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Effect of Temperature and Packaging Material on Vitamin A and Riboflavin in Milk

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EFFECT OF TEMPERATURE AND PACKAGING MATERIAL ON VITAMIN A AND RIBOFLAVIN IN MILK

BY

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ABSTRACT

The effect of different heating times and temperatures and packaging on vitamin A and riboflavin retention was studied in whole and skim milk. Refrigerated storage (4°C) in the dark for 15 days resulted in losses of vitamin A ranging from 9-12% in pasteurized whole milk, and 14% in pasteurized skim milk fortified with vitamins A and D. Riboflavin losses did not exceed 8%. Vitamin A was also more labile than riboflavin when stored at room temperature (23°C). Vitamin A was retained better in whole milk than in skim milk where there was no protection from milk fat. After 48 hours of storage at room temperature, pasteurized whole milk stored in a paperboard container lost 26.3% while that in plastic container lost 30%. Losses of vitamin A in skim milk stored in paperboard were 52%. In contrast, the losses or riboflavin of whole and skim milk were about 18%.

Boiling of milk at 100°C for 10 seconds resulted in more destruction of vitamin A than of riboflavin. Whole milk lost 10.2% of the vitamin A after a single boiling and 14.4% after twice boiling. Skim milk lost 17.4 and 28.5%, respectively. The riboflavin content remained very stable in both whole and skim milk. Losses did not exceed 1% for a single and 4% for twice boiling. Boiling

milk and holding it at 55°C was quite destructive to vitamin A, but had a smaller effect on riboflavin. After 12 hours of holding previously boiled milk at 55°C, 29% and 53% of the vitamin A were lost in whole and fortified skim milk, respectively. Riboflavin losses did not exceed 10-14% in whole or skim milk. A double cycle of boiling and holding milk at 55°C resulted in the largest losses of both vitamin A and riboflavin. After the second 12-hour holding at 55°C, vitamin A losses for whole milk were 30.4% to 33.1%. In fortified skim milk, the loss was 54.7%. Riboflavin losses for the double boiling and holding at 55°C were approximately 20% for whole or skim milk.

Packaging material such as paperboard and plastic containers did not greatly influence the losses of vitamin A or of riboflavin. However, vitamin A retention was better for whole than for skim milk.

As a result of these experiments, it is recommended that milk in Saudi Arabia continue to be packaged in paperboard containers. However, for maximum retention of vitamin A and riboflavin, milk handlers and consumers should be educated to stop heating milk and to store it in the dark under refrigeration.

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INTRODUCTION

As fluid milk is a universally accepted nutritious food for people of all ages, it is important that the milk be processed and packaged to maintain maximum high quality over a period of time. Quality factors which require careful control should include not only such parameters as microbiological content and organoleptic indices but also nutrient retention. Of the many vitamins present in pasteurized milk, it is well known that retention of Vitamin A and riboflavin can be influenced by exposure to different light intensities and wavelengths. The degree of loss is dependent on time and temperature and the type of milk package. Of importance are the properties of the packaging material, especially its light transmission characteristics. Type, size, and construction of the package can also affect vitamin retention.

Vitamin A is considered to be susceptible to destruction by oxidation which is enhanced by heat treatment. Riboflavin is more stable to heat treatment, but losses occur in the presence of light, with enhancement of losses during high temperatures.

As may be expected, a degree of protection, particularly from the deleterious effects of light, can be

obtained by proper selection of the milk package. Paperboard containers because of their low cost, availability and versatility are widely used. Plastic containers are also frequently used for bottling milk. The claim has been made that paperboard best prevents vitamin loss.

In Saudi Arabia milk drinking habits are influenced by traditional customs. People drink warm milk during breakfast and dinner at home and also in cafes and restaurants. Milk is initially boiled and then kept warm at around 55°C for two to eight hours in an open container. At the end of the day, any remaining milk is cooled down and refrigerated overnight. The next day, new milk is added to that stored milk, the milk boiled again and kept warm for two to eight hours. Because of the prolonged heating and exposure to air and light, there is concern over the retention of Vitamin A and riboflavin in milk.

The specific purposes of this study were:

1. To determine the effect of temperature and time on Vitamin A and riboflavin retention in milk treated in the manner traditional in Saudi Arabia.
2. To examine the effect of storage under various packaging materials (plastic vs paper) on Vitamin A and riboflavin retention in milk.

LITERATURE REVIEW

Types of Milk

Although milk is processed, it's not an engineered or fabricated food. It naturally has two major components: fat, including fat soluble vitamins, and non-fat solids which include proteins, carbohydrates, water soluble vitamins and minerals. The nutrients and other components of milk make it a food not duplicated by modern science. Some of the types of milk are (De Man 1980):

Whole milk: Whole milk contains not less than 3.25% milkfat. It also contains not less than 8.25% non-fat solids such as proteins, minerals, carbohydrates and water soluble vitamins.

Lowfat milk: Low fat milk contains 1% or 2% milk fat. It also contains at least 8.25% non-fat solids. It must contain 2,000 IU of vitamin A per quart. Vitamin A is added to offset its loss caused by removal of some of the milkfat.

Skim milk: Skim milk, also called nonfat milk, has had sufficient milkfat removed to bring the level to less than 0.5%. It must contain not less than 8.25% nonfat solids and must be fortified with vitamin A. If nonfat

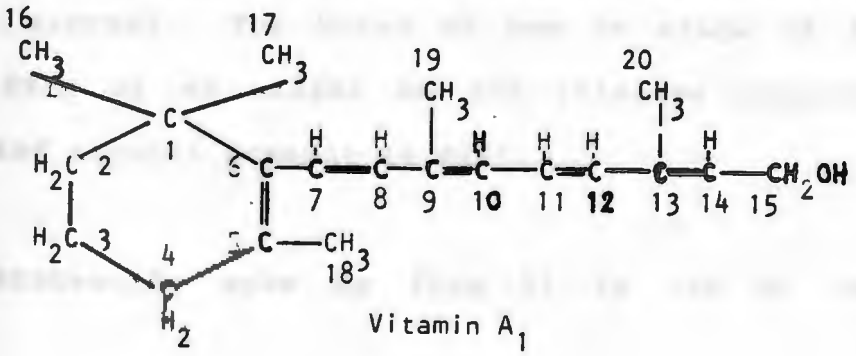
solids are added to reach 10% level it must be labeled as, "protein fortified" or "fortified with protein".

Pasteurization is a form of heat treatment (Peterson, 1978). In world-wide practice, one or more of four general temperature zones are in use for heat treating milk. These are: (1) 62.8°C for 30 minutes and/or 71.7°C for 15 seconds, (2) 79.4 - 90.6°C for 15 seconds or less, (3) 93.3-100°C and, (4) above 107.2°C momentarily up to 30 min. Pasteurization readily destroys the most heat resistent pathogens associated with milk. In addition, some properties of the milk are affected according to the duration and intensity of heat treatment. Pasteurization easily inactivates lipase and most other enzymes but in general does not impair the nutritive value of milk.

Vitamin A

Vitamin A, retinol, is the isoprenoid polyene alcohol also known as axeriphthol. It is a colorless compound, soluble in oils and fats, but practically insoluble in water (Atherton and Newlander, 1982).

Vitamin A has the following structure:



Vitamin A_1

Vitamin A as such, is not found in plants but is only of animal origin, and is present as vitamin A_1 in all animals and fish. (Hartman and Dryden, 1965). Vitamin A plays an essential role in regard to vision. Deficiency of vitamin A results in inhibition of growth, increased susceptibility to infection, loss of appetite, poor hair production, extensive keratinization of epithelial cells and mucuous membrane, loss of gland activities, multiple fetal abnormalities, night blindness, xerophthalmia and keratomalacia. The established signs of vitamin A deficiency in the human are the eye lesions, acne, senile vaginitis, atrophic rhinitis, anosmia and certain skin disorders. Hypervitaminosis A results in skin changes, hepatomegaly and painful joint swellings (Marks 1979, Webb et al 1983, Goodhart and Shils 1980).

Vitamin A in Milk

In normal milk and colostrum almost all vitamin occurs in the ester form. Only about 2 to 6% is present as the alcohol. The breed of cow or stage of lactation has little or no effect on the relative proportions of ester and alcohol present in milk.

Carotenoids make up from 11 to 50% of the total

vitamin A activity of milk, and can be converted to the vitamin in the animal body. The exact percentage depends upon the breed of the cow and the level of carotenoid intake (Reinart and Nesbitt, 1956). Since the intake of these substances is generally higher during the summer than during the winter, the fraction of total vitamin A activity due to the provitamin is greater in summer milk (Lord, 1945).

The yellow color of milk fat and of animal fat is due to the presence of carotenoids. The fat in Guernsey milk has a much more golden color than has that of Holstein milk because it has a higher content of carotene.

β-Carotene makes up the greatest fraction of the carotenoids in the milk (Strain, 1939). α-carotene, is generally absent from butterfat, but if cows are fed carrots, which contain about 25% of their total carotenoids in the form of α-carotene, this form of carotene will also appear in the milk (Hauge, 1942).

A part of the total carotenoids in milk may consist of compounds that are completely inactive as provitamin A. Inactive substances can range from 5 to 25% of the total carotenoids (Thompson and Kon, 1950).

The amount of vitamin A secreted into the milk and milk fat, either as vitamin A itself or a carotene, depends upon the level of carotene or vitamin A in the ration of the cow (Hibbes, et al, 1949). Large increases in the vitamin A content of milk can be obtained by supplementing the ration of the cow with concentrated sources of vitamin A such as fish liver oils. These oils contain only vitamin A no carotene, and the increase in the total vitamin A activity of the milk is all in the form of vitamin A. When carotene was sharply increased in the diet, vitamin A secreted in milk was increased very rapidly within 1-2 weeks (Thompson, and Ascarell, 1962).

A relationship between carotene and vitamin A and off-flavors in milk was reported by several researchers. The feeding of carotene but not of vitamin A was thought, in the beginning, to prevent the occurrence of oxidized flavor in milk (Whitnah, et al, 1937). Later, however, it was found (Trout and Gjessing, 1939) that it was not carotene but some other factor in carotene concentrates and carotene rich forages that was the effective substance.

The Committee on Biological Standardization of the World Health Organization has, as an international

standard, 1 IU of vitamin A equal to 0.30 micrograms of vitamin A alcohol or 0.6 micrograms of B-carotene. The levels of vitamin A in fluid whole milk in late winter are about 1,083 IU/Liter and, in summer, about 1,786 IU/ Liter. This difference was not due to the season but to differences between winter feed and summer pasture (Cary, et al 1947). The content of vitamin A in fluid whole milk, measured in International Units per quart, for winter and summer, in different countries, is shown in Table 1 (Hartman and Dryden, 1965).

In the United States, vitamin A palmitate is added to whole milk and skim milk at a concentration of 2,000 IU per quart. Vitamin A fortified skim milk is subject to decreases in vitamin A, because the vitamin is no longer protected by fat as it is in whole milk. In fluid skim milk, added vitamin A deteriorated gradually during normal storage of the milk at 4.4°C in the dark but was destroyed rapidly when the milk was exposed to sunlight in clear-glass bottles (Cox et al, 1957, and Birdsall, et al. 1958).

Table 1: Content of vitamin A in fluid whole milk.

Country	Winter Milk	Summer Milk	Annual
	IU/quart		
USA	1,025	1,690	1,425
Great Britain	880	1,380	-
Netherlands	1,220	1,870	1,620
New Zealand	1,990	1,600	1,670
India	-	-	1,535

Source: Hartman and Dryden, 1965

Effect of heat on Vitamin A in milk

Most researchers report that procedures such as pasteurization, sterilization, drying, or evaporation of milk cause little or no loss of the vitamin A or carotene (Fennema, 1977). In one study, however (Wagner, 1957), pasteurization lowered both the vitamin A and carotene content by 20% or more, and sterilization destroyed from 30 to 100% of the vitamin. In another study (Davidov and Kruglova, 1959), the process of evaporation and pasteurization involved in the preparation of condensed milk, decreased vitamin A by 20%, but no decrease was observed in carotene.

Although pasteurization of milk is not usually considered to result in vitamin A loss, exposure to light will result in loss. Vitamin A in whole milk and skim milk gradually deteriorated at 40°F in dark storage, and it was rapidly destroyed in clear glass bottles in sunlight, Prolonged heating of milk or butter at high temperatures with oxygen present, decreases vitamin A activity (Fragner et al, 1956). Vitamin A is relatively stable to heat in the absence of oxygen, however, losses may occur at high tempertures in the presence of oxygen (DeRitter, 1976).

The observation that added vitamin A is more stable in dry whole milk than in nonfat dry milk solids suggest thatthe fat in dry whole milk is involved in some manner in stabilizing vitamin A (Cox et al, 1957). Destruction of vitamin A was less in dried milk preheated to 180°F for 30 minutes than in those preheated to 145°F for the same period of time.

More vitamin A is destroyed during the boiling of reconstituted milk than during the boiling of fresh milk (Wilkinson and Conochie, 1958). With milk reconstituted from vitamin A-fortified skim milk powder, such losses ranged from 2 to 20% after 2 minutes of boiling and increased to 30% after 30 minutes of boiling. In another study, (Bauernfeind and Allen, 1963), decreases in potency were smaller; about 3 to 6% after 2 minutes and 6 to 9% after 30 minutes of boiling. When milk was heated at 220°C for 10 minutes, all vitamin A activity was destroyed (Hattianydi and Kanga, 1956).

Legge and Richards (1978) reported that vitamin A and beta-carotene did not show any significant change in concentration when human milk was heated to 86°C for 1 minute.

Effect of packaging on vitamin A in milk

The protection offered by a package is determined by the nature of the packaging materials and by the type of package construction. Packaging is a decisive factor in controlling nutrient retention in milk. There are three basic classes of packaging materials as related to light transmission a) transparent glass and clear plastic films, b) opaque-aluminum foil and laminates and c) translucent-paper, cardboard and plastic (Sattar and DeMan, 1975).

A very considerable proportion of packaged food is stored and distributed in packages made of paper-based materials . It seems probable that, because of its low cost, ready availability, and great versatility, paper will retain its predominant packaging position for some time to come. (Karel amd Heidelbaugh, 1975).

Paper cartons block out 98 percent of the destructive light. Paper gallon 2-paks are also easier to store, because they take up less space, fit in refrigerator doors and are easily disposed of in the home. (International Paper Company, 1982).

Other major types of milk containers on the market

today include flint glass, olefin-coated fiberboard, blow-molded polyethylene and the polyethylene pouch (Dimick, 1978; Siddall, 1957). Both the polyethylene and the polycarbonate containers have been shown to absorb contaminants, and to impart off odors into the product (Landsberg et al, 1977).

Barnard (1974), reported that 16% of the serious criticisms concerning the flavor of milk were due to oxidation. The majority were attributed to milk held in translucent or transparent containers, i.e. blow-molded plastic, glass, and the plastic pouch. Hankin and Dillman (1972), reported a similar study in which 31% and 33% of milk in glass and plastic containers were oxidized as compared to 4.4% in paper cartons. In conveying milk products from stores to home in transparent or translucent containers, a flavor problem could develop. A light-induced off-flavor is a function of the amount of radiant energy at the milk surface and the length of exposure (Bradley, 1980).

Sattar et al (1977), showed that losses of vitamin A and its precursor could be markedly reduced by limiting exposure of milk to energy below 465 nm. Destruction was not autocatalytic and followed zero-order kinetics. No

synergism was observed except that at β-carotene concentrations of greater than 2.5 ug/ml, a protective effect on vitamin A was observed.

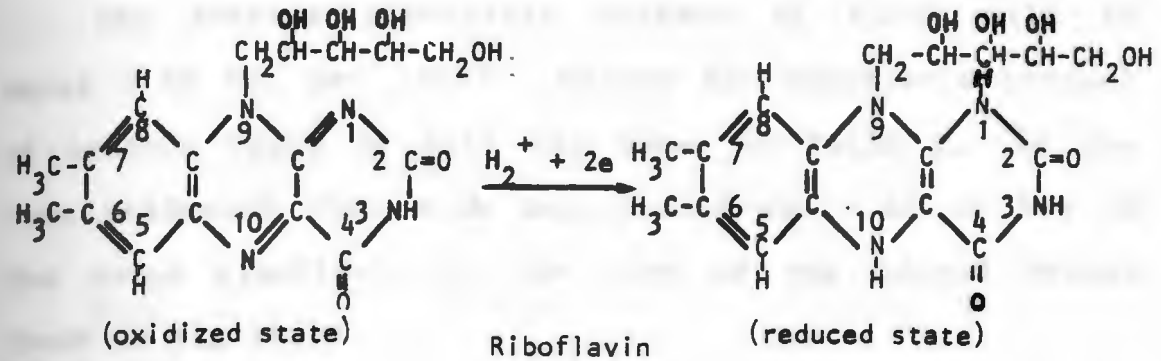
Hansen et al (1975), reported that homogenized milk packaged in polyethylene containers exposed to fluorescent light showed both flavor and vitamin deterioration. Vitamin A in whole milk packaged in plastic pouches (DeMan, 1980), dropped to 67.7% of its original content by 30 hours after exposure to 2.200 lx intensity fluorescent light and remained constant for a further 18 hours. In 2% milk, it dropped to 23.6% and in skim milk, to 4.2% of the original content. The 2% milk had an original Vitamin A content about twice as high as that of whole or skim milk.

In the fortification of low-fat fluid milks with Vitamin A (retinyl palmitate), Thompson and Erdody, (1974), demonstrated that this form of the vitamin was rapidly destroyed following fluroescent light exposure. Losses up to 60% occured in milk in glass following 200 feet-candles fluorescent light exposure for 3 hours. When milks were packaged in blow-molded polyethylene, 50% losses in total vitamin A occured in low fat milks supplemented with retinyl palmitate.

Cox et al, (1957), studied the effect of exposure of pasteurized whole and skim milk on retention of Vitamin A. The milk was packaged in amber glass bottle, plain glass bottles and waxed paperboard cartons and was exposed to diffused daylight and to short time irradiation with direct sunlight. Care was taken to avoid temperature changes. It was found that amber glass bottles and paperboard cartons afforded good protection, but plain glass bottles did not prevent considerable losses of the vitamin. Because of the highly unsaturated character of the molecules, it is susceptible to influence of light, and this includes sunlight as well as artificial light (Hartmand and Dryden, 1965).

Riboflavin

Vitamin B_2, riboflavin, lactoflavin or vitamin G is a yellow-orange to yellow-green pigment. It is a heat stable and one of the least soluble of water soluble vitamins. The riboflavin molecule consists of a D-ribitol unit attached to an isoalloxazine ring, as is shown in the following structures.



Riboflavin is a constituent of two coenzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (Hartman and Dryden, 1965). Riboflavin has a profound effect on such processes as tissue repair, tissue growth, reproduction and lactation.

Early symptoms of riboflavin deficiency may be related to oral or ocular lesions. Oral lesions include soreness and burning of the lips, mouth and tongue and cracking at the corner of lips. Ocular symptoms include photophobia, lachrymation, burning and itching of the eyes, visual fatique, blepharospasms and loss of visual acuity (Marks, 1979). Dermatitis is also a symptom.

Riboflavin content in milk

The average riboflavin content of fluid milk is about 1.75 mg. per liter. Values for riboflavin content of various types of milk are shown in Table 2. It has been estimated that milk contributes about 40 to 50% of the total riboflavin in the diet of the United States (Webb et al, 1983).

The riboflavin content of milk is usually higher during the spring or summer. This difference has been associated with a change in ration from indoor feeding to fresh pasture feeding (Kramer et al, 1939). Increases of 20% to 50% have been reported. In other instances, however, no significant differences were found between summer and winter rations (Gregory et al, 1958). Supplementation of the rate of the cow with crystalline riboflavin did not increase the content of riboflavin in milk (Marsh et al, 1947).

Forms of Riboflavin in milk

From 65 to 95% of the riboflavin in cow's milk is in the free form (Modi et al, 1959). According to Modi and Owen (1956) the rest is present as FAD, and there is none

Table 2: Riboflavin Content of Milk

MILK	AVERAGE (mg/kg)	RANGE (mg/kg)
Whole milk		
Fluid	1.75	0.81 - 2.58
Condensed	3.6	2.6 - 4.0
Evaporated	3.8	2.8 - 4.8
Dried	15.5	9.8 - 25.6
Skim milk		
Fluid	1.7	1.5 - 1.8
Dried	18.9	13.0 - 25.4

Source: Webb, et al, 1983.

as FMN. Funai (1955), however, reported 21% in the form of FMN, with 14% as FAD and the remainder in the free form. In skim milk, Nagasawa et al, (1961), found 94-95% of the riboflavin free, with the remainder bound about 60% as FMN and 40% as FAD.

The bound riboflavin in milk is, for the most part, attached to protein as part of an enzyme (Modi et al, 1959). The sites of binding between the vitamin and casein may be the tyrosine residues of the protein (Leviton and Pollansch, 1960).

Effects of Heat on Riboflavin in Milk

Losses of riboflavin due to heating are generally enhanced and complicated by the effects of light. In the absence of light, the riboflavin in milk is quite stable to heat.

No decrease in the vitamin occurred when pasteurized milk was heated for 22 hours at 37°C and similar results were obtained from raw milk (Sure and Fore, 1943). Heating milk in dark brown bottles at 100°C for 15 minutes caused only 5% loss in riboflavin (Funai, 1957).

Pasteurization caused only a negligible amount of destruction of the vitamin (Holmes, 1944), or none at all (Ford, et al, 1959). No loss was reported when milk was pasteurized under 8 atm oxygen pressure (Luck and Schillinger, 1959). When fresh skim milk was heated at 100°C for 45 minutes in the dark, only 5% destruction of riboflavin occured (Williams and Cheldelin, 1942). Homogenization of milk has no effect whatsoever on the riboflavin (Theophilus and Stamberg, 1945).

Stomberg and Theophilus (1944), found that the rate of destruction of riboflavin was related directly to milk temperature as well as the amount of light transmitted through the containers. This would mean greater loss in summer months when product temperature, in general, would be higher.

No changes in riboflavin were observed when human milk was heated at 62.5°C for 30 minutes; 72°C for 15 seconds; 83°C for 5 seconds and 100°C for 5 minutes (Goldsmith et al., 1983).

Effect of packaging on Riboflavin in Milk

The protection of riboflavin is of vital interest,

not only to the scientist but also to the consumer as well. Packaging material plays a significant role in preventing destruction of naturally occurring or fortified vitamins (Senyk and Shipe, 1981).

Hasken and Dimik (1979), have reported that the protection effect of milk containers is due to the light transmission properties of packaging material. Transmission characteristic of different milk containers are shown in Table 3.

The use of containers other than clear glass bottles increased the retention of riboflavin upon exposure to light. Brown, amber or ruby glass bottles or waxed paper cartons lessen considerably or eliminate its destruction (Dunkley et al 1962).

Sattar and DeMan (1973), observed that the paperboard carton and returnable plastic jug were both inadequate for protection of milk from loss of flavor and nutritional value. At 100 foot candles of light, the riboflavin losses were 7.1%, 11.1% and 22.3% for the carton, jug and clear package, respectively, compared to essentially no loss in the opaque pouch.

Table 3. Transmission of fluorescent light through various milk container materials.

MATERIAL	% TRANSMISSION
Clear flint glass	92
Clear polycarbonate	90
Tinted polycarbonate	75
non-returnable polyethylene	70
High density polyethylene	58
Unprinted fiberboard	4

Source: Hasken and Dimik, 1979

Dunkley et al, (1962) studied milk from three retail markets and found great variation in light caused damage between different samples. However, flavor protection and protection of riboflavin by opaque containers was evident.

Singh et al, (1975), reported that in whole milk stored in glass and regular blow-moulded polyethylene containers, losses of riboflavin increased significantly with increasing storage temperature.

Glass offers virtually no protection from the actinic rays of the sun or fluorescent light. Plastic containers provide little more protection than glass. The paper (fiber) containers provide the most protection against fluorescent light (Senyk and Shipe, 1981).

Among the most commonly used milk packages, this destruction is greatest in plastic containers (International Paper Company, 1982). Fluorescent store lights penetrate plastic, and within 24 hours, milk can lose up to 14% of its riboflavin and substantially more of its fortified Vitamin A. (Ms. Levey, 1982).

MATERIALS AND EXPERIMENTAL PROCEDURES

Sample Source

Raw milk was obtained from a local dairy farm in Kingston, Rhode Island. Whole pasteurized homogenized Vitamin D fortified milk in half gallon (1.89 L) paperboard containers, whole pasteurized homogenized Vitamin D fortified milk in one gallon plastic containers and pasteurized homogenized skim milk (nonfat) with Vitamins A and D added, in quart paperboard containers processed by East Greenwich Dairy Co. were purchased at a local market and then taken to the laboratory at the University of Rhode Island in a refrigerated case. All samples were kept in the dark under refrigeration.

Effect of Packaging and Storage Temperatures

Pasteurized, homogenized vitamin D fortified whole milk and skim milk containing added Vitamins A and D were held at one of two temperatures for varying lengths of time. Samples were held at room temperature (23°C) for 1, 2, 4, 6, 12, 24 and 48 hours. Other samples were refrigerated (4°C) for 5, 10, 15, 20, 25, 30, 40, 50, and 60 days.

Effect of Single or Double Heating at Various Times and Temperatures

As is customary in Saudi Arabia and other Arabic countries, samples of milk were initially heated to boiling. Then, they were cooled to 55°C. In one experiment, milk was held at 55°C for intervals of 2, 4, 6, 8, and 12 hours and then cooled and analyzed for vitamin A and riboflavin. (single heating). In another experiment, the milk was boiled for 10 seconds, cooled to 55°C and held at 55°C for 12 hours. The milk was refrigerated at 4°C for 24 hours. Then, the milk was boiled at 100°C for 10 seconds, cooled to 55°C and held for up to 12 hours. (twice boiled). After cooling to room temperature (23°C), vitamin A and riboflavin were determined on these samples at 2, 4, 6, 8 and 12 hours. Fig. 1 shows the experimental design for the single and double heating and holding experiments.

Equipment

A fluorometer A-4, Ferrand Optical Co., Inc., New York was used for all fluorometric analyses.

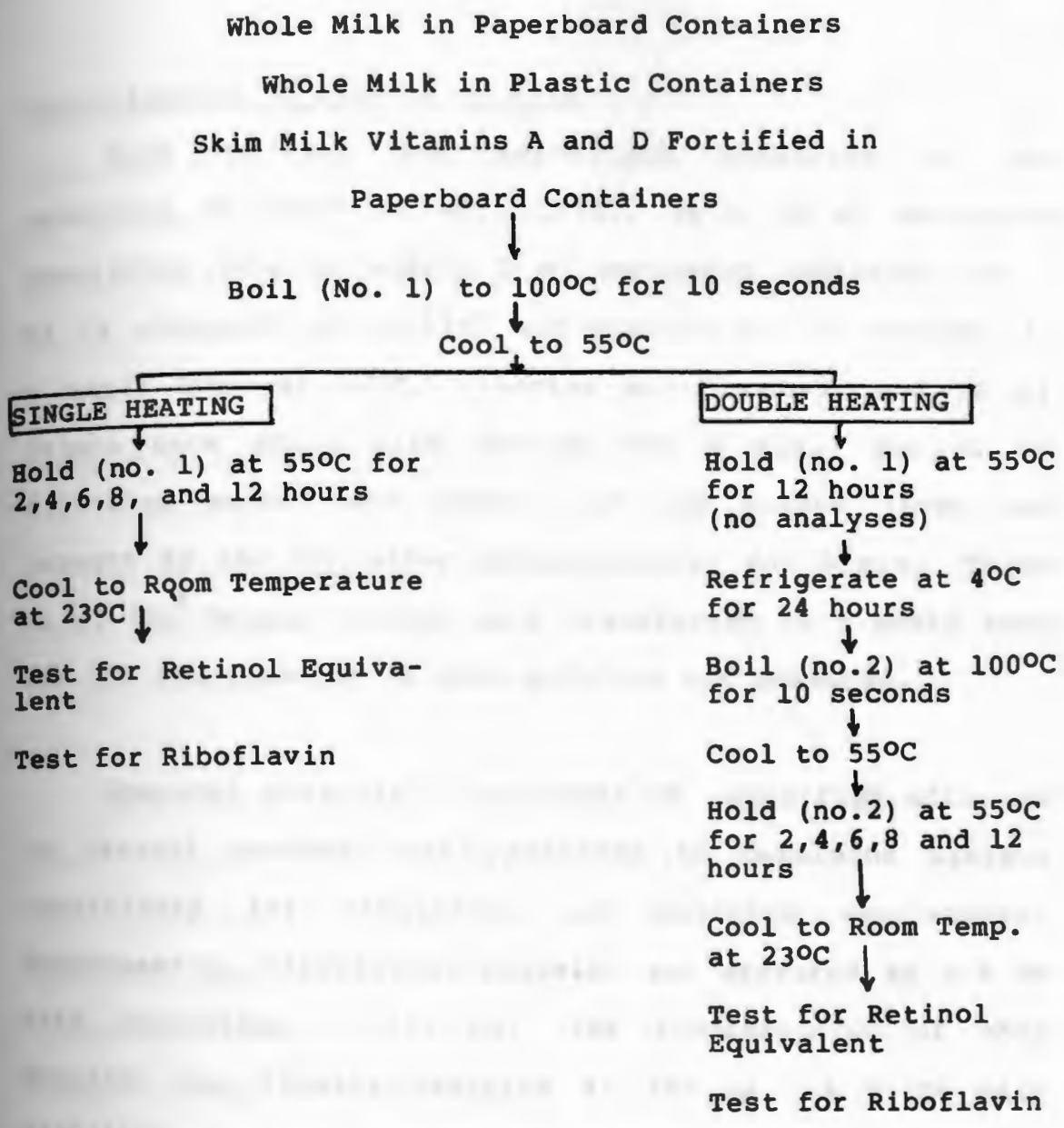


Fig. 1. Experimental design used to determine effect of heating and holding milk on Vitamin A and riboflavin retention.

Determination of Retinol in Milk

Milk (1 ml) was saponified according to the procedure of Senyk et al., (1974), in a 15 ml stoppered centrifuge tube by adding 2 ml potassium hydroxide and 2 ml 1% ethanolic pyrogallol and heating for 30 minutes in a water bath at 60°C. Samples were cooled, and 10 ml hexane were added with shaking for 2 min. Two ml of distilled water were added, and the hexane layer was brought to the top after centrifugation for 5 min. Three ml of the hexane extract were transferred to a small tube and the fluorescence of this solution was measured.

Spectral analysis of extracts of saponified milk and of retinyl acetate were performed to determine maximum sensitivity for excitation and emission wavelengths. Subsequently, fluorescent emission was measured at 475 nm with excitation at 330 nm. The fluoresecence of this solution was finally measured at 330 nm. A blank with distilled water or hexane was also read and subtracted from the sample reading. Senyk et al., (1974). Vitamin A was determined as retinol equivalents from a standard curve of retinyl acetate in hexane at concentrations 0, 4, 6, 8, 10, 14, and 16 ug/100 ml.

Retinol equivalent was calculated as the reading from the standard curve X 10 X 0.872 = ug/100 ml, where 10 is the dilution factor and the molecular weight of retinol (280)/molecular weight of retinol acetate (328) = 0.872.

Fluorometric Determination of Riboflavin in Milk

A standard curve was obtained using an intermediate concentration standard riboflavin solution containing 10 ug/ml diluted in 100 ml of 0.02N Acetic Acid to obtain concentrations of 0, 0.05, 0.1, 0.15 and 0.20 ug/ml.

Riboflavin was determined by the standard AOAC method (AOAC, 1980). Milk (5ml) was transferred to a 125 ml Erlenmeyer flask and covered with aluminum foil in a darkened room. Since riboflavin is light sensitive, all operations were performed in the absence of strong light. After adding 50 ml of 0.1N HCl to the flask, the sample was autoclaved for 30 minutes at 15 lbs. pressure.

After autoclaving, the sample was cooled and ajusted to pH 6.0 with NaOH. Since riboflavin is unstable in alkaline solution, the extract was swirled constantly during the addition of alkali to prevent a localized area of high pH. Immediately, 1 N HCl was added to bring the pH to 4.5.

The solution was diluted to 100 ml with water and filtered. Then, 1 N HCl was added dropwise to 50 ml aliquot of filtrate until no more precipitate was formed. This was followed by an equal number of drops of 1 N NaOH with constant shaking. The aliquot was diluted to 100 ml with water and filtered.

Ten ml of sample solution plus 1 ml of water was added to each of 2 test tubes and then mixed. Ten ml of sample solution and 1 ml of 0.5 ug/ml riboflavin working standard solution were added to each of 2 other test tubes and mixed. One ml of glacial acetic acid was added to all 4 tubes and mixed. To each tube, 0.5 ml of 3% $KMnO_4$ was then added and mixed and allowed to stand for exactly 2 minutes. After 2 minutes, 0.5 ml of 3% H_2O_2 was then added and mixed thoroughly. The red color disappeared within 10 seconds.

The fluorescence of extracts containing water was measured (reading A). Powdered $Na_2S_2O_4$ (20 mg) was added and within 10 seconds, fluorescence was again measured (reading C). Fluorescence of the extracts containing riboflavin standard was measured in the same manner (reading B). The riboflavin was calculated from

the following formula:

$$\frac{A-C}{B-A} \times \frac{\text{Amount of riboflavin added}}{\text{10-ml aliquot}} \times \text{dilution factor} \times \frac{1}{\text{sample wt.}} = \text{ug/gm}$$

Reading A = 10 ml filtrate and 1 ml water, Reading B 10 ml filtrate plus and 1 ml 0.5 ug riboflavin standard, and Reading C = blank of filtrate. Sample weight (5 ml) was 5.16 gm. The dilution factor was $\frac{100}{50}$ or 2.

Therefore riboflavin content of milk (ug/gm) =

$$\frac{A-C}{B-A} \times \frac{0.5}{10} \times \frac{100}{50} \times 100 \times \frac{1}{5.16\text{gm}} = \text{ug/gm}$$

[redacted]in, Fat, Moisture, Ash and Solids Determination

Percent moisture in the sample was determined by drying (105°C) a small portion to constant weight for 24 hours. Protein, fat and ash were determined by standard AOAC methods (AOAC, 1980). Total solids and nonfat solids were determined by calculation from the proximate analysis values.

[redacted]istical Analysis

Means and standard deviations were determined for retinol and riboflavin content of milk samples as

purchased and following experimental treatments. Each mean value for retinol and riboflavin represent a result obtained from 8 replicates.

Since it is known that there will be a loss of both retinol and riboflavin in storage and heating, the progression of this loss was presented graphically using linear regression. The slope of the line represents the change (decrease) in retinol or riboflavin content for a unit increase in storage or heating time. The straight linear relation between vitamin content and time can also be expressed by an equation generally written as $y = a - bx$, where y is the estimated retinol and riboflavin content, a is the retinol or riboflavin content line intercept, b is the slope of the line and x is the storage or heating time.

The closeness of the relation between retinol or riboflavin content and storage or heating time was determined by calculation of the correlation coefficient, (r).

Student's "T" Test was used to determine the significance of differences between means. The statistical procedures followed were as described by Bender et al., (1982).

RESULTS AND DISCUSSION

Proximate Composition of Raw and Processed Bovine Milk

Proximate analysis and values for non-fat solids and total solids of raw milk and pasteurized milk subjected to various treatments are shown in Table 4. The moisture content of the milk was slightly lower in the three samples that had been heated than in raw milk. This was true for the sample that was only pasteurized and also for the milk heated to 100°C for 10 seconds and the milk boiled at 100°C and subsequently held at 55°C for 2 hours. As expected, raw milk contained the most water (87.0%) and the least total solids (13.0%). In contrast, the sample which was heated to boiling and then held at 55°C for 2 hours contained the least water (84.6% and the most solids (15.2%).

Standard Curve for Vitamin A:

After calibration of the fluorometer, a standard curve of retinyl acetate was prepared. Table 5 shows the fluorescence units obtained for 6 concentrations of retinyl acetate in hexane ranging from 4 to 16 ug/100ml. The standard curves obtained at 330 nm and 360 nm are shown in Fig. 2. The standard curve obtained at 330 nm

was used to calculate the vitamin A values of the samples analyzed.

Recovery:

Recovery of added retinyl palmitate from pasteurized homogenized vitamin D milk is shown in Table 6. Retinyl palmitate was added at 25, 50, 75, and 100 ug/100 ml of milk. Average recovery was 99.7% with a range of 98.5 to 100%.

Effect of Refrigeration at 4°C on Vitamin A in Whole and Skim Milk:

This experiment measured the difference in vitamin A retention in whole milk stored under refrigeration at 4°C in plastic and in paperboard containers without exposure to light. A third sample consisted of skim milk containing added vitamins A and D, packaged in paperboard and also stored in the absence of light at 4°C. The vitamin A concentrations measured every 5 days over a total of 60 days are shown in Tables 7 and Fig. 3.

Vitamin A content of all samples decreased over the 60 day period. The vitamin A content was relatively stable over the first 5 days of storage in all samples of milk. The magnitude of the decrease was greater during

the 5 to 20 day period. Comparing the paperboard with plastic containers, the original samples of milk from plastic contained 41.78 ug retinol/100 ml and those in paperboard contained 43.16 ug retinol/100 ml. However, after 60 days of storage, the vitamin A content values were the same (30.04 ug retinol per 100 ml). Thus, there was no long term difference in vitamin A retention in milk packaged in either paperboard or plastic containers when held 30-60 days. Actual vitamin A loss over the 60 day period was 30.4% and 28.1% for paperboard and plastic containers, respectively. The similarity of the losses of vitamin A is also indicated by the quite similar slopes of the lines presented in Fig. 3, and correlation coeficients relating retinol content with time are given in Table 18.

Data for vitamin A loss over the 60-day period for skim milk containing added vitamins A and D are also given in Table 7. The samples contained 17.67 ug retinol/100ml at the start of the experiment and 8.84 ug retinol/100ml at the conclusion of the 60-day storage period. This is a loss of approximately 50%, and is greater than the losses found in the whole milk samples (28-30%). The loss is graphically shown in Figure 3. It is apparent that whole milk packaged in either paperboard

or plastic retained vitamin A better than skim milk stored in paperboard containers.

While measurement of quality was not an objective of this experiment, some observations relating to milk quality were made. After 15 day of storage, both closed paperboard and plastic containers started to swell indicating some gas production. When the containers were opened after 20 days, the milk samples in paperboard or in plastic containers had an unpleasant odor. After 25-30 days, some coagulation was noted with fluid rising to the top of the milk in the containers.

Effect of Storage at Room Temperature at 23°C on Vitamin A Whole and Skim Milk:

Table 8 shows the retention of vitamin A at intervals of 1, 2, 4, 6, 12, 18, 24, and 48 hours for whole milk packaged in either paperboard or plastic containers and stored at room temperature in the presence of room light. Retention is also shown for skim milk, containing added vitamins A and D, and packaged in paperboard. Originally, the milk had different concentrations of vitamin A. As shown in Table 8, the paperboard stored sample contained 43.96 ug retinol per

100 ml, and the plastic container stored sample contained 41.78 ug retinol per 100 ml. Losses of vitamin A in 12 hours were 14.3% and 15.3%, for paperboard and plastic containers, respectively. After 24 hrs, decreases were 18.2% and 23.1% for paperboard and plastic containers, respectively. At the end of the 48-hour storage period, milk in the paperboard container lost 26.3% of its vitamin A, while milk stored in plastic lost 30.1%. The two slopes are shown in Fig. 4, and correlation coefficient relating vitamin A content to time are given in Table 18.

Skim milk, containing added vitamins A and D, had a retinol content of 17.67 ug/100 ml at the start of the experiment. This decreased to 8.45 ug retinol per 100 ml at the end of the 48-hour experimental period. The losses were 31% at 12 hours, 49.0% at 24 hours and 52.2% for 48 hours. These are much higher than the losses found with whole milk. The retention is shown graphically in Fig. 4. However, the data obtained are not as linear as results from the other tests, as indicated by the lower correlation coefficient of -0.84 is shown in Table 18.

Both closed paperboard and plastic containers started to be inflated from gas production after 12-18 hours. The milk samples in both containers also had unpleasant odor

after 18-24 hours. Coagulative thickening was noted at 10-18 hours; water began to appear in the tops of both containers after 30-48 hours.

The results of this phase of the experiment indicate that vitamin A losses of whole milk stored at room temperature reached the 7-10% level at about 6 hours. The losses in whole milk were 14-15% in 12 hours, 18-23% in 24 hours and 26-30% in 48 hours. In all instances, the retention was 1 to 5% greater in paperboard. In fortified skim milk stored in paperboard, the losses were approximately double of those found with whole milk.

Effect of Holding Milk at 55°C After Boiling on Vitamin A Content:

Table 9 and Fig. 5 show the concentration of vitamin A at intervals of 2, 4, 6, 8 and 12 hours for whole milk obtained from paperboard or plastic containers and kept at 55°C in an open pan. Data are also shown for the skim milk containing added vitamins A and D obtained from paperboard containers. Originally, the milk had different concentration of vitamin A. As shown in Table 9, the milk from paperboard contained 42.21 ug retinol/100 ml, and from the plastic containers contained 41.78 ug retinol/100ml. After boiling for 10 seconds, the losses

were 6.4% and 9.6% for milk originally packaged in from paperboard and plastic containers, respectively. At the end of the 12-hour holding period, the milk from paperboard containers lost 29.0% of its vitamin A. Milk from plastic containers lost 29.3% of its original vitamin A content. The two slopes are shown in Fig. 5. Table 18 presents the correlation coefficients which relate retinol content to time.

Skim milk, containing added vitamins A and D, had an original retinol content of 17.67 ug retinol/100 ml which decreased 17% after boiling for 10 seconds. After 12 hours at 55°C, retinol content was reduced to 8.37 ug retinol/100 ml. The loss of retinol during this 12-hour period was 52.6%. This loss was much higher than that found for the whole milk samples. Vitamin A retention for the skim milk sample is shown graphically in Fig. 5. The correlation coefficient relating retinol content to storage time is shown in Table 18.

In this experiment, samples were observed at intervals for 12 hours. After 8-12 hours of holding at 55°C, the color of milk changed from white to light cream color.

This experiment showed that vitamin A losses of 6-10% were obtained from just boiling whole milk for 10 seconds. When held at 55°C in the presence of light in an open container for 12 hours, whole milk lost approximately 29% of its vitamin A content. Greater losses of vitamin A content were found with fortified skim milk. Losses due to boiling alone were 17%. After 12 hours holding at 55°C, the losses increased to 53%.

Effect of Twice Boiling and Then Holding Milk at 55°C on Vitamin A Content:

Table 10 and Fig. 6 show the concentration of vitamin A at intervals of 2, 4, 6, 8, and 12 hours for whole and fortified skim milk boiled twice, held at 55°C for 12 hours, boiled again, and then held again at 55°C for up to 12 hours. The milk was stored in the refrigerator between the two holding periods.

Originally, the milk had different concentration of vitamin A. As shown in Table 10, the milk obtained from paperboard contained 43.96 ug retinol/100 ml and the milk obtained from plastic packages contained 41.78 ug retinol/100 ml. After twice boiling and holding for 6 hours, vitamin losses were 26.3% and 27.5% for milk from

paperboard and plastic containers, respectively. After being held for 12 hours at 55°C milk from paperboard containers lost 30.4% of its vitamin A and milk obtained from plastic containers lost 33% of its original vitamin A content. The two slopes are shown in Fig. 6, and correlation coefficients relating retinol content to time are shown in Table 18.

Skim milk containing added vitamins A, originally contained 17.67 ug retinol/100 ml. This decreased 53% in 6 hours to a content of 8.32 ug retinol/100 ml. At the conclusion of the experiment, 12-hours, the retinol content was 8 ug/100 ml representing a loss of 54.7%. This loss was much higher than that found for the whole milk samples. Vitamin A retention for the skim milk sample is shown graphically in Fig. 6. A correlation coefficient for retinol content is given in Table 18.

The milk was examined visually during the second holding at 55°C for 12 hours. After 6 hours holding, the color of milk changed from white to cream color. Further holding at 55°C for 8-12 hours changed the color of the milk to light brown. The milk also began to increase in thickness because of water evaporation.

Comparison of vitamin A values between milk subjected to single or twice boiling and then holding at 55°C reveals that twice heating was slightly more detrimental. However, the losses attributed to twice holding at 55°C did not exceed 5% .

Effect of Boiling the Milk on its Vitamin A Content:

The effect of boiling at 100°C for 10 seconds on vitamin A in milk is shown in Table 11. The original vitamin A contents of raw, pasteurized whole milk and pasteurized skim milk were different. Retinol values ranged from 17.76 ug/100 ml for the skim milk to 51.05 ug/100 ml for the raw milk. Boiling these 3 samples of milk for only 10 seconds reduced vitamin A levels 1.4%, 11.4% and 17.4% in raw, whole and skim milk, respectively. A second boiling resulted in total losses of 13.8% , 15.5% and 18.5% in raw, whole and skim milk, respectively.

T-test analysis showed that the vitamin A content of raw milk, pasteurized whole milk and skim milk with added vitamins A and D (controls held at room temperature at 23°c) were significantly different ($P < 0.01$). There also was a significant difference between these three milks when held at room temperature and when treated with a single boiling. There was no significant difference

between single and twice heating at 100°C for 10 seconds for pasteurized whole milk and skim milk with vitamins A and D fortified. A significant difference ($P < 0.05$) was found in raw milk between samples subjected to single and twice heating to 100°C for 10 seconds. Thus, only in raw milk was there a difference between single and twice boiling. With pasteurized whole or skim milk, a second boiling did not result in further significant reductions in vitamin A content. These results indicate that the first boiling of milk has the most drastic effect on reducing vitamin A level.

Application of the Vitamin A Retention Results:

The results obtained on vitamin A retention confirm many reports in the literature on the stability of vitamin A under the influence of heat, light and packaging. Vitamin A was relatively stable after 5 days of storage in paperboard or plastic containers at a refrigeration temperature of 4°C. This would appear to be close to a normal holding time for milk in the U. S. or Saudi Arabia. However, when stored at 4° for 15 days, an unusually long storage time, vitamin A losses were 9% to 12% in whole milk and 14% for skim milk. The retention was slightly greater in paperboard than in plastic containers. These results agree with those of

(Cox et al., 1957) who reported that vitamin A in whole and skim milk gradually deteriorated at 40°F in dark storage.

Fragner et al (1956) indicated that exposure to light but not pasteurization will cause vitamin A loss. Bauernfend and Allen (1963) showed that depending on the time, boiling of milk can result in destruction of various amounts of vitamin A. Data from the review of (Hartman and Dryden, 1965) indicate that pasteurization can lower vitamin A by 20% or more, and that sterilization can destroy from 30% to 100% of the vitamin. In this experiment, a single boiling for only 10 seconds reduced vitamin A levels. The extent of loss was lowest (1.4%) in raw milk, intermediate in whole milk (10.2%) and greatest in vitamin A and D-fortified skim milk (17.0%). A second boiling for 10 seconds resulted in a further significant loss only in raw milk.

In Saudi Arabia, milk is often boiled more than once. A common handling procedure would be to boil the milk, hold it hot for a number of hours at 55°C in a pot, refrigerate at 4°C overnight, then reboil the milk and hold it hot at 55°C for a number of hours. As a duplication of the above, milk samples were given single

and twice heatings, and vitamin A loss measured. In this phase of the experiment, boiling for 10 seconds resulted in vitamin A losses of 6% for milk from paperboard and 10% for milk from plastic containers. During a 12-hours holding "hot" at 55°C, these two samples continued to decrease in vitamin A content until 29% of the original level was lost. The 12-hour period is not considered unusual.

A twice heating experiment consisting of two periods of boiling and holding the milk "hot" at 55°C attempted to duplicate a condition that can exist in Saudi food service establishments. The total losses of vitamin A from this procedure were as much as 28% in 6 hours and 33% after 12 hours. An important finding was that the second heating resulted in additional losses of only 1 to 5%. Apparently, after a certain loss under heating or storage, vitamin A becomes stabilized. This phenomenon was reported by Causeret et al.(1961)

While not recommended because of possible bacterial contamination, it is quite possible that milk may be stored at room temperature for many hours. In the experiment conducted, vitamin A losses were measured for

milk stored at 23°C for 1 to 48 hours. These losses were 7 to 10% in 6 hours, 14-15% in 12 hours, 18-23% in 24 hours and 26-30% in 48 hours. The lower value was for milk stored in paperboard containers. For skim milk stored in paperboard, the losses were approximately double those for whole milk. As reported by Birdsall et al. in (1958), vitamin A added to skim milk is susceptible to destruction because it is not protected by the fat, as in whole milk.

The obvious applications of this research are that the elimination of boiling and the hot or room temperature holding of milk would reduce vitamin A losses. As almost all Saudi milk is pasteurized and refrigerators are plentiful, these changes should cause no problems. Yet, it is apparent that an educational program is needed to get the dairies to put an expiration date on the milk and have the milk stored in the refrigerator except when used. Type of packaging presents no problem, as all of the milk in Saudi Arabia is packaged in paperboard. However, the practice of taking the milk out of the paperboard container, where it is somewhat protected from light, and storing it in an open pot container is to be discouraged.

A simple set of instruction suggested for the paperboard containers would read:

"Do not boil or heat. Keep in the refrigerator in the dark at 4°C when not in use."

Standard Curve for Riboflavin:

Table 12 shows the fluorescence units obtained for 4 concentrations of riboflavin in 0.02N acetic acid. The riboflavin concentrations were 0.05, 0.10, 0.15, and 0.20 ug/ml. The standard curve obtained at 330 nm is shown in Fig. 7.

Effect of Refrigeration at 4°C on Riboflavin in Whole and Skim Milk:

Table 13 and Fig. 8 show the riboflavin retention when pasteurized whole and skim milk were stored at 4°C for 1 to 60 days. The data show that there were no losses after 5 days of storage. After 10 days of refrigerated storage, the three milk samples lost similar amounts of riboflavin, precisely 5.7%, 5.8%, and 5.8%. At the end of the 60-day refrigeration period, losses were 15.3%, 16.0% and 15.4% for whole milk in paperboard, whole milk in plastic and skim milk in paperboard, respectively. These losses are presented graphically in

Fig. 8. Correlation coefficients relating riboflavin content with time are shown is Table 19.

The data obtained show that riboflavin in whole or skim milk stored under refrigeration in the dark in paperboard or plastic containers is relatively stable. Under practical conditions of refrigerated storage for 10 days losses of about 6% can be expected. For a longer refrigerated storage of 60 days, the losses of riboflavin can amount to about 16%.

A number of literature reports, including those by Josephson et al. (1946), Peterson et al (1944) and Stamberg and Theophilus (1945) confirm that little loss of riboflavin occurs when milk is stored in the dark. It was also reported by Burgwald and Josephson (1947), Stamberg and Theophilus (1945), and Theophilus and Stamburg (1947), that no riboflavin loss was found in milk stored in a refrigerator in the dark for 24 hours or longer. Burgwald and Josephson (1947) also reported that exposure of pasteurized milk to diffuse daylight for short periods of about 5 minutes each day for as many as 20 days did not affect riboflavin content.

[redacted]ct of Storage at Room Temperature at 23°C on [redacted]oflavin in Whole and Skim Milk:

Table 14 and Fig. 9 present riboflavin values for pasteurized homogenized whole milk, and skim milk stored at room temperature for 1 to 48 hours. After 12 hours of storage in the laboratory in the presence of light, losses of riboflavin were 10.2%, 10.3% and 9.6% for whole milk in paperboard, whole milk in plastic and skim milk in paperboard, respectively. At 48 hours, the losses for the above milk increased to 17.8%, 17.9% and 18.6%, respectively. The correlation coefficients which relate riboflavin content to time are given in Table 19.

These data verify that the riboflavin content of pasteurized milk is subject to only a small loss of about 10.2% when abused by storage at room temperature in the light for as long as 12 hours. After 48 hours at the above storage conditions, losses did not exceed 18.6%. It should be noted that there was no difference in riboflavin retention between whole milk packaged in either paperboard or plastic. The reason for this may be size and type of containers used. samples of whole milk and skim milk were packaged in paperboard, which offered [redacted]rotection from full intensity of the fluorescent and/or

sunlight. Another sample of whole milk was packaged in one gallon plastic containers. The size of this container reduced light exposure. On the other hand, the milks were never exposed to direct sunlight. As reported by Birdsall et al (1958), and Dunkley et al (1962) exposure to fluorescent light is less harmful than exposure to sunlight.

Effect of Holding Milk at 55°C After Boiling on Riboflavin Content:

Riboflavin values found in milks which had been boiled and then held at 55°C in a laboratory room for one to 12 hours are given in Table 15 and are shown graphically in Fig. 10. The data in Table 15 shows that boiling results in very little, if any, loss of riboflavin. After 6 hours at 55°C, riboflavin losses were 5.1%, 5.8% and 6.4% for whole milk from paperboard, whole milk from plastic and skim milk from paperboard, respectively. After 12 hours, the losses for the above milk increased to 10.2%, 11.5% and 13.5%, respectively. Correlation coefficients relating riboflavin content to time are shown in Table 19.

In this experimental phase, it is noteworthy that boiling to 100°C for 10 seconds did not alter riboflavin

content. Stamberg and Theophilus (1945) found that boiling milk for 30 minutes in a pan with a lid caused only a 1% decrease in riboflavin. As reported by Sure and Ford (1943) indicated that no decrease in riboflavin occurred when pasteurized milk was heated at 37°C for 22 hours. Experiments with raw milk gave similar results. In the present research, heating to 55°C for 12 hours resulted in riboflavin losses which ranged from 10.2% to 13.5%. Undoubtedly, the losses obtained in the milk kept hot at 55°C in an uncovered pot were influenced by the intensity of the existing light.

Effect of Twice Boiling and Then Holding Milk at 55°C on Riboflavin Content:

Table 16 and Fig. 11 show riboflavin content and two boilings and two holding periods at 55°C in an open vessel in a laboratory room. There was little loss of the vitamin even after two boilings. After 4 hours of holding at 55°C, the losses ranged from 10.2% to 11.9%. After 6 hours, losses were 13.4%, 14.1% and 14.1% for whole milk from paperboard, whole milk from plastic container and skim milk from paperboard container respectively. In 12 hours, these losses increased to 20.4%, 19.9% and 20.5%, for whole milk from paperboard,

whole milk from plastic containers and skim milk from paperboard containers, respectively. Correlation coefficient relating riboflavin content to time are given in Table 19.

In spite of the drastic treatment of twice boiling and two period of holding at the "hot" temperatures of 55°C for 12 hours the riboflavin loss did not exceed 20.5%. This attests to the good heat-stability of riboflavin.

A comparison can be made of the effect of a single vs. a double holding period at 55°C. Examination of the data in Tables 15 and 16 shows that double holding at 55°C approximately double the riboflavin loss. For example, comparing the 12 hour storage period for whole or skim milk from paperboard shows a 10% loss from a single holding and a 20% for the double holding. These results demonstrate that such treatment should be avoided if possible.

[redacted]t of Boiling Milk on Riboflavin Content:

A comparison of the effects of a single or double boiling to 100°C for 10 seconds is shown in Table 17. The raw milk, used as one of the controls, contained the

most riboflavin (2.57 ug/gm). The pasteurized whole and skim milk contained 1.56 - 1.57 ug/gm. The losses for raw milk amounted to 1.9% after the first boiling and 3.1% after the second boiling. Losses for both whole and skim milk were 1.3% after the first boiling. After the second boiling, the losses were 3.8% for whole milk and 3.2% for skim milk. Thus, the boiling reduced riboflavin to approximately the same extent in raw, pasteurized whole and pasteurized skim milk. While the magnitude of the losses did not exceed 2% for the first boiling or 4% after the second boiling.

Application of the Riboflavin Retention Results

The results obtained under different heating, storage, temperature and packaging conditions are in agreement with literature reports that light, but not heating temperature, has the most influence on retention of riboflavin in milk. As reported by a number of investigators including Hellstrom (1960) and Holmes et al. (1943), riboflavin in milk is quite stable to heat in the absence of strong light. In the experiments conducted, light exposure was limited to the use of paperboard containers, one gallon plastic containers, and a large laboratory room which excluded any direct sunlight. While there was exposure to fluorescent light,

it is less destructive to riboflavin than direct sunlight. It must also be mentioned that it was impossible to achieve precise control of light conditions.

Under usual conditions of handling and storage in the U.S., riboflavin losses obtained were not large and did not appear to be very much influenced by packaging in paperboard or in plastic containers. As a typical example, storage under refrigeration at 4°C for 15 days resulted in a riboflavin loss of approximately 7.0% in pasteurized whole or skim milk. Another example is that after storage for 12 hours at room temperature at 23°C, losses were 10.2%, 10.3% and 9.6% for whole milk in paperboard, whole milk in plastic and skim milk in paperboard, respectively.

Under conditions duplicating those practiced in Saudi Arabia, which include boiling and keeping the milk hot, the riboflavin content was remarkably resistant to destruction by heat. A single boiling of 100°C for 10 seconds of raw, pasteurized whole or pasteurized skim milk produced losses not over 2%. A double boiling of the above milks increased riboflavin loss to not more than 3.8%. Nevertheless, with pasteurization and refrigeration, boiling is unnecessary.

A more drastic treatment of milk, common in Saudi Arabia, is to boil milk and then hold it hot at about 55°C for as long as 12 hours. When done in the laboratory as part of this research, riboflavin losses ranged from 10.2% to 13.5%. These losses cannot be considered severe.

In Saudi Arabia it is possible for boiled and held "hot" at 55°C milk to be put away for the night in the refrigerator, and taken out in the morning and be re-boiled and held "hot' again for another 12 hours. Under these double boiling and holding at 55°C conditions, riboflavin losses did not exceed 20.5%. This double boiling and holding at 55°C resulted in about double the riboflavin losses obtained from a single boiling and holding at 55°C.

Practical measures to attain maximum retention of riboflavin content in Saudi Arabia are as follows:

1. Continue packaging of pasteurized milk in paperboard container containing an expiration date.

2. Attempt to educate all handlers and consumers that boiling and any further heating are unnecessary, as heating lowers vitamin content.
3. Attempt to educate consumers that milk is best stored in the refrigerator in the dark, and that light lowers vitamin content.

SUMMARY AND CONCLUSIONS

A series of experiments were conducted to determine the effect of different heating and storage temperatures, and packing on vitamin A and riboflavin retention in milk. The experiments were designed to determine the effects of a number of milk handling practices common in Saudi Arabia such as boiling milk and holding it hot for a number of hours.

Consideration of the results from a series of 5 experiments permits reaching the following conclusion:

1. Vitamins A and riboflavin are quite stable when stored under refrigeration. Storage under refrigeration at 4°C in the dark for 15 days resulted in losses of vitamin A ranging from 9-12 percent in pasteurized whole milk. Pasteurized skim milk, fortified with vitamins A and D, had a 14% loss of vitamin A. Riboflavin losses did not exceed 8%.

2. Vitamin A is more labile than riboflavin when stored at room temperature at 23°C in the presence of light and oxygen. After 12 hours of storage, pasteurized whole milk packaged in either paperboard or plastic lost 14-15% of its vitamin A content. Losses of vitamin A in skim milk in paperboard were 37%. In

contrast, the losses of riboflavin of whole and skim milk were about 10%.

3. Boiling of milk to 100°C for 10 seconds, a common practice in Saudi Arabia, resulted in more destruction of vitamin A than riboflavin. Boiling of pasteurized whole milk resulted in a vitamin A loss of 10.2% for a single boiling and a 14.4% loss for twice boiling. Losses for skim milk were 17.4% for a single boiling and 28.5% for twice boiling. Riboflavin content was much more resistant to heat destruction. Losses did not exceed 2% for a single boiling and 4% for twice boiling.

4. The Saudi practice of boiling milk and then holding it hot is quite destructive to vitamin A content but not as significant to riboflavin content. After 12 hours of holding in the heat at 55°C, vitamin A losses were 29% for whole milk from paperboard or plastic containers. Vitamin A loss in fortified skim milk amounted to 53%. On the other hand, riboflavin losses of whole milk 10-12% and skim milk did not exceed 14%.

5. A double cycle of boiling and holding milk hot at 55°C resulted in the largest losses of both vitamin A and riboflavin. After the second 12-hour holding period at 55°C, vitamin A losses for whole milk were 30-33%. In fortified skim milk the loss was 55%. This treatment resulted in losses of riboflavin from whole and skim milk of approximately 20%.

6. It is apparent that vitamin A was more susceptible to high temperature than riboflavin. In the absence of strong light, riboflavin is quite stable during heating. Under the experimental conditions used, the milk samples were not exposed to sunlight or to strong light. However, precise control of light was not possible.

7. While the literature gives evidence of the protective effect of paperboard containers over plastic containers, the results obtained in this experiment did not show any difference. This may have been due to the absence of strong light and the use of one gallon containers.

8. Recommendations for handling of milk in Saudi Arabia to maximize vitamins A and riboflavin retention include:

(1) Continue packaging of pasteurized milk in paperboard containers and provide an expiration date.

(2) Educate all consumers to stop boiling milk and that any further heating is unencessary because it lowers the vitamin content of the milk.

(3) Educate consumers that pasteurized milk is best stored in the refrigerator in the dark immediately after purchase because light and temperature lower the vitamin content in the milk.

9. Further experimentation is suggested which would include more precise control of light. The milks should also be evaluated by organoleptic and microbiological tests.

Table 4. Proximate Composition of Raw and Processed Bovine Milk

Sample	Water %	Fat %	Protein %	Carbohydrate %	Ash %	Non-Fat Solids %	Total Solids %
Raw Milk	87.0*	3.6	3.4	5.3	0.71	9.4	13.0
Whole Milk Stored at 23°C	86.0	3.5	3.5	6.3	0.71	10.5	14.0
Whole Milk after heating to 100°C for 10 seconds	85.7	3.4	3.6	6.5	0.72	10.8	14.2
Whole Milk after Boiling at 100°C; and heating at 55°C for 2 hr.	84.6	3.4	3.8	7.2	0.72	11.7	15.2

*Means are for 4 replicates.

Table 5. Fluorescence Units Obtained from Various Concentrations of Retinyl Acetate in Hexane

Concentration µg/100 ml	Fluorescence
Blank	--
4	0.12±0.00*
6	0.18±0.00
8	0.25±0.02
10	0.31±0.01
14	0.41±0.02
16	0.48±0.01

*Means are for 8 replicates measured at 330 nm excitation.

Table 6. Recovery of Added Retinyl Palmitate (Vitamin A Palmitate) from Pasteurized Homogenized Vitamin D Milk.

Retinyl Palmitate (Retinol) Added µg/100ml, milk	Total Found in Milk ug Retinol/100ml, Milk	Percent Recovery
0	42*	--
25	66	98.5
50	92	100.0
75	117	100.0
100	141	99.3

*Means are for 8 Replicates

Table 7. Effect of Refrigeration at 4°C on Vitamin A in Whole and Skim Milk.

Sample	µg Retinol/100ml - Days									
	0	5	10	15	20	25	30	40	50	60
Paperboard Container:										
Pasteurized Homogenized Vitamin D Whole Milk	43.16* ±1.38	42.51 ±0.75	40.32 ±0.93	37.96 ±0.78	35.59 ±0.85	33.95 ±1.03	32.32 ±1.01	31.59 ±0.85	30.59 ±1.40	30.04 ±1.48
Plastic Container:										
Pasteurized Homogenized Vitamin D Whole Milk	41.78 ±1.44	40.69 ±0.65	38.96 ±1.10	37.87 ±0.99	36.14 ±0.95	35.05 ±1.03	32.95 ±0.99	31.77 ±0.55	30.31 ±1.03	30.04 ±1.48
Paperboard Container:										
Pasteurized Homogenized Skim Milk (nonfat) Vitamins A and D Added	17.67 ±0.72	17.12 ±0.38	15.94 ±0.75	15.21 ±1.15	14.21 ±0.54	12.57 ±0.98	11.57 ±0.65	10.66 ±1.51	9.83 ±1.51	8.84 ±1.06

*Means are for 8 replicates.

Table 8. Effect of Storage in Room Temperature at 23°C on Vitamin A in Whole and Skim Milk.

Sample[1]	µg Retinol/100 ml								
	Hours								
	0	1	2	4	6	12	18	24	48
Paperboard Container:									
Pasteurized Homogenized Vitamin D Whole Milk	43.96[2] ±4.08	43.50 ±1.03	43.14 ±0.67	41.78 ±0.93	40.87 ±0.55	37.68 ±0.67	37.32 ±0.26	35.96 ±0.93	32.41 ±1.29
Plastic Container:									
Pasteurized Homogenized Vitamin D Whole Milk	41.78 ±1.44	41.10 ±1.10	39.96 ±0.93	38.32 ±0.95	37.50 ±1.54	35.50 ±1.23	32.86 ±1.10	32.13 ±1.03	29.22 ±1.10
Paperboard Container:									
Pasteurized Homogenized Skim Milk (nonfat) Vitamins A and D Added	17.67 ±0.72	17.03 ±1.21	15.48 ±0.99	14.30 ±0.67	14.12 ±0.93	11.12 ±0.82	9.57 ±0.67	9.02 ±0.01	8.45 ±0.01

1. Sample was kept in laboratory containing fluorescent light and window light. 2. Means are for 8 replicates.

Table 9. Effect of Holding Milk at 55°C After Boiling for 10 Seconds on Vitamin A Content

Sample	µg Retinol/100 ml						
	Hours						
	0[1]	single boiling[2]	2	4	6	8	12
Pasteurized Homogenized Vitamin D Whole Milk from Paperboard Container	42.21[3] ±1.15	39.50 ±1.43	36.96 ±1.10	34.77 ±1.02	32.13 ±1.03	31.59 ±0.85	29.95 ±1.56
Pasteurized Homogenized Vitamin D Whole Milk from Plastic Container	41.78 ±1.44	37.78 ±1.48	35.96 ±1.39	33.24 ±1.335	32.32 ±1.49	31.50 ±1.58	29.55 ±0.97
Pasteurized Homogenized Skim Milk (nonfat) Vitamins A and D added from Paperboard Containers	17.67 ±0.72	14.67 ±0.78	12.12 ±1.29	10.66 ±1.03	9.66 ±1.48	8.64 ±0.64	8.37 ±0.83

1. Before boiling. 2. After boiling to 100°C for 10 seconds. 3. Means are for 8 replicates.

Table 10. Effect of Twice Boiling and Then Holding Milk at 55°C for Various Times on Vitamin A Content

Sample	µg Retinol/100 ml						
	Hours						
	0[1]	After Twice boiling[2]	2	4	6	8	12
Pasteurized Homogenized Vitamin D Whole Milk from Paperboard Container	43.96[3] ±1.08	37.23 ±0.67	35.32 ±1.02	34.40 ±1.13	32.40 ±0.95	31.86 ±1.29	30.58 ±1.42
Pasteurized Homogenized Vitamin D Whole Milk from Plastic Container	41.78 ±1.44	35.94 ±1.27	32.77 ±1.13	31.86 ±0.67	30.31 ±1.03	29.22 ±1.01	27.94 ±0.75
Pasteurized Homogenized Skim Milk (nonfat) Vitamins A and D added from Paperboard Containers	17.67 ±0.72	12.69 ±1.31	11.23 ±0.36	9.57 ±0.84	8.32 ±0.36	8.11 ±0.00	8.00 ±0.01

1. Before boiling. 2. Samples were boiled for 10 seconds, refrigerated for 24 hours and reboiled for 10 seconds. 3. Means are for 8 replicates.

Table 11. Effect of Boiling at 100°C for 10 Seconds on Vitamin A in Milk

Sample	µg Retinol/100 ml		
	Control Held at 23°C	Single Heating to 100°C for 10 seconds	Double Heating to 100°C for 10 seconds[1]
Raw Milk	51.05 ± 1.00[2]	50.33 ± 1.05	43.96 ± 1.08
Pasteurized Homogenized Vitamin D Whole Milk	43.50 ± 0.67	39.05 ± 1.43	37.23 ± 0.67
Pasteurized Homogenized Skim Milk (nonfat) Vitamins A and D added	17.76 ± 0.75	14.76 ± 0.78	12.69 ± 1.31

1. Samples were boiled for 10 seconds, refrigerated for 24 hours and reboiled for 10 seconds. 2. Means are for 8 replicates.

Table 12. Fluorescence Unit Obtained From Various Concentrations Riboflavin in 0.02N Acetic Acid

Concentration µg/ml	Fluorescence
Blank	-
0.05	0.20 ± 0.01
0.10	0.41 ± 0.01
0.15	0.60 ± 0.01
0.20	0.80 ± 0.01

*Means are for 8 replicates, measured at 330 nm excitation.

Table 13. Effect of Refrigeration at 4°C on Riboflavin in Whole and Skim Milk.

Sample	µg/gm									
	Day									
	0	5	10	15	20	25	30	40	50	60
Paperboard Container:										
Pasteurized Homogenized Vitamin D Whole Milk	1.57* ±0.02	1.57 ±0.01	1.48 ±0.00	1.45 ±0.01	1.41 ±0.01	1.39 ±0.39	1.38 ±0.00	1.38 ±0.01	1.34 ±0.00	1.33 ±0.01
Plastic Container:										
Pasteurized Homogenized Vitamin D Whole Milk	1.56 ±0.01	1.54 ±0.01	1.47 ±0.01	1.45 ±0.01	1.40 ±0.01	1.39 ±0.01	1.37 ±0.01	1.35 ±0.01	1.33 ±0.01	1.31 ±0.01
Paperboard Container:										
Pasteurized Homogenized Skim Milk (nonfat) Vitamins A and D Added	1.56 ±0.02	1.55 ±0.01	1.47 ±0.01	1.45 ±0.01	1.41 ±0.01	1.39 ±0.01	1.37 ±0.01	1.35 ±0.01	1 .34 ±0.01	1.32 ±0.01

*Means are for 8 replicates.

Table 14. Effect of Storage in Room Temperature at 23°C on Riboflavin in Whole and Skim Milk.

Sample[1]	µg/gm								
	Hours								
	0	1	2	4	6	12	18	24	48
Paperboard Container:									
Pasteurized Homogenized Vitamin D Whole Milk	1.57[2] ±0.02	1.54 ±0.01	1.52 ±0.01	1.45 ±0.00	1.43 ±0.01	1.41 ±0.01	1.38 ±0.01	1.36 ±0.00	1.29 ±0.01
Plastic Container:									
Pasteurized Homogenized Vitamin D Whole Milk	1.56 ±0.01	1.52 ±0.02	1.49 ±0.01	1.45 ±0.01	1.43 ±0.01	1.40 ±0.01	1.37 ±0.01	1.35 ±0.01	1.28 ±0.02
Paperboard Container:									
Pasteurized Homogenized Skim Milk (nonfat) Vitamins A and D Added	1.56 ±0.02	1.53 ±0.01	1.50 ±0.01	1.45 ±0.01	1.43 ±0.01	1.41 ±0.01	1.39 ±0.01	1.35 ±0.01	1.27 ±0.01

1. Sample was kept in laboratory containing fluorescent light and window light. 2. Means are for 8 replicates.

Table 15. Effect of Holding Milk at 55°C After Boiling for 10 Seconds on Riboflavin Content.

Sample	µg/gm						
	Hours						
	0(1)	single boiling(2)	2	4	6	8	12
Pasteurized Homogenized Vitamin D Whole Milk From Paperboard Containers	1.57(3) ±0.02	1.54 ±0.01	1.53 ±0.75	1.51 ±0.93	1.49 ±0.93	1.46 ±0.01	1.41 ±1.03
Pasteurized Homogenized Vitamin D Whole Milk from Plastic Containers	1.56 ±0.01	1.53 ±0.01	1.51 ±0.72	1.49 ±0.38	1.47 ±1.15	1.44 ±0.98	1.38 ±0.65
Pasteurized Homogenized Skim Milk (nonfat) Vitamins A and D added from Paperboard Containers	1.56 ±0.02	1.54 ±0.01	1.51 ±0.38	1.48 ±1.15	1.46 ±0.98	1.41 ±1.03	1.35 ±1.05

1. Before boiling. 2. After boiling to 100°C for 10 seconds. 3. Means are for 8 replicates.

Table 16. Effect of Twice Boiling and Then Holding Milk at 55°C for Various Times on Riboflavin Content.

Sample	µg/gm						
	Hours						
	0[1]	after twice boiling[2]	2	4	6	8	12
Pasteurized Homogenized Vitamin D Whole Milk from Paperboard Containers	1.57[3] ±0.05	1.51 ±0.01	1.45 ±0.01	1.41 ±0.01	1.36 ±0.00	1.32 ±0.01	1.25 ±0.01
Pasteurized Homogenized Vitamin D Whole Milk from Plastic Containers	1.56 ±1.01	1.49 ±0.01	1.44 ±0.01	1.39 ±0.01	1.34 ±0.01	1.29 ±0.01	1.25 ±0.01
Pasteurized Homogenized Skim Milk (nonfat) Vitamins A and D added from Paperboard Containers	1.56 ±0.02	1.51 ±0.01	1.44 ±0.01	1.39 ±0.01	1.34 ±0.01	1.30 ±0.01	1.24 ±0.01

1. Before boiling. 2. Samples were boiled for 10 seconds, refrigerated for 24 hours, and reboiled for 10 seconds. 3. Means are for 8 replicates.

Table 17. Effect of Boiling at 100°C for 10 Second on Riboflavin in Milk

Sample	µg/gm		
	Control Held at 23°C	Single Heating to 100°C for 10 seconds	Double Heating to 100°C for 10 seconds[1]
Raw Milk	2.57 ± 0.01[2]	2.52 ± 0.00	2.49 ± 0.01
Pasteurized Homogenized Vitamin D Whole Milk	1.57 ± 0.02	1.55 ± 0.01	1.51 ± 0.01
Pasteurized Homogenized Skim Milk (nonfat) Vitamins A and D added	1.56 ± 0.02	1.54 ± 0.01	1.51 ± 0.01

1. Samples were boiled for 10 seconds, refrigerated for 24 hours and reboiled for 10 seconds. 2. Means are for 8 replicates.

Table 18. Linear Regression Analysis of Vitamin A in Milk

Sample	Slope B	Intercept A	Correlation Coefficient r
1. Storage after Refrigeration at 4°C			
Paperboard Container			
Whole Milk	-0.24	42.07	-0.93
Plastic Container			
Whole Milk	-0.21	40.96	-0.97
Paperboard Container			
Fortified Skim Milk Vitamins A & D added	-0.16	17.45	-0.98
2. Storage in Room Temperature at 23°C			
Paperboard Container			
Whole Milk	-0.24	42.56	-0.93
Plastic Container			
Whole Milk	-0.24	39.37	-0.92
Paperboard Container			
Fortified Skim Milk Vitamins A & D added	-.17	14.82	-0.84

Table 18. (Continued)

Sample	Slope B	Intercept A	Correlation Coefficient r
3. Holding Milk at 55°C after boiling			
Whole Milk From Paperboard Container	-0.80	38.43	-0.96
Whole Milk From Plastic Container	-0.73	37.47	-0.96
Fortified Skim Milk Vitamins A & D added From Paperboard Container	-0.51	13.41	-0.92
4. Twice Holding Milk at 55°C After Twice Boiling at 100°C for 10 Seconds			
Whole Milk From Paperboard Container	-0.55	36.59	-0.97
Whole Milk From Plastic Container	-0.63	34.71	-0.96
Fortified Skim Milk Vitamins A & D added From Paperboard Container	-0.40	11.79	-0.90

Table 19. Linear Regression Analysis of Riboflavin in Milk

Sample	Slope B	Intercept A	Correlation Coefficient r
1. Storage after Refrigeration at 4°C			
Paperboard Container			
Whole Milk	-0.01	1.53	-0.91
Plastic Container			
Whole Milk	-0.00	1.52	-0.94
Paperboard Container			
Fortified Skim Milk Vitamins A & D added	-0.01	1.52	-0.93
2. Storage in Room Temperature at 23°C			
Paperboard Container			
Whole Milk	-0.01	1.49	-0.92
Plastic Container			
Whole Milk	-0.01	1.48	-0.94
Paperboard Container			
Fortified Skim Milk Vitamins A & D added	-0.01	1.49	-0.95

Table 19. (Continued)

Sample	Slope B	Intercept A	Correlation Coefficient r
3. Holding Milk at 55°C after boiling			
Whole Milk From Paperboard Container	-0.01	1.55	-0.98
Whole Milk From Plastic Container	-0.01	1.53	-0.99
Fortified Skim Milk Vitamins A & D added From Paperboard Container	-0.01	1.54	-0.98
4. Twice Holding Milk at 55°C After Twice Boiling at 100°C for 10 Seconds			
Whole Milk From Paperboard Container	-0.02	1.50	-0.99
Whole Milk From Plastic Container	-0.02	1.48	-0.98
Fortified Skim Milk Vitamins A & D added From Paperboard Container	-0.01	1.49	-0.99

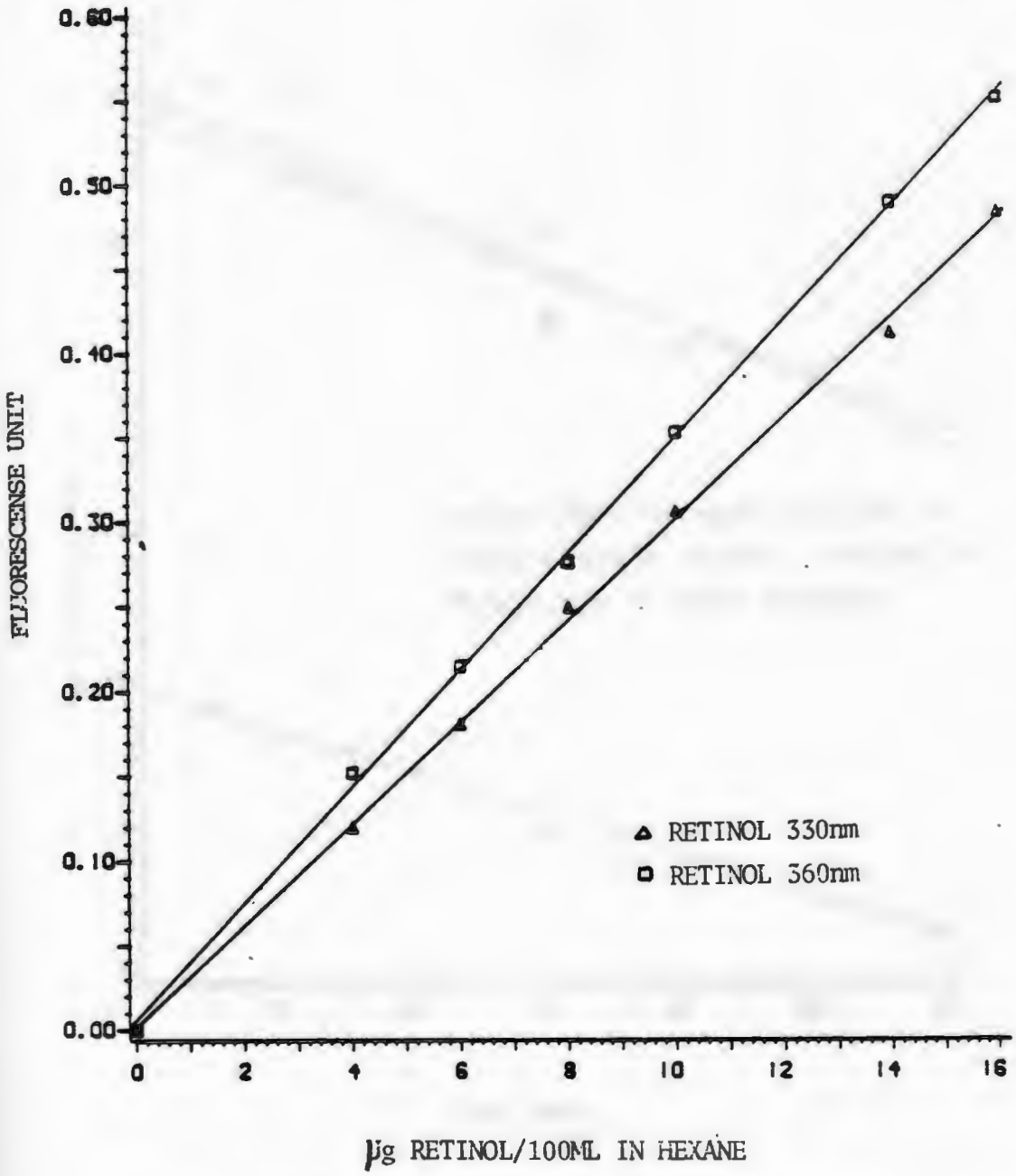


Fig. 2. Standard curve of retinol equivalents obtained from graded levels of retinyl acetate in hexane.

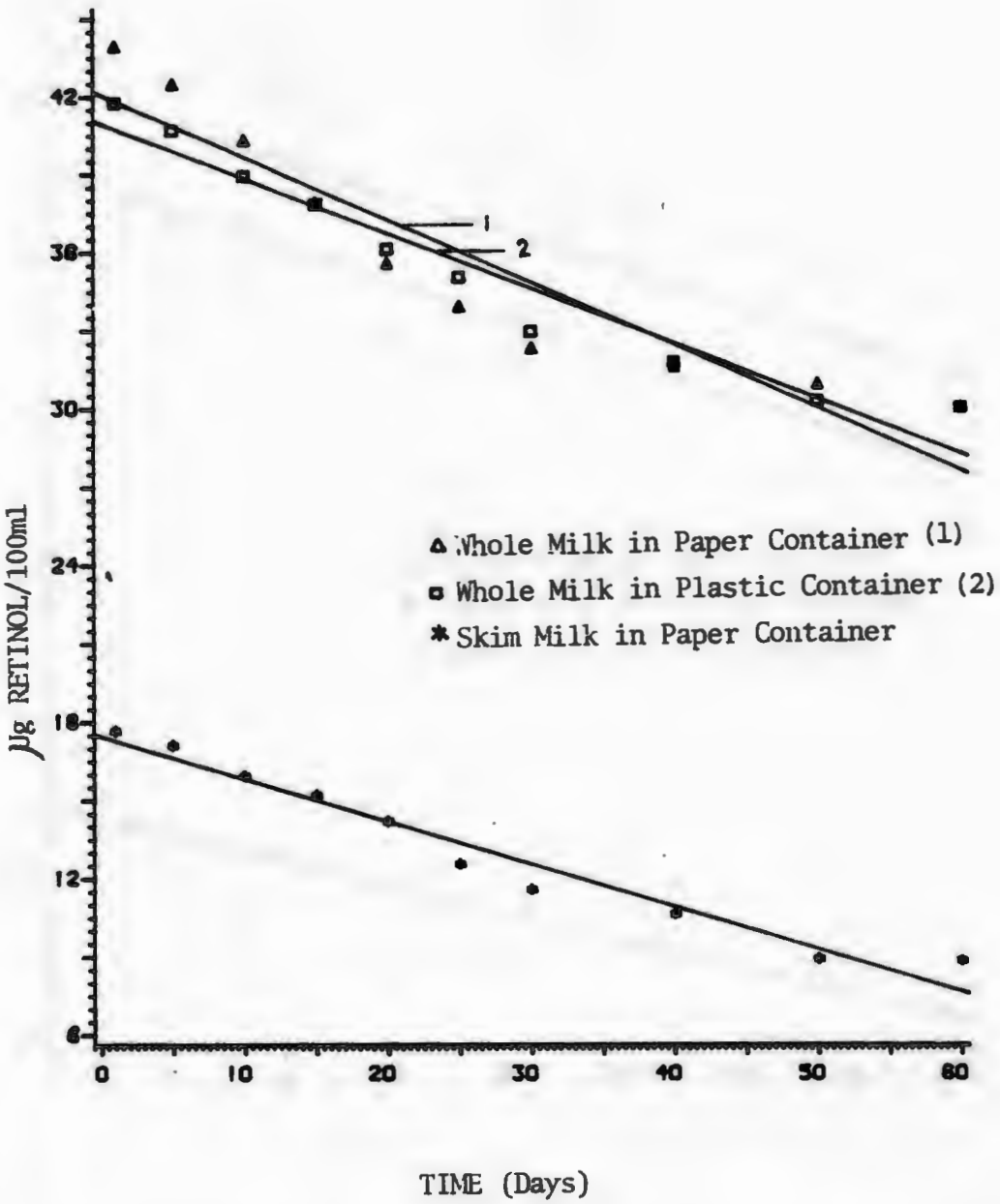


Fig. 3. Effect of refrigeration at 4°C on vitamin A in whole and skim milk.

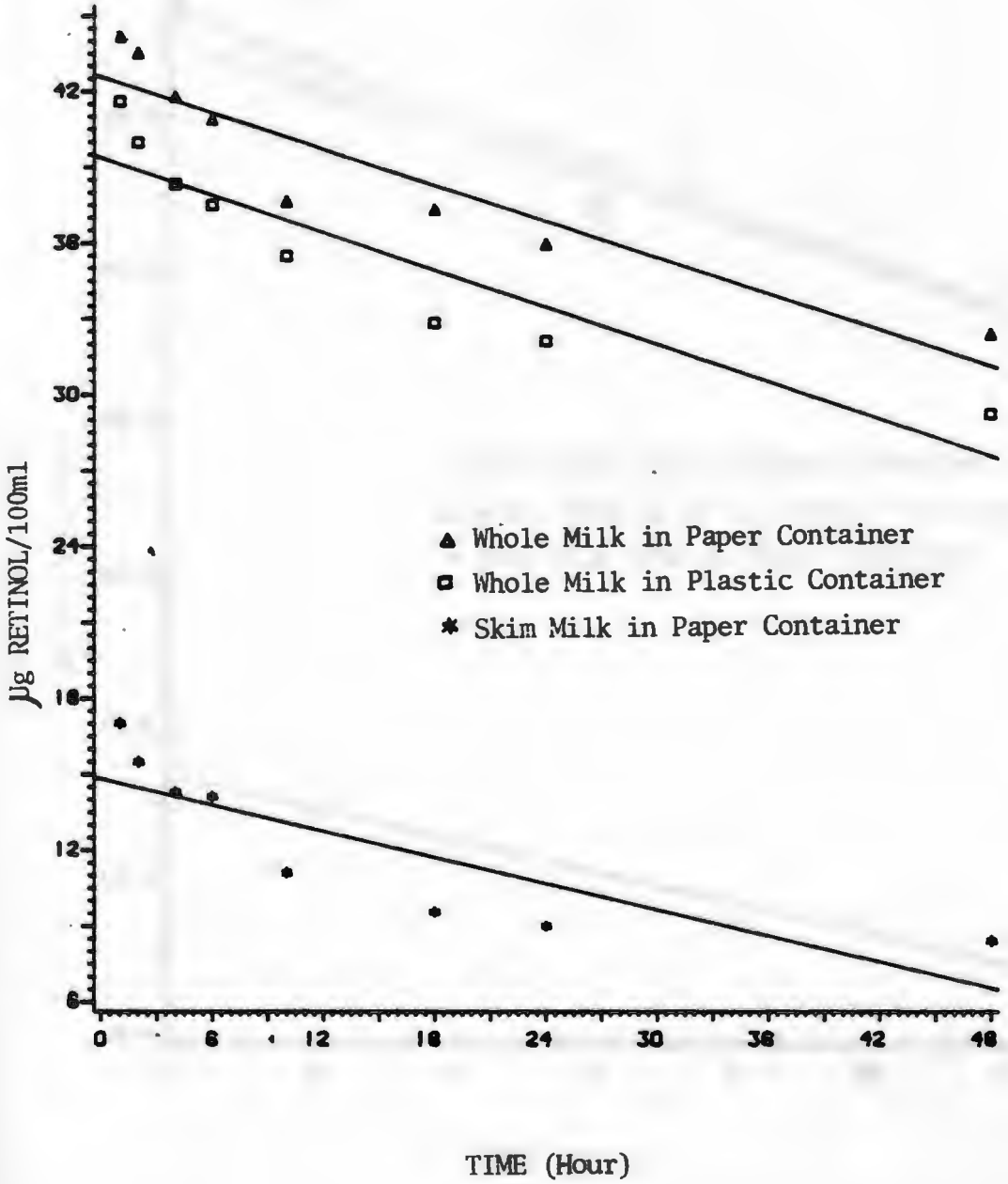


Fig. 4. Effect of storage at room temperature (23°C) on vitamin A in whole and skim milk.

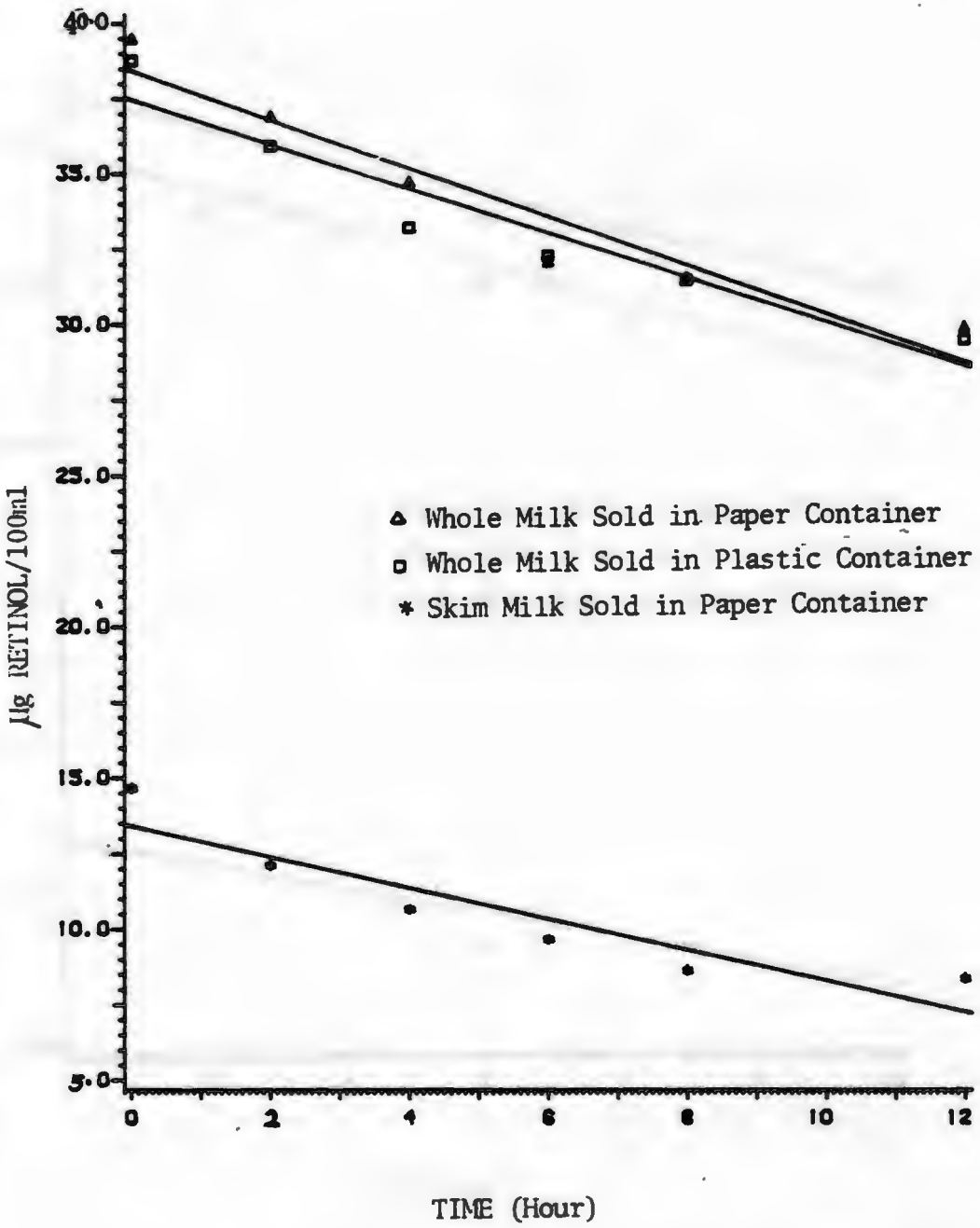


Fig. 5. Effect of holding milk at 55°C after boiling for 10 seconds on vitamin A content.

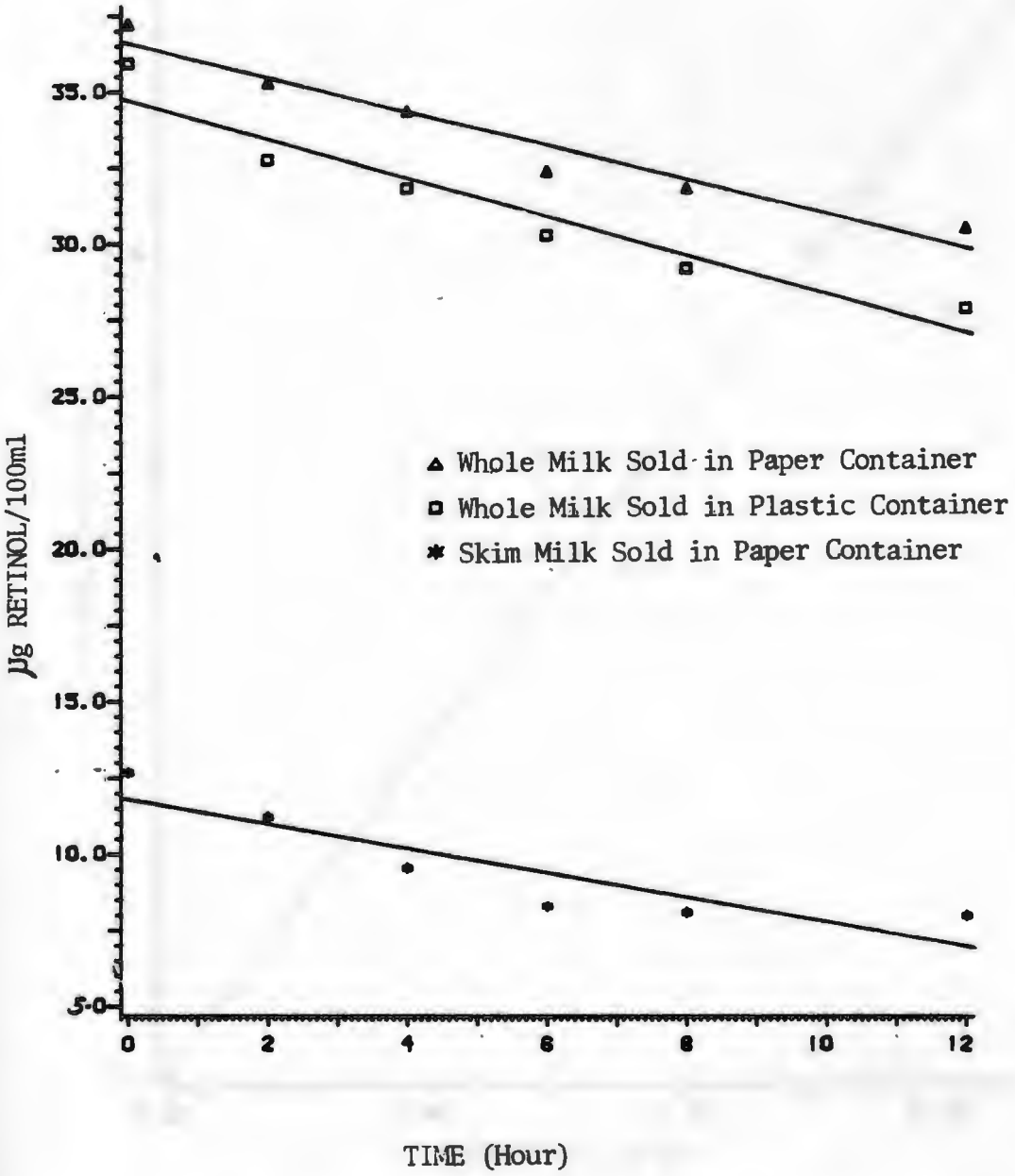


Fig. 6. Effect of twice boiling and then holding milk at 55°C on vitamin A content.

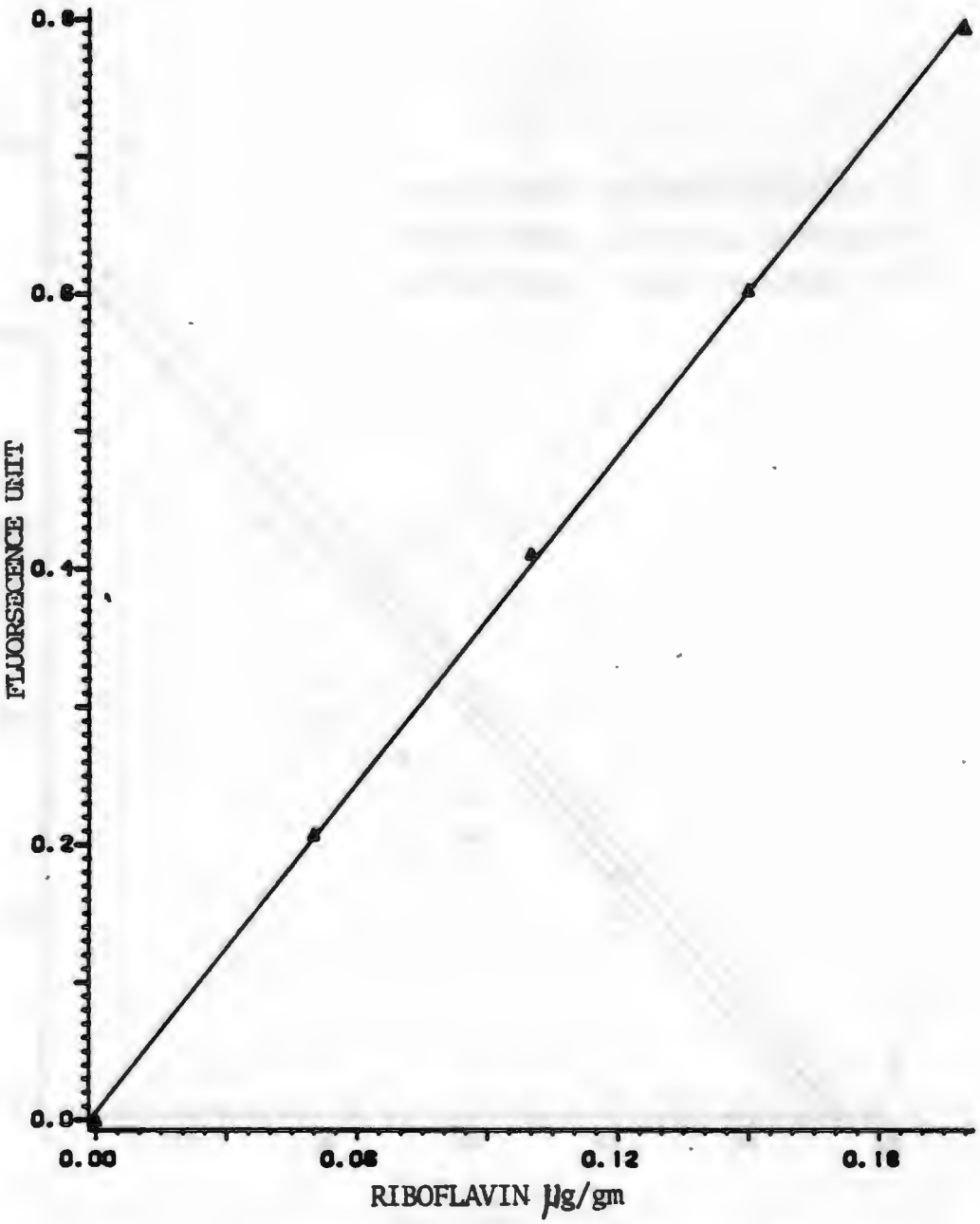


Fig. 7. Fluorescence Units Obtained from Various Concentrations of Riboflavin in 0.02N Acetic Acid (HOAC).

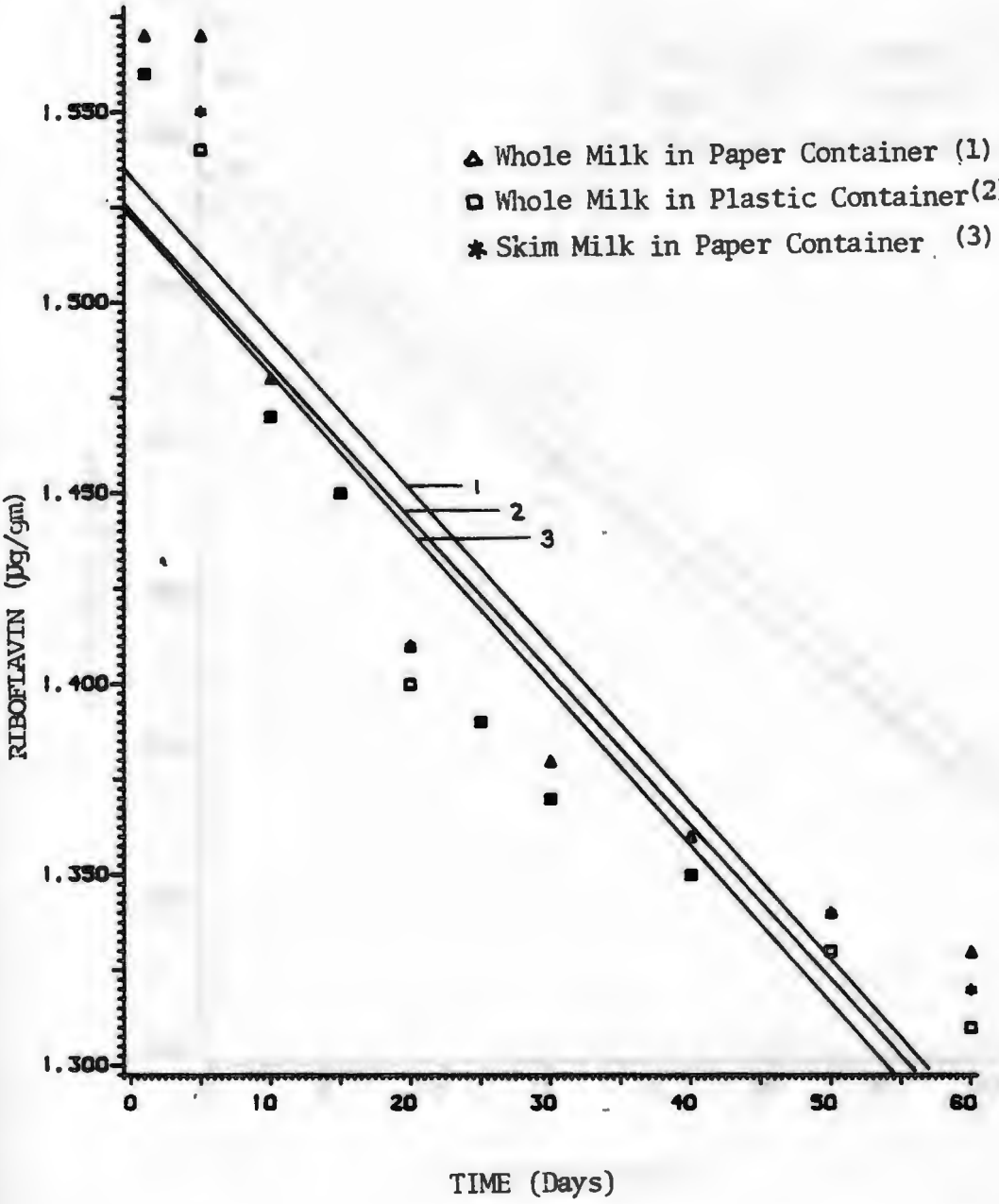


Fig. 8. Effect of refrigeration at 4°C on riboflavin in whole and skim milk.

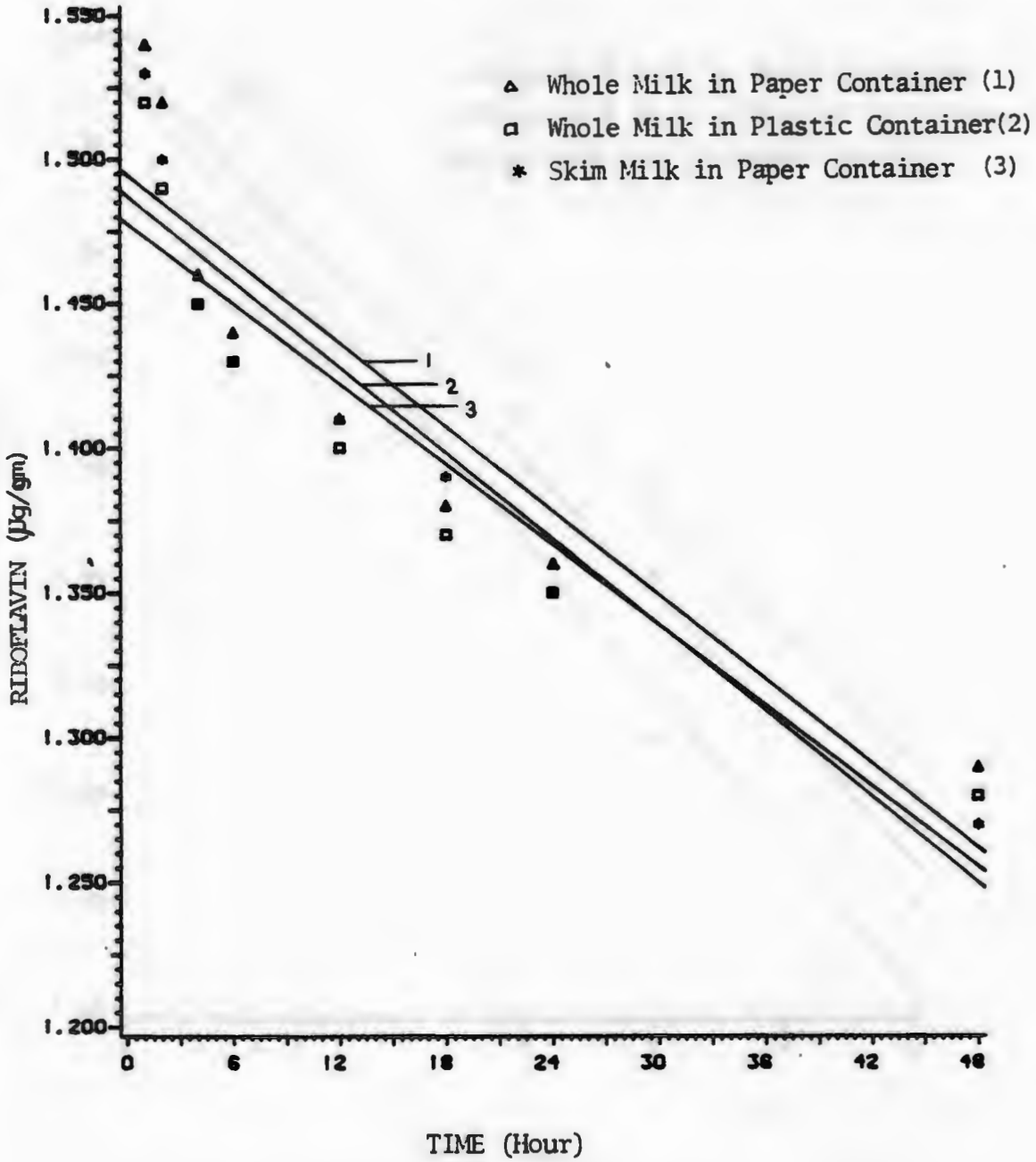


Fig. 9. Effect of storage at room temperature (23°C) on riboflavin in whole and skim milk.

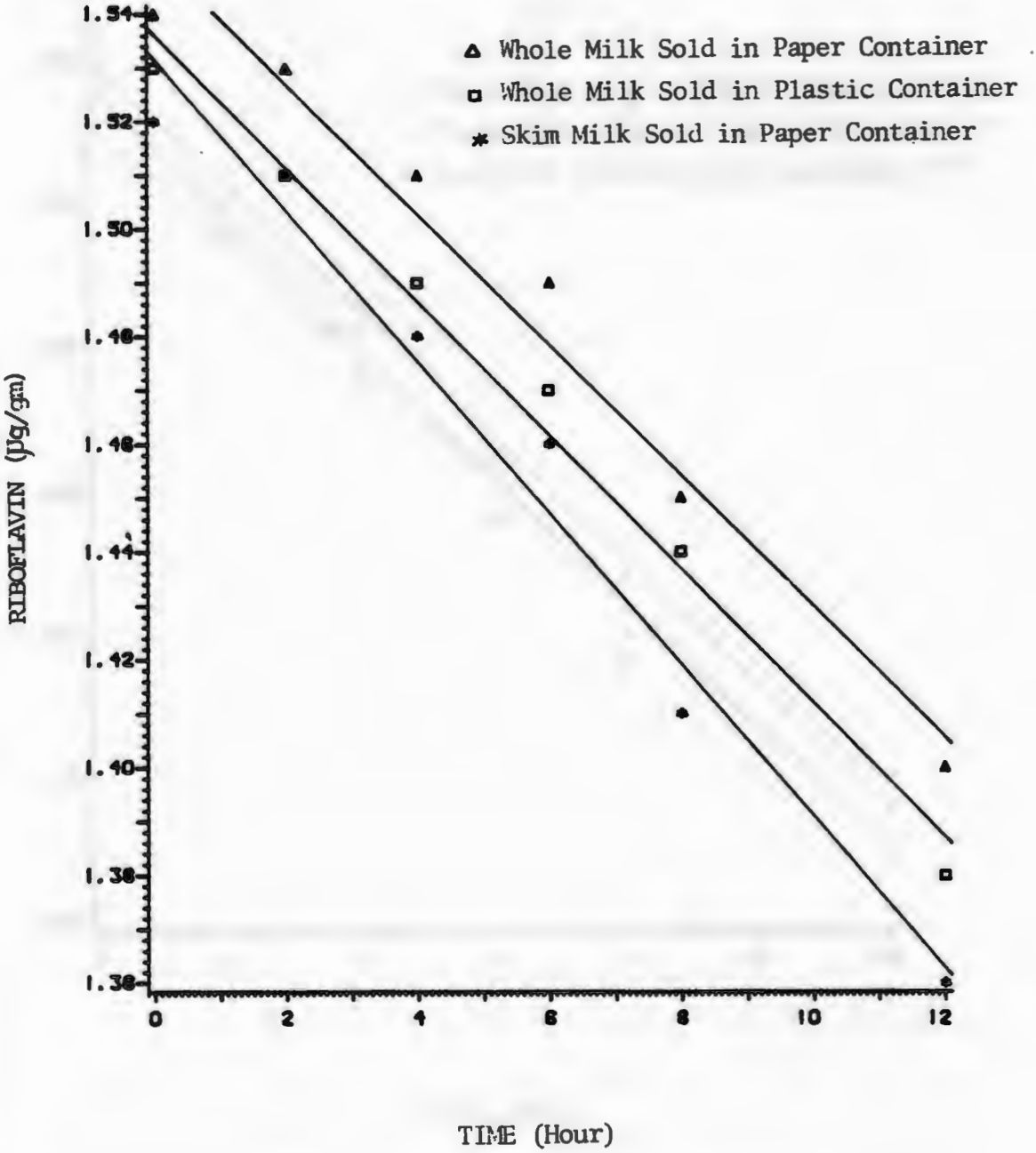


Fig. 10. Effect of holding milk at 55°C after boiling for 10 seconds on riboflavin content.

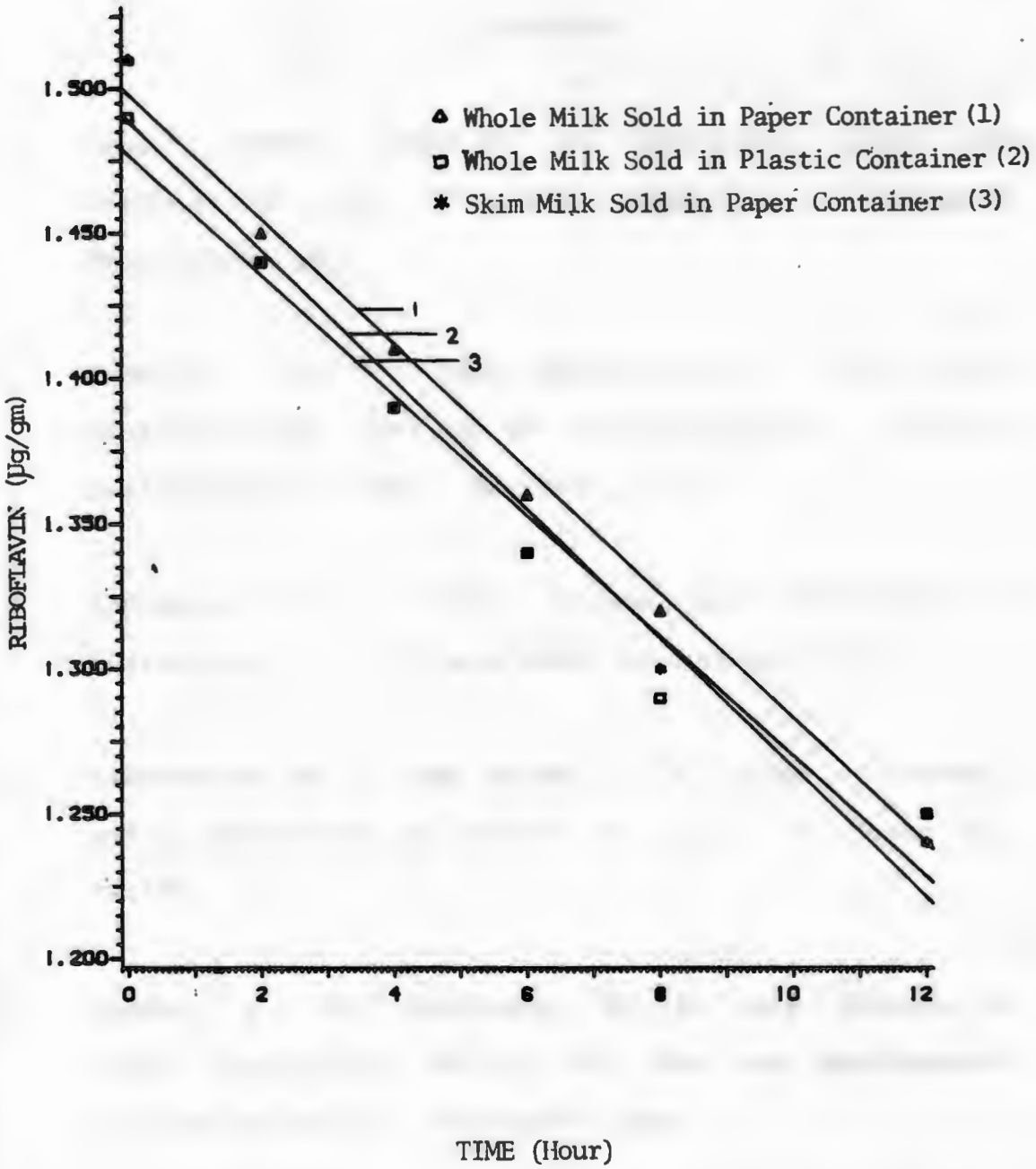


Fig. 11. Effect of twice boiling and then holding milk at 55°C on riboflavin content.

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