (42) studied the effect of 100 foot candles of fluorescent light on homogenized milk packaged in several different containers. Losses ranged from 10 to 17% after 72 hours of exposure. Lee and Harper (75) exposed whole milk to 200 foot candles of fluorescent light for 24 hours. Losses of riboflavin ranged from 12 to 18% for milk stored in plain plastic and glass. Singh et al. (107) observed a loss of 11% when milk was subjected to 300 foot candles

of fluorescent light packaged in blow molded containers for 48 hours. Senyk and Shipe (102) examined whole, 2%, 1% and skim milk exposed to 186 foot candles of fluorescent light. Riboflavin losses after 24 hours were 8, 10, 11 and 14% respectively.

In comparison to the literature, results from the experimental study show lower riboflavin losses. Most of the differences can be attributed to the higher light intensities. However, exposure time and positioning of the light source against the container face also play key roles. The experimental study coupled with the literature review, thus point out that riboflavin losses are expected from fluorescent light exposure.

Loss of riboflavin from unshielded fluorescent light exposure can be reduced through utilization of yellow colored tube shields. The protective capabilities of the shields were demonstrated in this work. A reduction in riboflavin of 2.45 to 4.55% was observed for both quarts and half gallons respectively in 24 hours. Hazelton Laboratories (14) also found that loss of riboflavin in milk was reduced by shielding fluorescent light. Shipe et al. (105) reported similar results.

The average number of hours milk sits on the store shelf was found to be ten (Appendix 1). Riboflavin losses after this time period did not significantly differ from those at 24 hours under shielded light (Table 7). Therefore, riboflavin degradation may reach a point in which further losses are practically non-existent when such protection is utilized. These results give additional support to the protective capabilities of tube shields.

Incorporation of yellow pigment into High Density Polyethylene bottles prevents fluorescent light from reaching milk. In a 24-hour

period, the riboflavin content did not change in any yellow pigmented container under either unshielded or shielded light. The protection provided by these bottles was also observed by other researchers.

Shipe and Senyk (104) noted that the yellow coloring added to plastic bottles effectively reduced riboflavin loss by 75%. Shipe et al. (105) observed that pigmented containers displayed protective capabilities similar to paperboard. Fanelli et al. (48) and Nelson and Cathcart (82) also reported the effectiveness of yellow pigmented containers in reducing riboflavin losses. Thus, under the conditions of this study, the yellow pigmentation completely prohibited loss of riboflavin.

Examination of riboflavin in the control and zero hour samples indicate that some variation exists between these initial amounts (Table 6). These variations may account for some of the riboflavin loss found during the study. Therefore, actual riboflavin losses may be slightly less than had originally been reported. Fanelli et al. (48) and Rivlin (91) indicate that such variation exists between these initial amounts.

Half gallon bottles were also exposed to high intensity fluorescent light to determine if an increase in riboflavin losses occurred. Samples were exposed to 200 foot candles of unshielded light and 170 foot candles of shielded light. A reduction in light intensity is noted due to shield utilization.

Loss of riboflavin over 24 hours amount to 10.73% and 5.85% for unshielded and shielded light respectively. These results were very similar to those in Table 7. Only a couple of percentage points separate these results, indicating that riboflavin deterioration may be similar

under different light intensities. The shields again provided excellent protection, allowing only a small portion of the riboflavin to degrade. The amount of riboflavin deterioration at 10 hours (5.37%) under shielded light was also approximately the same as that at 24 hours. This gives additional evidence that riboflavin loss reaches a point in which further degradation is practically absent under such protection.

In this study, a correlation was implicated between the photodegradation of riboflavin and the development of light activated flavor in milk. In most instances riboflavin acted as a catalysis resulting in the development of off-flavor in milk (Figure 11). This point was brought out by many researchers (85,97,120). The gallon bottles showed very little riboflavin loss (up to ten hours) in comparison with the amount of activated flavor development. This suggests that a relatively minor change in riboflavin content may cause the initiation of off-flavor in milk. Hansen et al. (61) noted that riboflavin was not destroyed in the formation of light-induced flavor. It also indicates that additional factors may cause the development of off-flavors in milk (106,112).

Loss of riboflavin exceeded the amount of flavor development for a few samples under the shielded light which indicates that shielding may interrupt the role riboflavin plays in the initiation of light activated flavor or that other factors may be involved in flavor changes (Figure 12). Thus, riboflavin can be a photosensitizer and play a secondary role in the development of light activated flavor in milk.

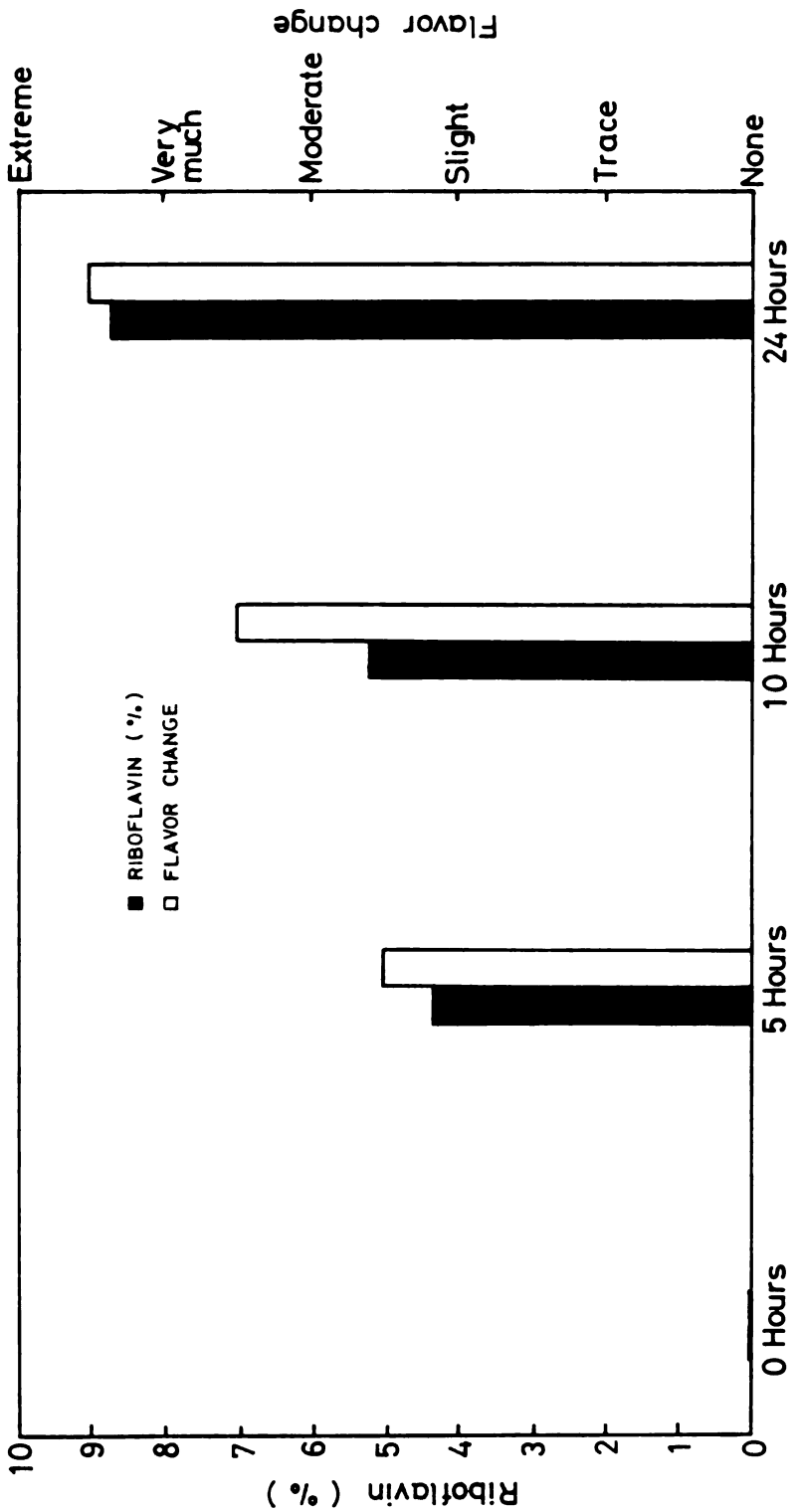



Figure 11. Riboflavin Loss and Activated Flavor Development for Half Gallon Milk Samples Exposed to Unshielded Fluorescent Light.

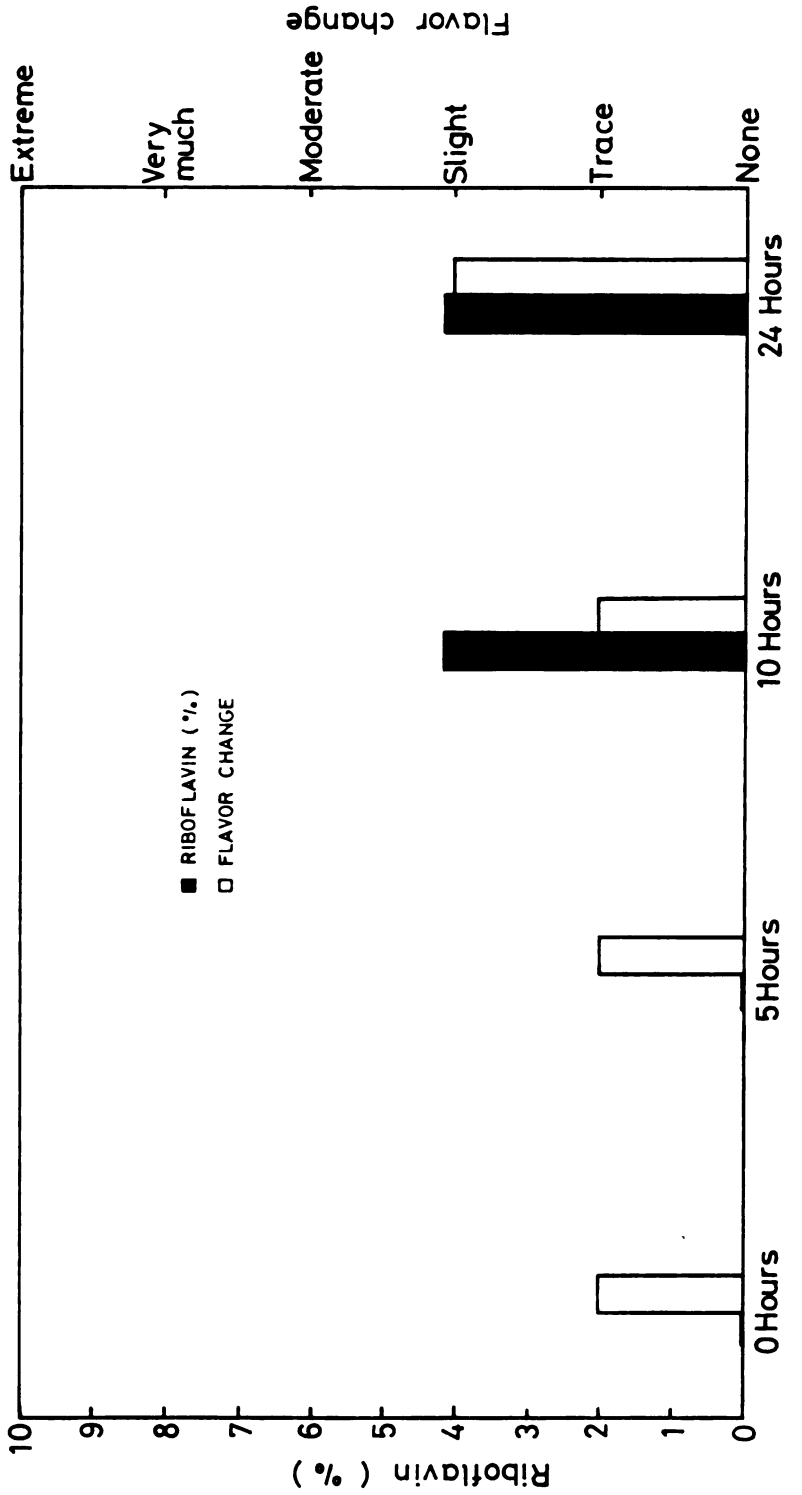



Figure 12. Riboflavin Loss and Activated Flavor Development for Half Gallon Milk Samples Exposed to Shielded Light.

In addition to the laboratory studies, actual "in-store" samples were obtained and analyzed for riboflavin loss. The study was conducted over a two-day period. Half-gallon containers exposed to both unshielded and shielded fluorescent light were obtained during each of these days. A total of eight samples were analyzed, two samples per day for each lighting condition. Both dairy cases were open displays with each fluorescent light parallel to the container tops. The milk bottles went several rows deep from their respective light sources. Average intensities were 91 and 89 foot candles for the unshielded and shielded fluorescent lights respectively.

Freshly produced milk, with the same code date as the store samples was used as a control. The control was produced five to six days before being stocked on store shelves. It was also analyzed for riboflavin content the same day it was produced. Store samples were then compared against the control to note any significant changes. A summary of the outcome is indicated in Table 8. The data in Table 8 show that loss of riboflavin under the shielded light was practically none, while those samples exposed to unshielded light experienced minimal loss of riboflavin. This is an indication that the milk was not on the shelf long enough to suffer significant loss of riboflavin due to fluorescent lighting. These results can be confirmed with the statistical analysis. During this study, the light by container type by exposure time interaction indicated that riboflavin loss would not be significant when subjected to these conditions. The "in-store" studies, thus give evidence for these results.

Table 8

Comparison of "In-store" Samples over a Two Day with a Freshly Produced Control

| Day | Sample | Riboflavin (mg/liter) | Percent Change |
|---|---|---|---|
| 1 | Control | 1.97 | - |
| 2 | Shielded Light | 1.96 | .51% |
| 3 | Shielded Light | 1.97 | 0% |
| 4 | Unshielded Light | 1.95 | 1.02% |
| 5 | Unshielded Light | 1.92 | 2.54% |

## Statistical Analysis

The photodegradation of riboflavin over time was subjected to a split-plot statistical program. The Manova application from Nie and Hull (83) and a Michigan State University Computer Laboratory Bulletin (79) determined the significance of interaction between light, container types and exposure times. The computer program and raw data file are presented in Appendices 4 and 5. Significance of the parameters are shown in Table 9.

In the above table the "F" and critical values help determine if the source parameters are significant at the specified level (.05). If the F-value is greater than this critical value, the source parameters are considered to have an important effect in the experimental study and thus on the riboflavin content of milk. In Table 9, exposure time by container type by light is the most important parameter from the split plot design. These three parameters were the influencing factors effecting riboflavin degradation during the study. This interaction determines if riboflavin loss is an important nutritional concern and therefore warrants further investigation. Results from the study indicate that riboflavin degradation is not significant when subjected to this set of parameters.

## Kinetics Study

An analysis was also conducted to determine first order kinetics for riboflavin degradation over time. A reaction of the first order is one in which the concentration of a substance at a given time is proportional

Table 9

A Manova Table Application to Determine if the Critical Value Indicates Source Parameters are Significant at the Specified Level (.05)

| Source | df | Sum of Squares | Mean Squares | F value | Critical Value (.05) |
|---|---|---|---|---|---|
| Light (2) | 1 | .12826 | .12826 | 16.44 | 4.17 |
| Container (5) | 4 | .31398 | .07850 | 10.06 | 2.69 |
| Light by Container | 4 | .03316 | .00829 | 1.06 | 2.69 |
| Error 1 | 30 | .23392 | .00780 | | |
| Exposures (4) | 3 | .11892 | .03964 | 12.27 | 2.72 |
| Exposure by Light | 3 | .00872 | .00291 | .90 | 2.72 |
| Exposure by Container | 12 | .10698 | .00892 | 2.76 | 1.88 |
| Exposure by Container by Light | 12 | .05310 | .00443 | 1.37 | 1.88 |
| Residual | 90 | .39071 | .00323 | | |

to the rate of disappearance of that reacting substance. This is represented by the following equation:

$$-\frac{dc}{dt} = kc$$

where c = riboflavin concentration

t = time

k = first order rate constant

Results from the quarts, half gallons and gallons were utilized to determine first order kinetics. Riboflavin levels in yellow pigmented containers showed no significant changes in riboflavin degradation and were therefore excluded from further analysis.

Riboflavin decreased over time from exposure to fluorescent light (Table 6). This data was subjected to linear regression analysis (Tables 10 and 11).

Plots of these points (time vs concentration) were made through application of the Spectra-Physics SP 4200 computing integrator (Spectra-Physics Autolab Division, San Jose, California, 95134). A "best-fit" system is employed among the data points (Figure 13).

The graphs suggest photodegradation of riboflavin over time follows first order kinetics. Other authors have also indicated the importance of first order kinetics in riboflavin degradation. Singh et al. (107) and Satter et al. (98) reported similar results. The authors found that losses of riboflavin could be described by first order kinetics. It was also indicated that riboflavin loss increased with a rise in storage temperature.

Table 10

Linear Regression Analysis ($\ln R_n/Ro$) for Riboflavin Concentration ($*10^{-2}$ mg/liter) Under Unshielded Fluorescent Light

| Hours | Quarts | Half Gallons | Gallons |
|---|---|---|---|
| 0 | 0 | 0 | 0 |
| 5 | -.0272 | -.0354 | 0 |
| 10 | -.0598 | -.0490 | -.0047 |
| 24 | -.0693 | -.0862 | -.0334 |

Table 11

Linear Regression Analysis ($\ln R_n/Ro$) for Riboflavin Concentration ($*10^{-2}$ mg/liter) Under Shielded Fluorescent Light

| Hours | Quarts | Half Gallons | Gallons |
|---|---|---|---|
| 0 | 0 | 0 | 0 |
| 5 | -.0143 | 0 | 0 |
| 10 | -.0434 | -.0420 | 0 |
| 24 | -.0434 | -.0372 | -.0339 |

Ro = 0 hours

Rn = 5, 10 and 24 hours

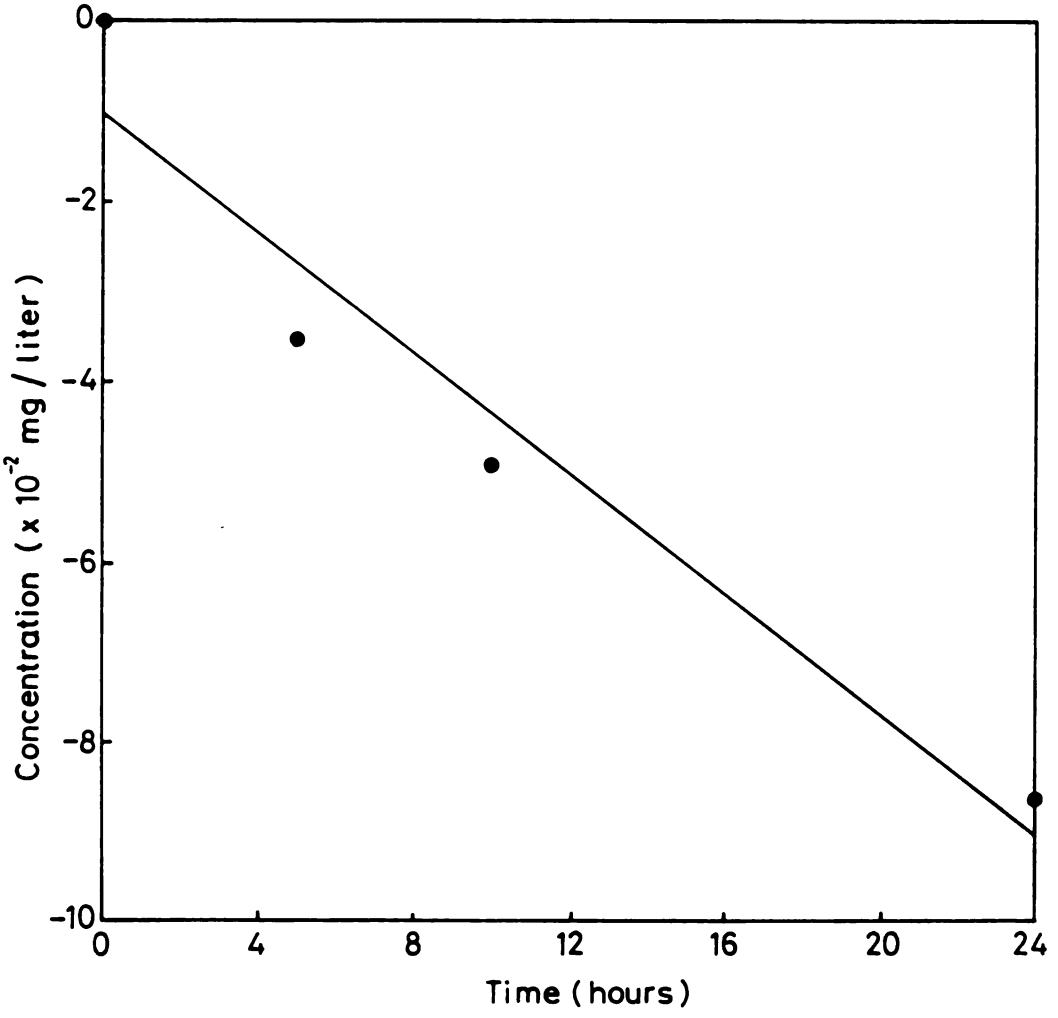



Figure 13. A Typical Plot Showing the Degradation of Riboflavin Concentration over Time Through First Order Kinetics and Linear Regression Analysis.

Table 12

Volume/Area Ratios for the High Density Polyethylene Containers

| Container | Volume (ml) | Area ($cm^2$) | Volume/Area ($ml/cm^2$) |
|---|---|---|---|
| Quart | 946 | 637 | 1.49 |
| Half Gallon | 1892 | 930 | 2.03 |
| Gallon | 3784 | 1397 | 2.71 |

Table 13

K ($hours^{-1}$) Values for the High Density Polyethylene Containers

| Container | Unshielded Light | Shielded Light |
|---|---|---|
| Quart | .00270 | .00175 |
| Half Gallon | .00334 | .00168 |
| Gallon | .00150 | .00150 |

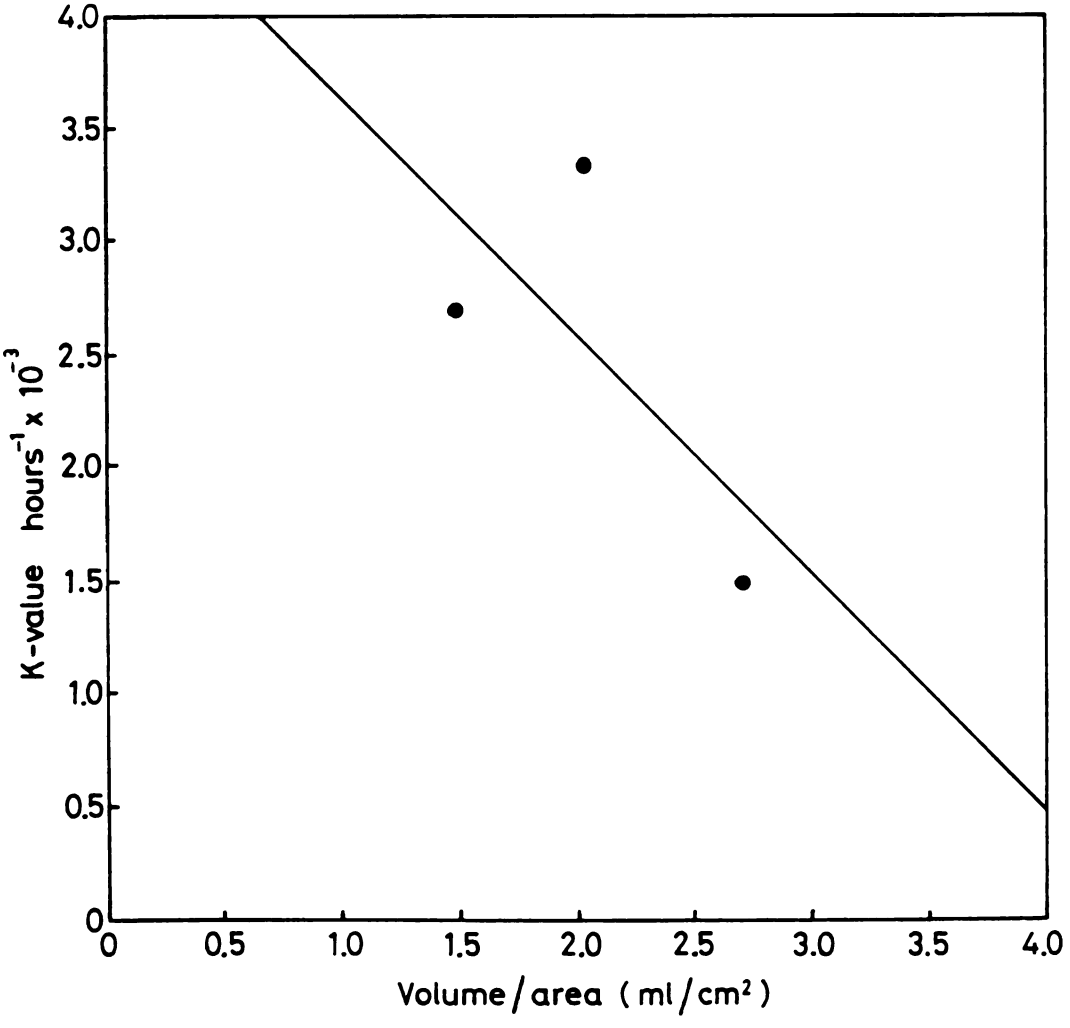



Figure 14. Rate Constant vs. Volume/Area Plot for High Density Polyethylene Containers Exposed to 100 Foot Candles of Unshielded Fluorescent Light.

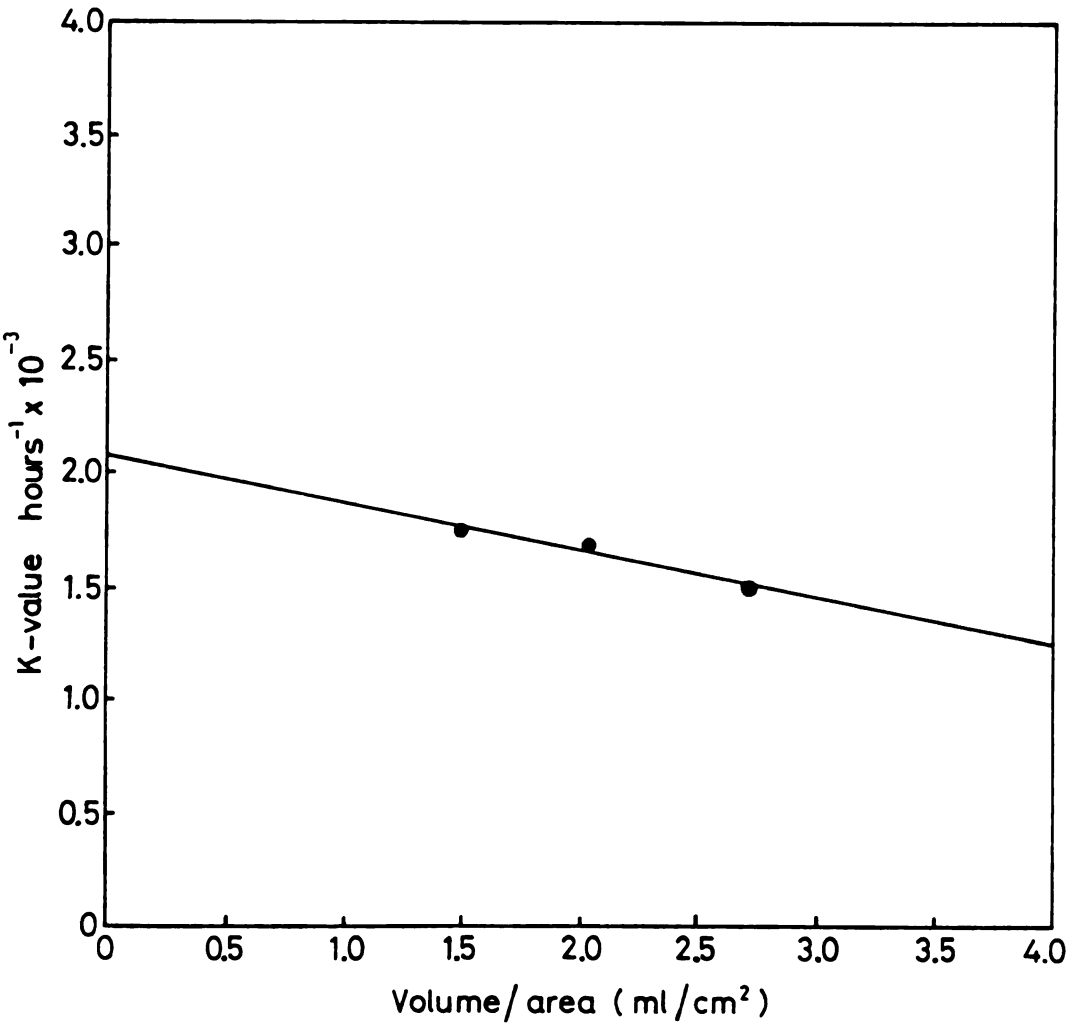



Figure 15. Rate Constant vs. Volume/Area Plot for High Density Polyethylene Containers Exposed to 90 Foot Candles of Shielded Fluorescent Light.

Results drawn from this study show that first order kinetics can be assumed, but more data is needed to solidify this conclusion.

The rate constant k (hours$^{-1}$) obtained from the Spectra-Physics integrator was then plotted against volume/area. The volume, area, volume/area and k-value are presented in Tables 12 and 13. The graphs are shown in Figures 14 and 15. From these results, a decreasing volume/area ratio corresponds to an increased rate constant. Riboflavin therefore degrades quicker in quart containers than in half gallons and faster in half gallon bottles than in gallons. This point is brought out by Farrer (49) who indicates small volume/area ratios are more prone to degradation than larger ones. The reason being that a smaller amount of milk is present to "dilute" the light. Fluckiger (52), Bradley (31) and Mottar (80) have also reported on the disadvantage of small volume/area ratios.

Conclusions drawn from the kinetics study indicate agreement with the literature. Combination of the rate constant and volume/area ratios clearly bring this out.

## Sensory Evaluation

Consumer acceptance for milk is largely influenced by its flavor. According to Thomas (112) fresh normal milk as produced should have a pleasing slightly sweet flavor, little aroma, and a pleasant mouthfeel and aftertaste.

Biologically produced, milks flavor is affected by genetic and a number of other factors from production to consumption. These deviations in flavor can be sensed by the consuming public.

In this study, panel members marked coded milk samples on a graduated scale designed to determine the degree of light activated flavor (Appendix 3). Responses were then assigned numerical values (Appendix 6). These values were then recorded as the scores for the samples under study. Larmond (74) notes from the scores, variation between samples is evident. The degree of light activated flavor for each set of samples and perception of that flavor by panel members was determined to be significant or non-significant through a statistical analysis at the .05 level (Appendix 6). Results from the statistical design indicate perception of light activated flavor in milk between the panelists was not significant. However, activated flavor among the samples varied under given experimental conditions (Tables 14, 15 and 16).

Many researchers have observed light activated flavor in milk. Coleman (38), Hansen et al. (61) and Hoskin and Dimick (70) noted significant off-flavor development from fluorescent light exposure. Data in Tables 14-16 indicate samples varied considerably between the two light sources. Light activated flavor was less evident under shielded light than unshielded light. The shields protect milk from critical wavelengths, namely those in the blue-violet region of the visible spectrum.

Only the quarts and gallons experienced a significant flavor change over 24 hours (Table 16). However, the half gallon containers did not develop a significant degree of light activated flavor over the 24 hour time period. This may be due to slight changes in milk quality during the experimental study. For all of the containers under the shielded light, flavor changes did not occur until after the milk had been

Table 14

Degree of Light Activated Flavor for Each Sample Subjected to Both Unshielded and Shielded Fluorescent Light

| Container | 0 Hours | 5 Hours | 10 Hours | 24 Hours |
|---|---|---|---|---|
| **Unshielded Fluorescent Light** | | | | |
| Quarts | none | slight | moderate | very much |
| Half-Gallons | none | slight/ moderate | moderate/ very much | very much/ extreme |
| Gallons | trace | trace | moderate | moderate |
| Pigmented Gallons | none | trace | trace | slight |
| **Shielded Fluorescent Light** | | | | |
| Quarts | trace | trace | slight | moderate |
| Half-Gallons | trace | trace | trace | slight |
| Gallons | trace | trace/ slight | slight | moderate |
| Pigmented Gallons | none/ trace | trace | trace | slight |

Table 15

Milk Samples Indicating a Significant Difference in Light Activated Flavor from Exposure to Unshielded Fluorescent Light

| Container | Significant Flavor Change Over 24 Hours | Samples Denoting a Difference in Flavor (Hrs) |
|---|---|---|
| Quarts | Yes | 24 and 0 |
| | | 24 and 5 |
| | | 10 and 0 |
| Half-Gallons | Yes | 24 and 0 |
| | | 24 and 5 |
| | | 24 and 10 |
| | | 10 and 0 |
| | | 10 and 5 |
| | | 0 and 5 |
| Gallons | Yes | 24 and 0 |
| | | 24 and 5 |
| | | 10 and 0 |
| | | 10 and 5 |
| Pigmented Gallons | Yes | 24 and 0 |

Table 16

Milk Samples Indicating a Significant Difference in Light Activated Flavor from Exposure to Shielded Fluorescent Light

| Container | Significant Flavor Change Over 24 Hours | Samples Denoting a Difference in Flavor (Hours) |
|---|---|---|
| Quarts | Yes | 24 and 0 |
| | | 24 and 5 |
| Half Gallons | No | - |
| Gallons | Yes | 24 and 0 |
| Pigmented Gallons | No | - |

exposed for 24 hours.

The literature also notes the importance of shields. Shipe et al. (105) reported fluorescent light shields reduced flavor degradation during the first 8 hours of exposure. Bradley (32) and Hansen et al. (60,61) also noted the protective capabilities of shields.

The yellow pigmented containers also provided excellent protection against light activated flavor development. The only significant change occurred under unshielded light. A slight variation in flavor was noted between the samples at 0 and 24 hours. This indicates that flavor changes do occur with pigmented containers. However, these changes are slight and occur only after prolonged exposure.

Shipe and Senyk (104) reported on the effectiveness of yellow pigmented High Density Polyethylene (HDPE) bottles. Shipe et al. (105) also observed yellow pigmented containers to be an effective barrier against light induced flavor changes. The yellow colored containers, like the tube shields provide protection for milk by blocking out harmful fluorescent light.

Milk in the translucent HDPE bottles exposed to unshielded fluorescent light had noticeable flavor change during the study. Significant variations were observed for all containers over the 24 hour period. Differences in flavor quality for these containers were also noted after 10 hours of exposure. This suggests that the store display time for milk of 10 hours (Appendix 1) will also develop a light induced flavor.

Variations in activated flavor development over the 24 hour period could result from sensory evaluations being conducted over several weeks. Since fresh milk was obtained for each taste panel session, slight

variations in production and processing may have occurred to influence results. Comments by panelists indicate fluctuations in flavor quality during the testing period. Judgements by the panelists may have also influenced results. Depending on personnel circumstances, preception of activated flavor may have varied for any one day of testing.

## Conclusions

The current controversy between the manufacturers of plastic and paperboard milk containers will continue. Whether it is a case of economics, protection or convenience each producer is convinced of the superiority of their own container. This experimental study was unique in that High Density Polyethylene bottles were exposed to shielded and unshielded fluorescent light in both the laboratory and store dairy cases.

Riboflavin losses were greater in the quarts and half gallons than in the gallons. Degradation also tended to be slower under shielded than unshielded light. The riboflavin content of the milk in the yellow pigmented containers did not change under either lighting condition. Similar results were observed during the taste panel analysis. Light activated flavor developed more slowly under shielded light.

The protection provided by the tube shields and pigmented containers is evident. Their ability to block out harmful fluorescent light namely those wavelengths in the blue-violet region of the visible spectrum is shown by the reduction in vitamin degradation and light induced flavor over time.

In the absence of other protection, loss of riboflavin in unpigmented containers was minimal under unshielded fluorescent light. The "in-store" studies confirmed the laboratory work in showing that

riboflavin losses in milk are small when exposed to both shielded and unshielded fluorescent light.

Even though some losses did occur from light exposure, riboflavin can be found in many other foods we consume to meet recommended daily allowances. Stores can also be more responsible by providing adequate protection for milk through tube shields, pigmented containers, turning off dairy case lighting and consumer education.

Therefore, claims by the paperboard companies that High Density Polyethylene is not an adequate package for milk should not be considered valid, until all advantages and disadvantages of the container are considered.

## Recommendations for Future Research

Recommendations for future research include pooling milk before running any experimental tests or taste panels. Pooling of milk involves mixing approximately 15-20 gallons of freshly produced milk in a single container under subdued lighting conditions. This ensures that initial riboflavin amounts for all containers are similar. However, all testing would have to be conducted within the limits of the code date for reliable results.

Examination of the containers under both shielded and unshielded light at higher light intensities would determine if similar amounts of riboflavin are lost over the same time period. In addition, exposing milk to these intensities would give further support to the protective capabilities of the shields and pigmented containers.

An in-depth "in store" survey would provide a better idea of the display life of milk. The study encompasses marking newly stocked containers in a dairy case and determining how many bottles remain after a specified time. Comparisons could then be made with the calculations determined from turnover times (Appendix 1).

Another study could be conducted to determine if delivery from the dairy to the store has any effect on milk quality. Items that could be investigated include the length of time milk sits on the delivery dock or in the store's cooler before being stocked in the dairy case.

It would also be interesting to compare different milk types with 2% milk under the same experimental conditions.

# APPENDICES

# APPENDIX 1

Store Parameters Utilized as Part of the Experimental Study

A survey was taken of several food store dairy cases to help determine the parameters utilized in this study. These results were then included in the experimental procedure.

The light type used in each store was a 40 watt cool white fluorescent bulb.

A Bi-Temperature Probe (Taylor Instrument, Adren, North Carolina, 28704) was placed in various parts of the dairy cases to obtain an average reading for the research work. An average temperature of 5.6$^{o}$C was found in the survey.

A Gossen Panlux electronic light meter (Gossen GMBH, West Germany) was utilized to determine light intensities of the dairy case surveyed. To obtain an accurate measurement, the meter was placed next to the milk samples and then pointed directly at the light source. An average of 91 foot candles was observed. The results of the survey are indicated below.

Light Intensity Readings (in foot candles)

| Store | Number of Readings | Percent of Total | Minimum Intensity | Maximum Intensity | Average Intensity | Adjusted Average to Percent Value |
|---|---|---|---|---|---|---|
| A | 10 | 6.7% | 9 | 125 | 50.5 | 90.9 |
| B | 20 | 13.4% | 4 | 155 | 70.1 | 90.7 |
| C | 21 | 14.1% | 1.9 | 152 | 62.6 | 90.7 |
| D | 23 | 15.4% | 1 | 300 | 37.8 | 90.7 |
| E | 23 | 15.4% | 50 | 310 | 155.3 | 91.1 |
| F | 52 | 35.0% | 40 | 480 | 113.1 | 90.7 |

$$\text{Overall Average} \sim \frac{\text{Total Light Intensities}}{\text{Number of Readings}} = 13540.3/149 = 90.8 \simeq 91 \text{ foot candles}$$

The adjusted average relates to the number of readings taken at each store to the overall total. Surprisingly, all the stores averaged about

the same light intensity.

The average number of hours each milk bottle spent in the dairy case was approximately 10 hours. The data and calculations follow. The number of hours dairy case lights were on, the turnover rate and the gallons sold per week were obtained through personal communications with store managers. The rest of the data was acquired by observations and calculations.

| Store | Number of Hours Dairy Case Lights on/Week | Turnover Rate Gallons/ 2 Days | Gallons Sold Per Week | Gallons Per Hour | Number of Gallons/ Shelf |
|---|---|---|---|---|---|
| 1 | 93 | 480 | 1440 | 15.5 | 96 |
| 2 | 87 | 96 | 288 | 3.3 | 48 |
| 3 | 96 | 340 | 1020 | 10.6 | 85 |

Example Calculations for Store 1:

Number of Hours Dairy Case Lights are on Per Week: 93 hours/week

Store Turnover Rate: 480 gallons/2 days

Number of Gallons Sold Per Week:

480 gallons x 3 delivery days = 1440 gallons/week

Number of Gallons Sold Per Hour:

$$\frac{1440 \text{ gallons/week}}{93 \text{ hours/week}} = 15.5 \text{ gallons/hour}$$

Amount of Time Milk is Exposed to Light/Shelf

$$\frac{96 \text{ gallons/shelf}}{15.5 \text{ gallons/hour}} = 6.19 \text{ hours/shelf}$$

| Store | Number of Hours Milk is Exposed to Light per Shelf |
|---|---|
| 1 | 6.19 hours |
| 2 | 14.55 hours |
| 3 | 8.01 hours |
| Average | 9.6 ∿ 10 hours |

# APPENDIX 2

## Acetate-Buffer Composition

A .2 M, pH 4 acetate buffer* was prepared and used for the standard and eluting solutions. The buffer was prepared as follows:

| Percentage | Solution Mixture |
|---|---|
| 50 | Distilled water |
| 41 | 11.5 ml glacial acetic acid in 1000 ml distilled water |
| 09 | 27.2 grams sodium acetate ($C_2H_3O_2Na.3\ H_2O$) in 1000 ml distilled water |

To ensure a pH of 4 ± 5%, the final mixture was measured with an Orion Research Model 301 Analog pH meter (Orion Research Incorporated, Cambridge, Massachusetts, 02139).

*Walpole, G.S., Journal of the Chemical Society. 1914. 105:2501.

# APPENDIX 3

Evaluation of Light Activated Flavor in Milk

Date _____ Name _____

Please evaluate the milk samples for any light activated flavor. Indicate the amount of off-flavor in each sample on the scales below. Please feel free to comment on the flavor quality of each sample (i.e. oxidized, metallic, bitter, feed etc.) in the space provided. Thank you for your participation.

| Sample No. _____ | Sample No. _____ | Sample No. _____ |
|---|---|---|
| ____ none | ____ none | ____ none |
| ____ trace | ____ trace | ____ trace |
| ____ slight | ____ slight | ____ slight |
| ____ moderate | ____ moderate | ____ moderate |
| ____ very much | ____ very much | ____ very much |
| ____ extreme | ____ extreme | ____ extreme |

| Sample No. _____ | Sample No. _____ | Sample No. _____ |
|---|---|---|
| ____ none | ____ none | ____ none |
| ____ trace | ____ trace | ____ trace |
| ____ slight | ____ slight | ____ slight |
| ____ moderate | ____ moderate | ____ moderate |
| ____ very much | ____ very much | ____ very much |
| ____ extreme | ____ extreme | ____ extreme |

| Sample No. | Comments |
|---|---|
| | |
| | |
| | |
| | |
| | |
| | |

# APPENDIX 4

Statistical Program Utilized for the Experimental Study

Data on riboflavin from the photodegradation study was statistically analyzed. The split-plot design from Nie and Hull (83) and a Michigan State University Computer Laboratory Bulletin (79) was used to determine the significance of interaction between light, container types, exposure times and riboflavin amounts (Appendix 5). This information was then entered into a file and the following Manova program was utilized.

```
*JOBCARD*, CM250000, JC1000, RG2, L100.
Attach, Data, FCOdatamilklight
Hal, SPSS9, D=Data.
*EOS
Variable List   Light Carton Bottle Expose Ribos.
N of Cases      Unknown
Input Format    Fixed (F1.0,1X,F1.0,1X,F1.0,1X,F1.0,1X,F4.2)
Manova          Ribos By Light (1,2), Carton (1,5), Bottle (1,4),
                Expose (1,4)/
                Design = Light, Carton, Light by Carton vs 1,
                Bottle Within Light By Carton = 1, Expose,
                Expose By Light, Expose By Carton,
                Expose By Light By Carton/
```

# APPENDIX 5

## Data File for the Statistical Problem

Data for the split plot program was entered into the computer under the following format:

| | Column 1 | Column 3 | Column 5 | Column 7 | Column 9-12 |
|---|---|---|---|---|---|
| Line | Light | Carton | Bottle | Expose | Ribos |
| 1 | 1 | 1 | 1 | 1 | 2.26 |

Key:

Line: Data Points 1 - 160

Light: 1 - Unshielded Light

2 - Shielded Light

Carton: 1 - Quarts

2 - Half Gallons

3 - Gallons

4 - Pigmented Gallons

5 - Foil Wrapped Controls

Bottle: Represents the container under study during the 24 hour period.

Expose: 1 - 0 hours

2 - 5 hours

3 - 10 hours

4 - 24 hours

Ribos: Riboflavin amounts from extracted milk samples.

The complete data file is represented on the next few pages.

| Line | Column 1 Light | Column 3 Carton Type | Column 5 Bottle | Column 7 Exposure Time | Column 9-12 Riboflavin |
|---|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1 | 2.26 |
| 2 | 1 | 1 | 1 | 2 | 2.20 |
| 3 | 1 | 1 | 1 | 3 | 2.13 |
| 4 | 1 | 1 | 1 | 4 | 2.07 |
| 5 | 1 | 1 | 2 | 1 | 2.23 |
| 6 | 1 | 1 | 2 | 2 | 2.16 |
| 7 | 1 | 1 | 2 | 3 | 2.10 |
| 8 | 1 | 1 | 2 | 4 | 2.10 |
| 9 | 1 | 1 | 3 | 1 | 2.26 |
| 10 | 1 | 1 | 3 | 2 | 2.22 |
| 11 | 1 | 1 | 3 | 3 | 2.07 |
| 12 | 1 | 1 | 3 | 4 | 2.12 |
| 13 | 1 | 1 | 4 | 1 | 2.20 |
| 14 | 1 | 1 | 4 | 2 | 2.15 |
| 15 | 1 | 1 | 4 | 3 | 2.13 |
| 16 | 1 | 1 | 4 | 4 | 2.05 |
| 17 | 1 | 2 | 1 | 1 | 2.29 |
| 18 | 1 | 2 | 1 | 2 | 2.26 |
| 19 | 1 | 2 | 1 | 3 | 2.26 |
| 20 | 1 | 2 | 1 | 4 | 2.13 |
| 21 | 1 | 2 | 2 | 1 | 2.30 |
| 22 | 1 | 2 | 2 | 2 | 2.24 |
| 23 | 1 | 2 | 2 | 3 | 2.20 |
| 24 | 1 | 2 | 2 | 4 | 2.11 |
| 25 | 1 | 2 | 3 | 1 | 2.32 |
| 26 | 1 | 2 | 3 | 2 | 2.18 |
| 27 | 1 | 2 | 3 | 3 | 2.18 |
| 28 | 1 | 2 | 3 | 4 | 2.11 |
| 29 | 1 | 2 | 4 | 1 | 2.32 |
| 30 | 1 | 2 | 4 | 2 | 2.16 |
| 31 | 1 | 2 | 4 | 3 | 2.13 |
| 32 | 1 | 2 | 4 | 4 | 2.10 |
| 33 | 1 | 3 | 1 | 1 | 2.11 |
| 34 | 1 | 3 | 1 | 2 | 2.19 |
| 35 | 1 | 3 | 1 | 3 | 2.13 |
| 36 | 1 | 3 | 1 | 4 | 2.06 |
| 37 | 1 | 3 | 2 | 1 | 2.20 |
| 38 | 1 | 3 | 2 | 2 | 2.14 |
| 39 | 1 | 3 | 2 | 3 | 2.14 |
| 40 | 1 | 3 | 2 | 4 | 2.07 |

| | | | | | |
|---|---|---|---|---|---|
| 41 | 1 | 3 | 3 | 1 | 2.06 |
| 42 | 1 | 3 | 3 | 2 | 2.14 |
| 43 | 1 | 3 | 3 | 3 | 2.15 |
| 44 | 1 | 3 | 3 | 4 | 2.07 |
| 45 | 1 | 3 | 4 | 1 | 2.14 |
| 46 | 1 | 3 | 4 | 2 | 2.14 |
| 47 | 1 | 3 | 4 | 3 | 2.06 |
| 48 | 1 | 3 | 4 | 4 | 2.03 |
| 49 | 1 | 4 | 1 | 1 | 2.03 |
| 50 | 1 | 4 | 1 | 2 | 2.02 |
| 51 | 1 | 4 | 1 | 3 | 2.11 |
| 52 | 1 | 4 | 1 | 4 | 2.01 |
| 53 | 1 | 4 | 2 | 1 | 2.01 |
| 54 | 1 | 4 | 2 | 2 | 2.09 |
| 55 | 1 | 4 | 2 | 3 | 2.06 |
| 56 | 1 | 4 | 2 | 4 | 2.06 |
| 57 | 1 | 4 | 3 | 1 | 2.07 |
| 58 | 1 | 4 | 3 | 2 | 2.13 |
| 59 | 1 | 4 | 3 | 3 | 2.14 |
| 60 | 1 | 4 | 3 | 4 | 2.08 |
| 61 | 1 | 4 | 4 | 1 | 2.07 |
| 62 | 1 | 4 | 4 | 2 | 2.08 |
| 63 | 1 | 4 | 4 | 3 | 1.82 |
| 64 | 1 | 4 | 4 | 4 | 2.06 |
| 65 | 1 | 5 | 1 | 1 | 2.26 |
| 66 | 1 | 5 | 1 | 2 | 2.22 |
| 67 | 1 | 5 | 1 | 3 | 2.20 |
| 68 | 1 | 5 | 1 | 4 | 2.16 |
| 69 | 1 | 5 | 2 | 1 | 2.28 |
| 70 | 1 | 5 | 2 | 2 | 2.29 |
| 71 | 1 | 5 | 2 | 3 | 2.27 |
| 72 | 1 | 5 | 2 | 4 | 2.29 |
| 73 | 1 | 5 | 3 | 1 | 2.12 |
| 74 | 1 | 5 | 3 | 2 | 2.15 |
| 75 | 1 | 5 | 3 | 3 | 2.16 |
| 76 | 1 | 5 | 3 | 4 | 2.15 |
| 77 | 1 | 5 | 4 | 1 | 2.05 |
| 78 | 1 | 5 | 4 | 2 | 2.15 |
| 79 | 1 | 5 | 4 | 3 | 2.03 |
| 80 | 1 | 5 | 4 | 4 | 2.14 |
| 81 | 2 | 1 | 1 | 1 | 2.13 |
| 82 | 2 | 1 | 1 | 2 | 2.11 |
| 83 | 2 | 1 | 1 | 3 | 2.09 |
| 84 | 2 | 1 | 1 | 4 | 2.12 |
| 85 | 2 | 1 | 2 | 1 | 2.14 |

| | | | | | |
|---|---|---|---|---|---|
| 86 | 2 | 1 | 2 | 2 | 2.11 |
| 87 | 2 | 1 | 2 | 3 | 1.98 |
| 88 | 2 | 1 | 2 | 4 | 2.08 |
| 89 | 2 | 1 | 3 | 1 | 2.11 |
| 90 | 2 | 1 | 3 | 2 | 2.07 |
| 91 | 2 | 1 | 3 | 3 | 2.21 |
| 92 | 2 | 1 | 3 | 4 | 2.06 |
| 93 | 2 | 1 | 4 | 1 | 2.10 |
| 94 | 2 | 1 | 4 | 2 | 2.08 |
| 95 | 2 | 1 | 4 | 3 | 1.85 |
| 96 | 2 | 1 | 4 | 4 | 1.98 |
| 97 | 2 | 2 | 1 | 1 | 2.21 |
| 98 | 2 | 2 | 1 | 2 | 2.27 |
| 99 | 2 | 2 | 1 | 3 | 2.11 |
| 100 | 2 | 2 | 1 | 4 | 2.11 |
| 101 | 2 | 2 | 2 | 1 | 2.24 |
| 102 | 2 | 2 | 2 | 2 | 2.14 |
| 103 | 2 | 2 | 2 | 3 | 2.16 |
| 104 | 2 | 2 | 2 | 4 | 2.06 |
| 105 | 2 | 2 | 3 | 1 | 2.15 |
| 106 | 2 | 2 | 3 | 2 | 2.16 |
| 107 | 2 | 2 | 3 | 3 | 2.16 |
| 108 | 2 | 2 | 3 | 4 | 2.15 |
| 109 | 2 | 2 | 4 | 1 | 2.15 |
| 110 | 2 | 2 | 4 | 2 | 2.23 |
| 111 | 2 | 2 | 4 | 3 | 1.96 |
| 112 | 2 | 2 | 4 | 4 | 2.13 |
| 113 | 2 | 3 | 1 | 1 | 2.07 |
| 114 | 2 | 3 | 1 | 2 | 2.20 |
| 115 | 2 | 3 | 1 | 3 | 2.21 |
| 116 | 2 | 3 | 1 | 4 | 2.02 |
| 117 | 2 | 3 | 2 | 1 | 2.07 |
| 118 | 2 | 3 | 2 | 2 | 2.06 |
| 119 | 2 | 3 | 2 | 3 | 2.08 |
| 120 | 2 | 3 | 2 | 4 | 2.01 |
| 121 | 2 | 3 | 3 | 1 | 2.09 |
| 122 | 2 | 3 | 3 | 2 | 2.09 |
| 123 | 2 | 3 | 3 | 3 | 2.08 |
| 124 | 2 | 3 | 3 | 4 | 1.97 |
| 125 | 2 | 3 | 4 | 1 | 2.15 |
| 126 | 2 | 3 | 4 | 2 | 2.09 |
| 127 | 2 | 3 | 4 | 3 | 2.06 |
| 128 | 2 | 3 | 4 | 4 | 2.10 |
| 129 | 2 | 4 | 1 | 1 | 2.13 |
| 130 | 2 | 4 | 1 | 2 | 1.98 |

| | | | | | |
|---|---|---|---|---|---|
| 131 | 2 | 4 | 1 | 3 | 2.04 |
| 132 | 2 | 4 | 1 | 4 | 2.13 |
| 133 | 2 | 4 | 2 | 1 | 2.07 |
| 134 | 2 | 4 | 2 | 2 | 2.04 |
| 135 | 2 | 4 | 2 | 3 | 2.05 |
| 136 | 2 | 4 | 2 | 4 | 1.97 |
| 137 | 2 | 4 | 3 | 1 | 1.91 |
| 138 | 2 | 4 | 3 | 2 | 1.98 |
| 139 | 2 | 4 | 3 | 3 | 2.00 |
| 140 | 2 | 4 | 3 | 4 | 2.08 |
| 141 | 2 | 4 | 4 | 1 | 1.95 |
| 142 | 2 | 4 | 4 | 2 | 2.08 |
| 143 | 2 | 4 | 4 | 3 | 2.04 |
| 144 | 2 | 4 | 4 | 4 | 2.05 |
| 145 | 2 | 5 | 1 | 1 | 2.03 |
| 146 | 2 | 5 | 1 | 2 | 2.18 |
| 147 | 2 | 5 | 1 | 3 | 2.18 |
| 148 | 2 | 5 | 1 | 4 | 2.02 |
| 149 | 2 | 5 | 2 | 1 | 2.23 |
| 150 | 2 | 5 | 2 | 2 | 2.22 |
| 151 | 2 | 5 | 2 | 3 | 2.10 |
| 152 | 2 | 5 | 2 | 4 | 2.04 |
| 153 | 2 | 5 | 3 | 1 | 2.05 |
| 154 | 2 | 5 | 3 | 2 | 2.16 |
| 155 | 2 | 5 | 3 | 3 | 2.11 |
| 156 | 2 | 5 | 3 | 4 | 2.06 |
| 157 | 2 | 5 | 4 | 1 | 1.96 |
| 158 | 2 | 5 | 4 | 2 | 2.04 |
| 159 | 2 | 5 | 4 | 3 | 2.08 |
| 160 | 2 | 5 | 4 | 4 | 1.88 |

APPENDIX 6

## Statistical Analysis

The following statistical analysis is designed to determine if differences between the samples and judges are significant.

Initial Parameters:

Number of Judges: 7

Light Type: White fluorescent

Container Type: Gallons

Each level of light activated flavor from the scoring sheet was given a numerical value. The values ranged from 0 points for "none" to 5 points for "extreme". The ratings assigned by the judges for each sample are shown below.

| Judges | Samples | | | | |
|---|---|---|---|---|---|
| | 0 Hours | 5 Hours | 10 Hours | 24 Hours | Total |
| A | 1 | 0 | 3 | 3 | 7 |
| B | 0 | 2 | 3 | 4 | 9 |
| C | 1 | 0 | 3 | 4 | 8 |
| D | 0 | 1 | 3 | 3 | 7 |
| E | 1 | 0 | 3 | 5 | 9 |
| F | 0 | 1 | 1 | 3 | 5 |
| G | 3 | 0 | 4 | 2 | 9 |
| Total | 6 | 4 | 20 | 24 | 54 |
| Mean | .86 | .57 | 2.86 | 3.43 | |

These results were then applied to an analysis of variance.

## Analysis of Variance

Correction Factor (CF)

$= 54^2/28$

$= 104.14$

Sum of Squares, Samples: $(6^2+4^2+20^2+24^2)/7 - CF$

$= (36+16+400+576)/7 - 104.14$

$= 1028/7 - 104.14$

$= 146.86 - 104.14$

$= 42.72$

Sum of Squares, Judges: $(7^2+9^2+8^2+7^2+9^2+5^2+9^2)/4 - CF$

$= (49+81+64+49+81+25+81)/4 - 104.14$

$= 430/4 - 104.14$

$= 107.5 - 104.14$

$= 3.36$

Sum of Squares, Total: $(1^2+0^2+...+3^2+2^2) - CF$

$= (12+6+62+88) - 104.14$

$= 168 - 104.14$

$= 63.86$

Analysis of Variance Table

| Source of Variation | df | ss | ms | F-value |
|---|---|---|---|---|
| Samples | 3 | 42.72 | 14.24 | 14.38 |
| Judges | 6 | 3.36 | .56 | .57 |
| Error | 18 | 17.78 | .99 | |
| Total | 27 | 63.86 | | |

The level of significance at (.05) for both samples and judges were 3.16 and 2.66 respectively, Chart 3, Larmond (74). When the F-value is

greater than the (.05) value significance is indicated.

Thus...

Significance is indicated for the samples when 14.38 > 3.16 and not shown for the judges since .57 < 2.66.

Turkey's T Test is next utilized to determine which samples and judges are significantly different from each other.

Samples

Standard Error;

$$SE = \sqrt{.99/7}$$

$$= \sqrt{.14}$$

$$= .38$$

From Chart 4 of Larmond (74);

4 samples, 18 df = 4.00

Least significant difference = 4.00 x .37 = 1.48

| Sample Means; | 0 | 5 | 10 | 24 |
|---|---|---|---|---|
| | .86 | .57 | 2.86 | 3.43 |

The means are arranged according to magnitude

| 24 | 10 | 0 | 5 |
|---|---|---|---|
| 3.43 | 2.86 | .86 | .57 |

The means are then compared with each other to determine if the difference is greater than 1.48. If the subtracted value is greater than 1.48 significance is indicated.

24 - 5 = 3.43 - .57 = 2.86 > 1.48

24 - 0 = 3.43 = .86 = 2.57 > 1.48

24 - 10 = 3.43 - 2.86 = .57 < 1.48

10 - 5 = 2.86 - .57 = 2.29 > 1.48

10 - 0 = 2.86 - .86 = 2.00 > 1.48

0 - 5 = .86 - .57 = .29 < 1.48

24 has significantly more activated flavor than at 0 and 5 hours.

24 and 10 hours are not significant.

10 has significantly more activated flavor than the samples at 0 and 5 hours.

The samples at 0 and 5 hours are not significantly different from each other.

Letters are used on the above results to indicate differences:

| 24 | 10 | 0 | 5 |
|---|---|---|---|
| 343a | 2.86ab | .86c | .57c |

Any two means not followed by the same letter are significantly different at the 5% level.

Turkeys test is also used to determine which judges differ significantly.

Judges

Standard Error:

$$SE = \sqrt{.99/4}$$

$$= \sqrt{.25}$$

$$= .50$$

From Chart 4 of Larmond (1977); 7 judges, 18 df = 4.67

Least significant difference = 4.67 x .50 = 2.34

The mean for each judge is determined next.

Judges

| A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|
| Totals ∿ (from sample table) | | | | | | |
| 7 | 9 | 8 | 7 | 9 | 5 | 9 |
| Means (Total/4) | | | | | | |
| 1.75 | 2.25 | 2.00 | 1.75 | 2.25 | 1.25 | 2.25 |

Means are arranged in order of magnitude.

| B | E | F | C | A | D | G |
|---|---|---|---|---|---|---|
| 2.25 | 2.25 | 2.25 | 2.00 | 1.75 | 1.75 | 1.25 |

The means are then compared with each other to determine if the difference is greater than 2.34

JB - JG = 2.25 - 1.25 = 1.0 < 2.34

JE - JG = 2.25 - 1.25 = 1.0 < 2.34

JF - JG = 2.25 - 1.25 = 1.0 < 2.34

JC - JG = 2.00 - 1.25 = .75 < 2.34

JA - JG = 1.75 - 1.25 = .75 < 2.34

JD - JG = 1.75 - 1.25 = .50 < 2.34

Since all the above results are less than 2.34, no significance is indicated between the judges and their ability to distinguish light activated flavor in milk.

*This statistical analysis was taken from: Larmond, Elizabeth. 1977. Laboratory Methods for Sensory Evaluation of Food, Publication 1637. Research Branch, Canada Department of Agriculture.

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