THE EFFECT OF BAKING ON THE RETENTION OF THIAMINE, RIBOFLAVIN AND NIACIN IN ARABIC BREAD

By

SHAWKY DAGHER

A THESIS

Submitted to the

AMERICAN UNIVERSITY OF BEIRUT

In partial fulfilment of

the requirement for the

degree of

MASTER OF SCIENCE IN

AGRICULTURE

June 1966

THE EFFECT OF BAKING ON THE RETENTION OF THIAMINE, RIBOFLAVIN AND NIACIN IN ARABIC BREAD

By

SHAWKY DAGHER

Approved:

Morteza Maleki

Morteza G. Maleki: Assistant Professor of Food Technology.
In Charge of Major.

James W Cowan

James W. Cowan: Associate Professor of Food Technology and Nutrition.

Nuhad J. Daghir

Nuhad J. Daghir: Assistant Professor of Poultry Science.

Raja I. Tannous

Raja I. Tannous: Assistant Professor of Food Technology and Nutrition.

W. W. Worzella

W.W. Worzella: Professor and Chairman of Graduate Committee.

Date Thesis is presented: June 1, 1966.

VITAMIN RETENTION IN BREAD

DAGHER

ACKNOWLEDGMENTS

The author wishes to extend his thanks to Dr. Morteza Maleki, for his help, advice and suggestions during the course of this study. He is also indebted to the Nutrition Department of the School of Medicine for the use of their laboratory equipment.

AN ABSTRACT OF THE THESIS OF

Shawky Dagher for M.S. in Food Technology and Nutrition

Title: The effect of baking on the retention of thiamine, riboflavin and niacin in Arabic bread.

Bread is the principal staple food in most human diets and a primary source of the B vitamins, in particular thiamine. Previous studies on European-type bread showed that thiamine was destroyed during baking and that more destruction occurred if the bread were toasted. It was also shown that riboflavin and niacin are not affected by the baking process, though some of the the riboflavin may be lost during storage of the bread.

In this study, the effect of different baking conditions of Arabic bread on thiamine, riboflavin and niacin was investigated. A muffle furnace was regulated to bake Arabic bread at 400, 450 and 500°C for different time intervals. A micro-baking technique, representing the normal operations of commercial bakeries of Beirut, was adopted. Brown, white and vitamin enriched breads were prepared and analyzed with their respective doughs for the three vitamins. Thiamine was determined by the thiochrome method, riboflavin and niacin were determined by microbiological assay.

The results showed that thiamine was destroyed during baking; the amount lost seemed to be directly proportional to the intensity of heating. More destruction of thiamine occurred in brown than in white Arabic bread baked under identical conditions. A uniform loss of riboflavin was observed in white, as well as in brown Arabic bread; though the percent retention was greater in the white than in the brown bread. Negligible loss of niacin occurred under all baking conditions of both white and brown Arabic bread.

In vitamin enriched experimental samples, retention values of riboflavin were better than in the case of unenriched bread. The niacin added was not affected by baking and was completely retained. The addition of 1 mcg of riboflavin, 5 mcg of thiamine and 30 mcg of niacin per gr of white flour, was sufficient to increase its vitamin content to that of brown flour.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

Cereals have long been regarded as an important constituent of the human diet, especially in the case of low income people. Because of the low economic standard of the majority of the population in the Middle East, and due to the availability of cereals in the area, the Middle Eastern diet is basically a cereal diet. Wheat is the major cereal consumed, generally in the form of bread. It has been estimated that in Jordan, 46-63% of the total calorie intake is derived from bread (2, p.71) while in Lebanon, bread contributes 47% of the total energy intake (3, p.63).

Different kinds of bread are consumed in different Middle Eastern countries. In Lebanon three kinds of bread are commonly consumed: (i) The flat circular Arabic bread; (ii) the thin sheets of Markouk or mountain bread and (iii) the cylindrically shaped French bread or "Khubz Franji". Of importance in this study was the Arabic bread or "Khubz Arabi".

Arabic bread, is the most popular kind of bread in Lebanon, and it is consumed more than any other type. It is circular in shape and composed of two layers; each layer is less than half a centimeter in thickness, and ranges in diameter from 10 to 30 cm. Two kinds of Arabic bread are in common use in Lebanon: (i) the brown Arabic bread, which is generally consumed in the rural areas and is made of brown

flour of about 80-90% extraction, and (ii) the White Arabic bread, made of white flour of about 60-70% extraction (zero flour). The latter is largely consumed in urban areas.

The consumption of white Arabic bread is gradually increasing in Lebanon. Modern mills that produce refined white flours have replaced recently many of the old fashioned stone mills that produce only whole wheat flour. Because of the differences in vitamin content between the two grades of flour, namely brown and white flour, in some countries, the latter is often enriched with thiamine, riboflavin and niacin as well as some minerals to replace nutrients which are partially lost in the various stages of processing. Nutrition surveys (2,p. 182, 3,pp. 41-42) report marginal or low levels of intake of these vitamins in the Middle East, in particular riboflavin.

Although bread is the most important constituent of the daily diet in this area, little research has been conducted to study its nutritional value and possibilities of its enrichment. In the present study, emphasis was put on vitamin losses during the baking of white, brown and vitamin-enriched, white Arabic bread, baked at different temperatures for different time intervals. Particular interest was given to thiamine, riboflavin and niacin since wheat and wheat products are considered a primary source of these nutrients.

II. REVIEW OF LITERATURE

The Preparation of Arabic Bread or "Khubz Arabi" in Beirut[1]

Bakeries purchase the flour from the local mills. Refined flour of 60-70% extraction, commonly referred to as zero flour, is used in the preparation of white Arabic bread; brown flour of 80-90% extraction is used for brown bread.

Before the dough is mixed, the flour is sifted to remove chaff, insects and other foreign matter. About 1.5 kg of salt are dissolved in a few liters of water and about 750 gr of compressed yeast[2] are then suspended in this brine. This mixture is added in small successive portions to 100 kg of sifted flour while the flour is being mixed. More water is added while mixing is continued. The volume of water is not measured prior to mixing in most of the bakeries but determined by the consistency of the dough during mixing. Usually, the amount of water ranges from 45-70 liters for every 100 kg of flour. Mixing time differs depending on the absorption qualities of flours. A period of 12-20 minutes mixing is usually enough to bring about uniform mixing of the ingredients.

Fermentation starts shortly after mixing is completed and continues until the yeast is killed by the heat of the oven.

---

1. This information was obtained by the author who surveyed 12 bakeries operating in Beirut during September 1965.

2. This amount of yeast is used during the hot periods of the year. In winter 1000 gr of compressed yeast are used for 100 kg of flour.

The two main end products of yeast fermentation are carbon dioxide and alcohol. The dough rises due to the production of carbon dioxide gas which is caught in the gluten network. The first period of fermentation is usually about ten minutes, after which the dough mass is divided into balls, with smooth unbroken surface skin. To permit sufficient swelling of the individual pieces, the balls are coated with a thin layer of dusting flour, and allowed another ten-minute fermentation period.

With the help of a wooden roller and with the application of dusting flour, the rounded dough balls are molded into flat circular sheets, about $\frac{1}{2}$ cm thick. The dough sheets are placed on wooden boards and allowed a final fermentation time of 45 minutes in the hot atmosphere of the bakery, usually on elevated stands.

Shortly before baking, the dough sheets are turned over; after two minutes they are transferred to the baking oven with a wooden spatula. The heat in the compact, room-like oven, is generated by the combustion of black fuel or diesel oil. Pelshenke (39, p.19) reported that the baking temperature is 450-500°C. When exposed to the heat of the oven, the flat pieces of dough puff up, and separate into two layers. Baking is continued for 40 to 60 seconds, after which the loaves are removed and cooled.

Thiamine

The thermolabile character of thiamine accounts for the destruction of this vitamin during processes involving heating. Bottomley *et al.*, (9) working on Australian bread, reported an average loss of 29.5% of thiamine during baking; Coppock *et al.* (14) found 20% loss of thiamine in bread baked at 475°F for 30 minutes. However the same authors (15), using a more refined technique for the measurement of thiamine found that the overall average loss of thiamine on baking was nearer to 15% rather than 20%.

The destructive effects of baking appears to be largely due to the losses in the crust; the thinner the bread is, the greater the heat penetration and the higher the destruction. In a study on bread baked at a relatively low temperature (212°F), Martin (27) found that the crust contained only about two thirds as much thiamine as the crumb. Morgan *et al.* (35) using a rat growth method to estimate thiamine, showed that there was a definitely lower level of thiamine in the crust of whole wheat bread than in the crumb; the maximum difference was 35%. Meckel *et al.* (29) studied different kinds of U.S. Army bread including the "garrison $1\frac{1}{4}$ lb" loaf and the "garrison 10 lb sheet", baked at 440°F for 35 and 44 minutes respectively. In the $1\frac{1}{4}$ lb loaves, there was 17% loss of thiamine, whereas in the latter type, the loss was 14%. These workers interpreted this difference to be due to the relatively greater amount of crust in the $1\frac{1}{4}$ lb loaves.

Morgan et al. (35) showed that loaf size had a small but demonstrable effect on thiamine retention. Loaves weighing $1\frac{1}{2}$ lb baked under identical conditions with 1 lb loaves, had slightly higher thiamine content. However, variations in baking temperature as represented by 350°F and 446°F caused no consistent effect upon the retention of this vitamin. In contrast, Zaehringer and Personuis (51) reported that pale, medium and dark bread baked at 400°F for 30, 40 and 50 minutes, retained 83.1, 80.4 and 73.8% of thiamine respectively. Similar work showed that in dinner rolls baked at the same temperature for 15, 20 and 25 minutes thiamine retention was 89.5, 84.5 and 77.5%. These workers concluded that the percentage loss of thiamine was greater between dark and medium colour crust bread than between medium and pale crust bread. These results indicate that thiamine destruction occurrs more rapidly as the baking time is increased.

Meckel et al. (28) baked enriched Zwieback dough for 27 min, and toasted it for a further 19 minutes. The overall loss of thiamine was 38% attributable almost equally to the baking and toasting operations.

The composition of the bread formula seems important to thiamine retention during baking. Dawson et al. (16) reported that only 11% of the thiamine, added as special enriched yeast, was lost during baking of bread made of 73% extraction flour. Also, the results of Golberg (19) on South African bread, made of 95-100% extraction flour, showed a 22.8% loss as compared

to 16.8% when British flour of 85% extraction was used. He explained that this difference was possibly due to the longer time required for the baking of whole wheat bread.

Arny et al. (4) baked muffins at 400°F for 27 minutes, with tartarate baking powder, butter-milk, whole wheat flour and whole wheat flour plus butter-milk; they found 85.3, 84.9, 92.6, 96.3 and 99.1% retention of thiamine respectively. They concluded that whole wheat flour retained most of its thiamine in baking, especially when non butter-milk was added.

Destruction of thiamine during baking was shown by several workers to depend on pH. For example Brackman (10) baked biscuits enriched with thiamine at 450°F for 15 minutes and observed that at lower pH there was better recovery of the added thiamine. In the same study, about 85% of the added thiamine survived the baking process when the pH of the baked product was 7.1 or lower. Similar observations were reported by Lincoln et al. (25) in the baking of farina, and by Pace (38) in working with corn bread. Furthermore, Beadle et al. (7) showed that the stability of thiamine to heat is a function not only of pH, but also of the electrolyte system involved.

Riboflavin

Riboflavin is sensitive to visual and ultraviolet light, but is stable to heat (50). Andrews et al. (1) found out that if bread samples were sliced and allowed to dry on benches in

the laboratory they lost as much as half of their riboflavin content, even in the absence of direct sunlight.

Loy and co-workers (26) reported no loss of riboflavin in bread stored for 3 days in the dark, or under artificial light; however, they noted appreciable loss after 7 days. When bread was placed in sunlight there was a significant loss of riboflavin in bread during the first day of exposure, and more than half the original amount was lost after 7 days. Partially baked rolls, packaged in commercial cellophane or wax paper lost a significant amount of riboflavin during the first seven days of storage under light of 100 foot candle intensity. When the rolls were wrapped in aluminum foil (8), no loss occurred. Stephens et al. (46) found that, when partially baked rolls were exposed to three 8-hr periods under 60 foot candles artificial light, 16.5, 13.5 and 1.7% of the original riboflavin was lost respectively when clear, yellow and orange cellophane wrappings were used. However, t he effect of cellophane cover was not so pronounced in the case of bread. Morgareidge (37) working on white bread found that, regardless of the type of the wrapper, there was no loss of riboflavin when bread displays were subjected to normal intermittent illumination for periods up to 5 days.

Refined flour seems to have lower capacity for retaining its riboflavin during baking. Auerman and coworkers (5) reported that flour of 72-85% extraction retained 64-88% less of its original riboflavin than did whole wheat bread

during breadmaking. In the drying of sliced bread, Andrews et al. (1) estimated riboflavin losses in whole wheat bread to be considerably less than in white bread; in one of their experiments, 40% loss occurred in white bread and only 10% in whole wheat bread.

Niacin or Nicotinic Acid

Whole wheat is relatively high in niacin. Barton-Wright (6) found that English wheat contained 48 mcg niacin per gram of wheat but 85% extraction flour from the same wheat contained only 10.5 mcg per gr.

Many workers have agreed on the relatively high stability of niacin during breadmaking. For example, no loss of niacin occurred during baking of bread at 425°F for 30 minutes (44); in another study about 95-100% of the original niacin was retained under normal baking conditions (30). Similar results were obtained using different kinds of flour (5) and when the flour was enriched with synthetic niacin (20).

Chaudhuri et al. (12) observed that the niacin of the bran portion of cereals is entirely in a bound form, and is not available to the rat unless released by alkali treatment. Kodicek (24) isolated the bound form of nicotinic acid in the cereal bran and found its gross molecular formula to be $C_{94}H_{142}O_{66}N_2$, a compound which he named niacytin. He emphasized that niacin can be liberated from niacytin only by alkali hydrolysis.

Working on bound niacin in dietary wheat products

Clegg et al.(13) found that whole meal scones containing 0.75 gr of sodium bicarbonate and baked at 265°F for 0, 5, 7, 10, 15 and 20 minutes, contained respectively 61.9, 57.0, 56.0, 58.0, 50.2 and 47.0% of their niacin in the bound form. They concluded that under these conditions, niacin was released from its bound form only when baking time exceeded 10 minutes.

Milling and Enrichment

The differences in composition between wheat and flour result primarily from the separation of the endosperm from other structural parts of the wheat kernel, mainly germ and bran.

There is a general distribution of thiamine, riboflavin and niacin throughout the kernel before maturity; at maturity, however, these vitamins tend to localize in certain portions of the grain (40). Thiamine is concentrated in the aleurone layer, scutellum and embryo, whereas riboflavin is concentrated mainly in the embryo with lower concentrations in the aleurone and scutellum (34, p. 4, 35, 45). Niacin is found in the aleurone layer, scutellum and embryo (85) (34, P.4).

Several studies have been made on the vitamin content of flour as compared to that of wheat. Schultz et al. (43) observed a reduction in thiamine content with increase in flour refining. Coppock et al. (14) reported a loss of 92.5, 82.5 and 45.0% of the original thiamine content in flours of 41, 70

and 80% extractions respectively. In addition, they reported 70.6, 58.8 and 52.9% loss of riboflavin and 87.3, 85.5 and 80.0% loss of niacin in flours of 41, 70 and 80% extraction respectively.

The enrichment of white flour to restore the vitamins lost in milling has been recommended by many workers and different forms and combinations have been proposed. For example, Dawson et al. (16) and Free (18) recommended the addition of dried yeast or special thiamine enriched yeast as supplementary sources of these vitamins. Mitchell et al. (33) found that white bread, enriched with non-fat milk solids was superior in its nutritive value to white bread enriched with thiamine, riboflavin, iron and niacin. However, Westerman (48), pointed out the nutritional superiority of vitamin enriched bread and Robertson (41) claimed that the best nutritional bread is that made from white flour fortified with thiamine, riboflavin, niacin and iron. Different levels of enrichment have been adopted in different countries. Menden et al. (31) reviewed the methods and levels of enrichment in various countries.

III. MATERIALS AND METHODS

Materials

All chemicals used in the vitamin assayswere reagent grade. The base exchange tubes for the thiamine determination were prepared by sealing a 15 cm length of 6 mm internal diameter glass tubing to the bottom of a 50 ml test tube. A capillary tube 3 cm long, with a base of 0.3 mm was sealed to the lower end of the tube. The bottom of the base exchange tube was plugged with a small piece of glass wool, and the column filled with distilled water. About 3 gr of activated Decalso (Fisher Scientific Co., Pittsburgh, Pa.) were added to each tube in 10 small portions, and allowed to settle by gravity. The tubes were allowed to drain and only those with a flow rate of 3 ml per minute were retained.

The working thiamine standard solution was prepared fresh for each analysis by diluting 2 ml of stock thiamine solution in a flask containing 5.0 ml of 2.5 M sodium acetate solution and 750 ml of 0.1 N sulfuric acid; the volume was then adjusted to 1 liter with distilled water. For the preparation of stock thiamine solution 100mg of desiccated crystalline thiamine hydrochloride were dissolved in 25% ethanol and diluted to one liter with the same solvent.

The ferricyanide oxidizing agent was also prepared the day to be used by dissolving 1 gr of potassium ferricyanide

in 100 ml of distilled water and then diluting 3 ml of this solution to 100 ml with 15% sodium hydroxide. For the working niacin standard solution, 50 mg of desiccated crystalline niacin were dissolved in 500 ml of 25% ethyl alcohol; this mixture was called solution A. One hundred ml of solution A were then diluted to 1 liter with 25% ethyl alcohol to make solution B. On the day of use 1 ml of solution B was diluted up to 100 ml with distilled water.

The riboflavin working standard solution was prepared by dissolving 50 mg of desiccated riboflavin in 2000 ml of 0.02 N acetic acid; this reagent, known as solution A, was stored under toluene in low actinic glassware. The riboflavin solution B was prepared by diluting 100 ml of solution A to 1 liter with 0.02 N acetic acid; this solution was also protected from light and stored under toluene. On the day of use, the working standard solution was prepared by diluting 10 ml of solution B to 100 ml with distilled water.

For the microbiological determination of niacin and riboflavin, assay media were obtained from DIFCO Laboratories (Detroit, Michigan); the peptonized milk, tryptone and agar used in the preparation of the culture media, were also obtained from the same source.

Methods

Preparation of the bread samples:

The two kinds of flour used in this experiment, namely the white or zero flour of 65% extraction and the brown flour of 85% extraction were obtained from a local bakery in Beirut.

The straight dough was prepared as described on pages 3-4. For mixing, a small Crypto electric mixer (Model EB 12 Crypto Ltd, London) was used; the following ingredients were added:

Flour	1000 gr
Water	550 gr
Salt	12.5 gr
Yeast[1]	7.5 gr

The mixing was continued for 12 minutes; the rest of the operation was carried out as follows:

First fermentation	8 minutes
Rounding	-
Second fermentation	10 minutes
Rolling (circular dough sheets of 8 cm diameter and 4 mm thickness)	-
Third fermentation	45 minutes

A small muffle furnace (Type 1500, Thermolyne Corp. Dubuque, Iowa) was used as the baking oven. To prevent

---

1. Compressed fresh yeast imported from Holland, not more than one week old.

direct contact between the dough sheets and the hearth of the furnace, a smooth 1 cm thick asbestos sheet elevated by two porcelain crucibles was used as a baking floor.

Baking time and temperature were according to the following table:

Table 1. Baking time and temperature used in the baking of Arabic bread.

Temperature (°C)	Time (sec)
400	30
400	60
400	90
450	30
450	45
450	60
500	15
500	30
500	45

Twelve loaves of bread were baked at each time-temperature combination and the appearance of each group was compared with that of commercial prepared Arabic bread using a scoring scale 0-10. After baking, the loaves were sliced and air dried in the dark at room temperature for 72 hr. The crisp bread pieces were ground to 20 mesh sieve in a Thomas mill (Model ED 5, A. Thomas Co., Pgh. Pa.) the ground samples were stored at -18°C in glass containers made light-proof by wrapping tightly with aluminum foil. Just before baking, a sample of dough was taken and stored under the same conditions. In addition, samples of white and brown bread and their respective doughs were obtained from local bakeries in Beirut and stored with

the experimental samples until the time of analysis.

Vitamin-enriched bread was prepared exactly the same way as white Arabic bread except that the water used contained the added vitamins. The enriched dough was baked at 450°C for 45 seconds. For enrichment, the levels used are summarized in Table 2.

Table 2. Levels of vitamin enrichment of white Arabic bread.

Vitamin	Enrichment Levels (mg per kg flour) Level I	Level II	Level III
Thiamine	2	5	10
Riboflavin	1	3	6
Niacin	10	40	60

Moisture determination:

Water content of the different experimental samples was determined according to the Official Methods of Analysis of the Association of Official Agricultural Chemists (23, p. 169).

Thiamine Assay:

The thiochrome method was used in the estimation of thiamine. Except for certain modifications, the method described by Mickelsen and Yamamoto (23, pp.191-259) was followed.

Duplicate samples of about 5 gr of brown or 6 gr of white Arabic bread were weighed accurately and transferred to 250 ml erlemeyer flasks. Each sample was mixed thoroughly with 75 ml of 0.1 N hydrochloric acid and autoclaved at 15 psi for 15 min. After the hydrolyzate was cooled to room temperature,

the pH was adjusted to 4.5 by the addition of 2.5 M sodium acetate in which 0.3 gr of Taka - Diastase (Parke-Davis and Co.) enzyme was suspended. The flasks were shaken and the mixture was quantitatively transferred to a 100 ml volumetric flask. One drop of toluene was added, and the mixture was incubated at 47°C for 2 hours.

After incubation, the extract was allowed to cool to room temperature, and was then diluted to volume with distilled water. The flasks were shaken thoroughly and the solids allowed to settle before the contents were filtered through Whatman No. 1 filter paper. The first 10 ml of the filtrate were discarded.

Twenty five ml of the filtrate were pipetted into the reservoir of a base exchange tube; .0 ml of working thiamine standard solution were transferred into another tube. The filtrate was discarded and the Decalso washed with three successive 10 ml portions of boiling distilled water. The washings were discarded and the thiamine was eluted with three portions of 10, 10 and 5 ml of hot acidified potassium chloride solution. The eluate was collected in a 25 ml volumetric flask and made up to volume with acidified potassium chloride solution.

Four 5-ml aliquots of the eluates from the column of the standard thiamine solution were pipetted into four test tubes. To two of the test tubes, 3 ml of 0.03% ferricyanide oxidizing agent were added and mixed gently. This was done with the help of an automatic syringe, and without touching the sides

of the tube. Three ml of 15% sodium hydroxide solution were added to the other two test tubes. Immediately, 15 ml of isobutanol were added to all the tubes, and shaken vigorously for 90 seconds by bubbling a stream of filtered air through the mixture. Aliquots of 5 ml of the assay solution were pipetted into another set of 4 similar tubes and treated exactly as above.

The reaction vessels were allowed to stand for a few minutes until the two layers separated, the lower aqueous layer was then siphoned out. About 1-2 gr of anhydrous sodium sulfate were added to the isobutanol and the tubes were shaken well. The contents of the tubes were then transferred to centrifuge tubes and centrifuged at 2000 rpm for 5 min.

The fluorescence of the clear, colourless isobutanol extract was measured with the Farrand Spectrofluorimeter (Farrand Optical Co. Inc., New York,) using 365 mu for the exciting radiation, and 435 mu to measure the emitted fluorescence.

Readings were made within 15 seconds after exposure in the fluorimeter and the thiamine content of each sample, expressed in mcg per gr, was calculated according to the following formula:

$$\text{mcg } B_1/\text{gr} = \frac{A-B}{S-D} \times \frac{20}{\text{Weight of sample}}$$

Where:

A = Fluorescence of the unknown.

B = Fluorescence of the unknown blank.

C = Fluorescence of the standard, interpolated for 1 mcg.

D = Fluorescence of the standard blank.

To avoid vitamin destruction,direct sunlight, or other sources of ultraviolet light were carefully avoided after oxidizing the thiamine to thiochrome.

Riboflavin and niacin assay:

With the exception of certain modifications, niacin and riboflavin were determined by the microbiological assay procedure as described by the Association of Official Agricultural Chemists (2.3 PP. 667-668 and PP. 669-670).

For the determination of niacin, samples of 1 gr of brown or 1.5 gr of white Arabic bread were weighed accurately and mixed thoroughly with 100 ml of 1N sulfuric acid in a 250 ml beaker. The mixture was autoclaved at 15 psi for 30 minutes, and allowed to cool to room temperature. The extract was adjusted to pH 6.0-6.5 with 1N sodium hydroxide,and then by dropwise addition of O.1N Hydrochloric Acid the pH was adjusted to 4.5. The hydrolyzate was allowed to stand for one hour, then filtered through Whatman No. 1 filter paper. The filtrate was neutralized to pH 6.8 with dilute sodium hydroxide, and the volume made up to 250 ml with distilled water.

For the riboflavin assay, samples of 4 gr of brown or 6.5 gr of white Arabic bread were added to 100 ml of 0.1N hydrochloric acid. Hydrolysis was performed at 15 psi for 30 minutes after which the pH was adjusted to 4.5 by the addition of 2.5 M sodium acetate. The hydrolyzate was then filtered through Whatman No. 4 filter paper, and the pH

adjusted to 6.8 with 0.1 N sodium hydroxide; the volume was adjusted to 250 ml with distilled water.

The assay organisms used were: (i) Loctobacillus arabinosus - ATCC no. 8014 (American Type Cultures Collection Washington, D.C.) for niacin and (ii) Lactobacillus arabinosus ATCC no. 7469 for riboflavin. The lyophilized culture of these organisms were revived as described by Sakr (42, pp. 14-15). Weekly stab transfers were made into a solid agar medium made up of the following ingredients:

Peptonized milk	10 gr
Tryptone	10 gr
Agar	10 gr
Tomato juice	200 ml
Distilled water	800 ml

Aliquots of 10 ml of this medium were transferred to test tubes, plugged with non-absorbant cotton, and autoclaved at 15 psi for 15 minutes. The medium was then allowed to solidify in a refrigerator.

The day before use, culture tubes containing sterilized growth medium[1], were inoculated from the stab cultures and incubated at 37°C for 18 hours. One drop of this suspension was used to inoculate each of the assay tubes.

For niacin determination, aliquots of 0.0, 1.0, 2.0, 3.4, 4.0 and 5.0 ml of the niacin working standard solution

---

1. This is prepared the same way as the stock agar medium, but without the addition of agar to the nutrient solution.

were transferred into a series of test tubes. Aliquots of 1.0, 2.0 and 3.0 ml of the hydrolyzate prepared for niacin determination were added to another set of tubes.

For riboflavin determination aliquots of 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 ml of the riboflavin standard solution were added, and into another set of tubes aliquots of 1.0, 2.0 and 3.0 ml hydrolyzate, prepared for riboflavin determination, were added. Sufficient distilled water was added to bring the volume in each tube to 5.0 ml. The basal medium for the assay of the respective vitamin was added at the rate of 5.0 ml per tube.

The samples and the standards were assayed under the same experimental conditions; all assays were performed in triplicate. The tubes were placed in a wire rack covered tightly with aluminum foil and autoclaved at 15 psi for 10 minutes. After cooling to room temperature, each was aseptically inoculated with one drop of the inoculum delivered through a sterile syringe fitted with a 20-gauge needle. After inoculation, the tubes were incubated at 38°C for 72 hours. The lactic acid produced was titrated with sodium hydroxide, using bromo-thymol blue as indicator.

The standard curve for each vitamin was drawn by plotting the average volume of sodium hydroxide used for titrating each set of triplicate tubes against the vitamin content of the tubes. The amount of vitamin in each tube containing the sample aliquots was obtained by extrapolation from the standard curve and calculated as mcg per gr dry matter as follows:

$$\text{mcg vitamin per gr dry sample} = \frac{\text{Average mcg per ml x 250}}{\text{Dry weight of sample (gr)}}$$

Calculation of percent retention:

The vitamin content of bread samples and their respective doughs were calculated as mcg per gr dry matter. The percent retention was calculated on the basis that the vitamin content of dough is 100%. This percent retention of any baked sample is a comparison between its vitamin content after baking and that of its corresponding dough.

IV. RESULTS AND DISCUSSION

Moisture was determined on all samples of bread and dough used so that values for vitamin content could be expressed on dry weight basis; the data for moisture are shown in Table 3. Because, even in the air-dried bread samples there was 9-12% moisture, all samples were stored at -18°C to minimize possible chemical reactions. Also, the dough samples were stored under the same conditions at their original moisture content of 40% to avoid vitamin losses by drying or chemical reactions within the dough.

The results presented in Table 4 describe the appearance of the bread loaves prepared by different baking procedures. Out of many combinations of baking time and temperature used the ones reported yielded bread of varying degrees of acceptability. Bread samples similar in appearance to the commercially baked samples were obtained in the laboratory when baked at 400, 450 and 500°C for 60, 45 and 30 seconds respectively. Brown bread seemed to resist heat effect more than white bread. When samples were underbaked, brown bread loaves were relatively lighter in color than the white loaves; the same effect was noticed on overbaked samples.

The data shown in Table 5 indicate the destructive effect of heat on thiamine. In the underbaked samples of white Arabic bread most of the thiamine was retained during baking.

Table 3. Moisture content of experimental samples

Treatment	White Bread	Brown Bread
	%	%
Baked at 400° for 30 sec.	11.86	11.63
400° for 60 sec.	11.67	9.97
400° for 90 sec.	10.78	9.27
Baked at 450° for 30 sec.	11.97	12.14
	11.40	8.99
	10.27	8.82
Baked at 500° for 15 sec.	12.69	9.65
	11.43	9.51
	10.79	9.00
Dough used in preparing above bread	41.02	41.47
Commercially prepared bread	10.40	10.08
Commercially prepared dough	38.03	38.77
Flour	13.92	13.39
Bread enriched with low vitamin level	10.12	
Dough enriched with low vitamin level	40.34	
Bread enriched with medium vitamin level	9.89	
Dough enriched with medium vitamin level	40.84	
Bread enriched with high vitamin level	9.34	
Dough enriched with high vitamin level	41.37	

Table 4. Scoring evaluation of the appearance of different bread samples.

	White Arabic Bread		Brown Arabic Bread	
Baking Method	Score	Description	Score	Description
Commercial bread	10	Choice	10	Choice
Baked at 400°C for 30 sec.	2	Raw, underbaked	1	Raw, underbaked
Baked at 400°C for 60 sec.	10	Choice	10	Choice
Baked at 400°C for 90 sec.	3	Dark brown, overbaked	5	Overbaked
Baked at 450°C for 30 sec.	4	Slightly raw, underbaked	3	Raw, underbaked
Baked at 450°C for 45 sec.	10	Choice	10	Choice
Baked at 450°C for 60 sec.	4	Brown, overbaked	6	Slightly brown, overbaked
Baked at 500°C for 15 sec.	1	Raw, underbaked	0	Raw
Baked at 500°C for 30 sec.	10	Choice	10	Choice
Baked at 500°C for 45 sec.	0	Charred, overbaked	1	Highly overbaked

Scoring: Choice = 10, Very Good = 9, Good = 8, Fair = 7, Acceptable = 6, Borderline = 5, Poor = 0 - 4.

Table 5. Effect of baking time and temperature on thiamine retention in Arabic bread.

Baking Method	White Bread		Brown Bread	
	Thiamine Content	Thiamine Retention	Thiamine Content	Thiamine Retention
	mcg/gr dry matter	%	mcg/gr dry matter	%
Baked at 400°C for 30 sec.	2.13	99.57	10.49	95.58
Baked at 400°C for 60 sec.	1.61	75.37	6.07	57.02
Baked at 400°C for 90 sec.	1.31	61.10	4.43	41.64
Baked at 450°C for 30 sec.	1.99	93.43	8.32	78.22
Baked at 450°C for 45 sec.	1.62	75.75	6.98	65.57
Baked at 450°C for 60 sec.	1.31	61.10	5.85	54.94
Baked at 500°C for 15 sec.	2.12	99.26	8.55	80.34
Baked at 500°C for 30 sec.	1.68	76.41	7.33	68.85
Baked at 500°C for 45 sec.	1.31	61.34	3.94	37.03
Unbaked dough	2.14	100.00	10.64	100.00
Baked at commercial bakery	2.28	92.68	8.06	105.30
Unbaked dough prepared at commercial bakery	2.46	100.00	7.66	100.00
Flour	2.38		9.17	

The results with brown bread compared with those of the white showed a greater loss of thiamine. As seen in table 5, this loss in white bread baked at various baking conditions was 0.5-39.0%, while that in brown bread was 4.5-63.0%; this effect was more pronounced on bread overbaked at 500°C for 45 seconds; under such treatment, white bread retained 61% of its original thiamine while the respectively treated brown bread retained only 37% of its original thiamine.

These findings which are illustrated in Fig. 1, are in agreement with results reported by Hoffman et al. (22) who showed considerable losses in thiamine due to toasting of brown bread, compared to negligible losses incurred in toasting white bread.

Reports on work with European-type bread show that 15-20% thiamine is destroyed during normal baking (17,44). In the present work, the baking conditions used to produce loaves of Arabic bread of normal appearance (400°C for 60 sec., 450°C for 45 sec. and 500°C for 30 sec.), resulted in 25% loss of thiamine in white bread in all three treatments and 43, 34 and 31% loss in brown Arabic bread respectively. Though the high temperature-short time treatment resulted in the retention of more thiamine in brown bread than did other baking treatments, there seemed to be a generally greater loss of this vitamin in Arabic bread than in the European-type bread. This difference is due probaly to the high temperature used

28

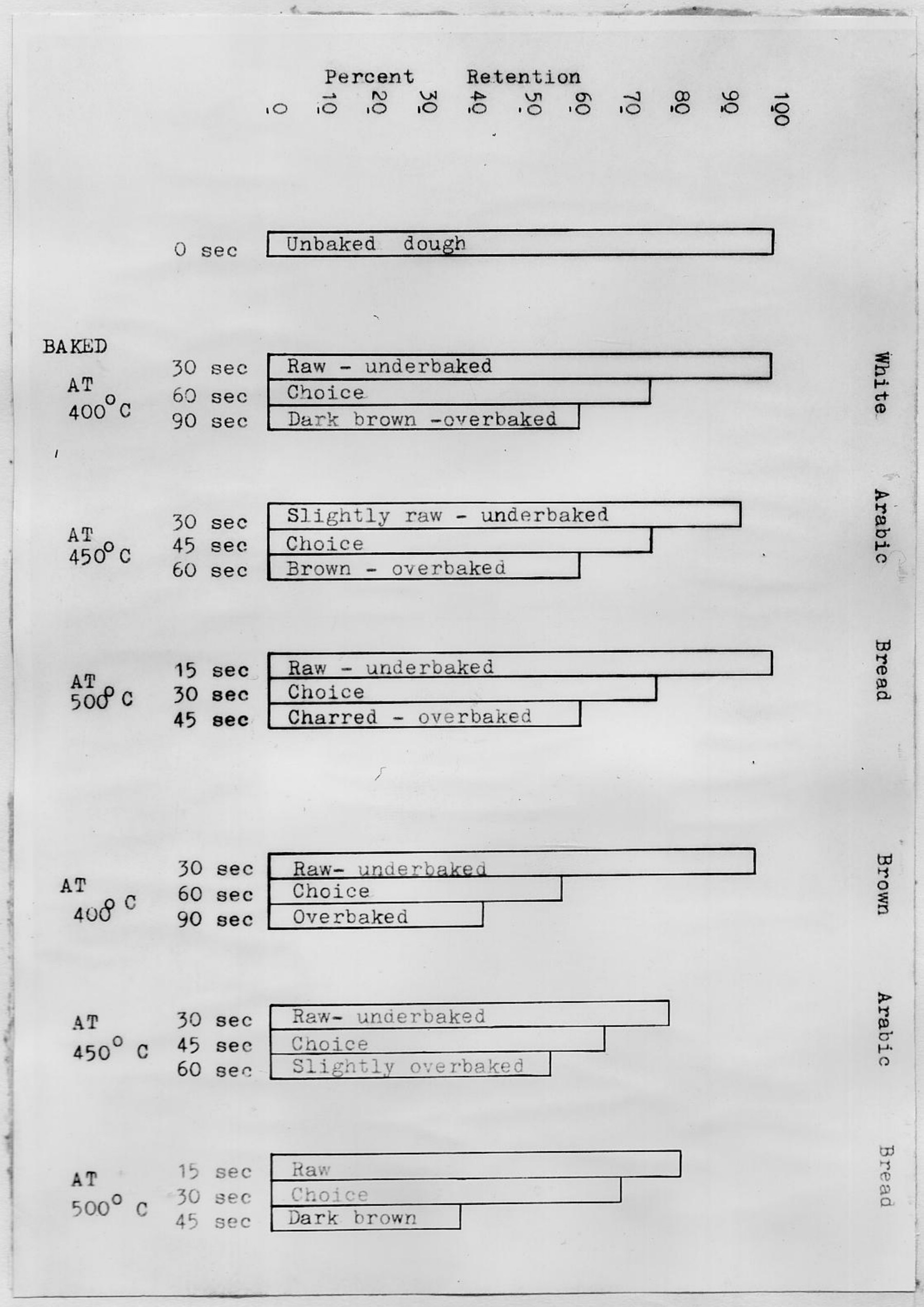

Figure 1. The effect of baking on thiamine retention in Arabic bread.

and the greater penetration of heat into the thin loaf of Arabic bread, as compared to the thicker loaf of European bread.

White Arabic bread prepared in a commercial bakery retained 92% of its thiamine, whereas no loss occurred in brown bread (Table 5); in both cases, the retention was better than in experimental samples from the present work. This discrepancy may have been due to the differences in baking conditions such as temperature, radiant heat and relative humidity between the commercial oven and the muffle furnace used as the experimental oven.

As shown in Table 6 and Fig. 2, the effect of baking on riboflavin retention in bread seemed to be less severe than on thiamine. These data show that, with one exception, all the white Arabic bread samples lost 10-20% of their original riboflavin. In brown bread, greater losses occurred during baking (20-26%). The commercially baked brown bread showed slightly higher riboflavin retention than the laboratory samples. According to one report (44) normal baking of European bread caused no loss of riboflavin; in other cases (29) there was an increase of up to 21% of this vitamin. Increase in riboflavin content of European bread and decrease of this vitamin in Arabic bread, could be due to differences in the nature of heating. In ovens used for European bread there is no significant light radiation, while in ovens used for baking Arabic bread there is more radiation effect, due to much higher temperature. It is known that riboflavin is photosensitive and is easily destroyed by radiation. Therefore it can be concluded that even though

Table 6. The effect of baking time and temperature on riboflavin retention in Arabic bread.

Baking Method	White Bread		Brown Bread	
	Riboflavin Content	Riboflavin Retention	Riboflavin Content	Riboflavin Retention
	mcg/per gr dry matter	%	mcg/per gr dry matter	%
Baked at 400°C for 30 sec	0.49	87.5	1.03	74.8
Baked at 400°C for 60 sec	0.51	89.8	1.06	77.0
Baked at 400°C for 90 sec	0.49	86.3	1.09	79.7
Baked at 450°C for 30 sec	0.40	71.4	1.04	76.2
Baked at 450°C for 45 sec	0.46	81.3	1.06	77.0
Baked at 450°C for 60 sec	0.45	79.8	1.06	77.0
Baked at 500°C for 15 sec	0.49	87.5	1.03	74.8
Baked at 500°C for 30 sec	0.46	81.4	1.02	74.1
Baked at 500°C for 45 sec	0.46	81.1	1.02	74.1
Unbaked dough	0.56	100	1.37	100
Prepared and baked at a commercial bakery	0.70	79.9	1.24	85.3
Unbaked dough prepared at a commercial bakery	0.88	100	1.45	100
Flour	0.50		1.30	

31

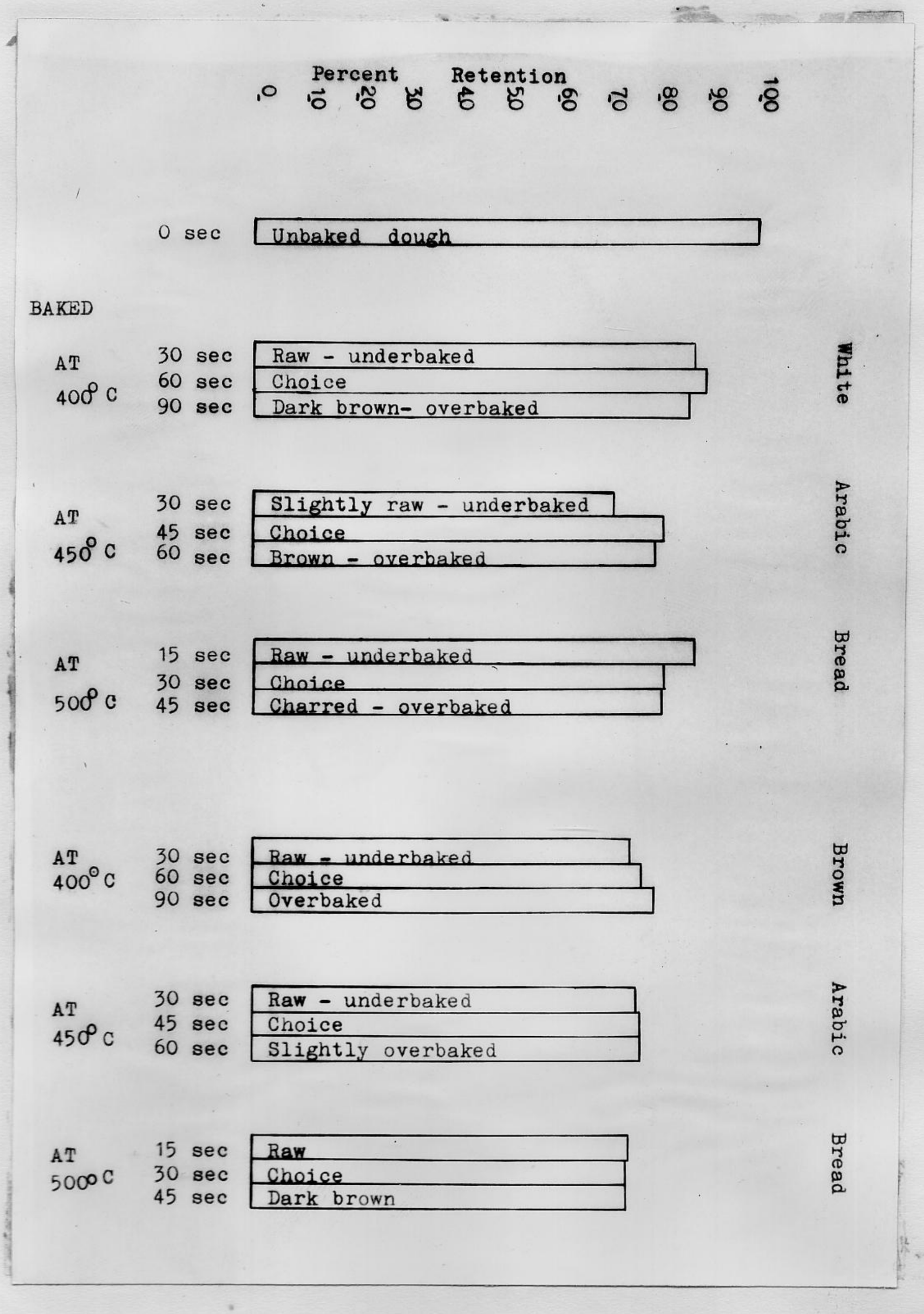

Figure 2. The effect of baking on riboflavin retention in Arabic bread.

there may be some increase in riboflavin content in Arabic bread due to heat, as is the possible case in European bread, the destructive effect of light from radiation would counteract this effect.

The niacin content of the experimental samples is reported in Table 7 and illustrated in Fig. 3. These results indicate that 95-100% of the niacin was retained in all samples of both white and brown Arabic bread, whether prepared in the laboratory or in the commercial bakery. These data confirm the high stability of niacin to heat, light and other baking factors as reported by other workers (11, 30, 44). The slight increase in niacin content in the white or brown flours compared to their corresponding doughs is probably due to the contribution of the yeast added prior to fermentation.

The results presented in Table 8 indicate that enrichment of flour with synthetic thiamine at the levels of 5 and 10 mcg per gr flour caused an increase in the thiamine content of the corresponding dough greater than could be attributed by the added vitamin. A similar increase was noted during the fermentation of dough from brown flour (Table 6) which is relatively high in thiamine. These increases may be explained by the multiplication of the yeast cells during fermentation. In the work of Hintzer (21), it was shown that the presence of synthetic thiamine in flour had a slight stimulating action on fermentation; also, Williams (49) found

Table 7. The effect of baking time and temperature on niacin retention in Arabic bread.

Baking Method	White Bread		Brown Bread	
	Niacin Content mcg/per gr dry matter	Niacin Retention %	Niacin Content mcg/pergr dry matter	Niacin Retention %
Baked at 400°C for 30 sec.	29.77	94.90	40.69	98.30
Baked at 400°C for 60 sec.	30.50	97.13	41.89	101.45
Baked at 400°C for 90 sec.	30.33	96.50	41.44	100.24
Baked at 450°C for 30 sec.	31.96	101.59	41.83	101.21
Baked at 450°C for 45 sec.	30.74	97.77	43.09	104.36
Baked at 450°C for 60 sec.	31.58	100.64	42.55	102.90
Baked at 500°C for 15 sec.	30.52	97.13	42.24	102.18
Baked at 500°C for 30 sec.	30.82	98.09	41.89	101.45
Baked at 500°C for 45 sec.	30.63	97.45	40.07	97.09
Unbaked dough	31.38	100.00	41.27	100.00
Prepared and baked at a commercial bakery	31.71	98.75	40.52	101.76
Unbaked dough prepared at a commercial bakery	32.12	100.00	39.77	100.00
Flour	29.50		39.76	

34

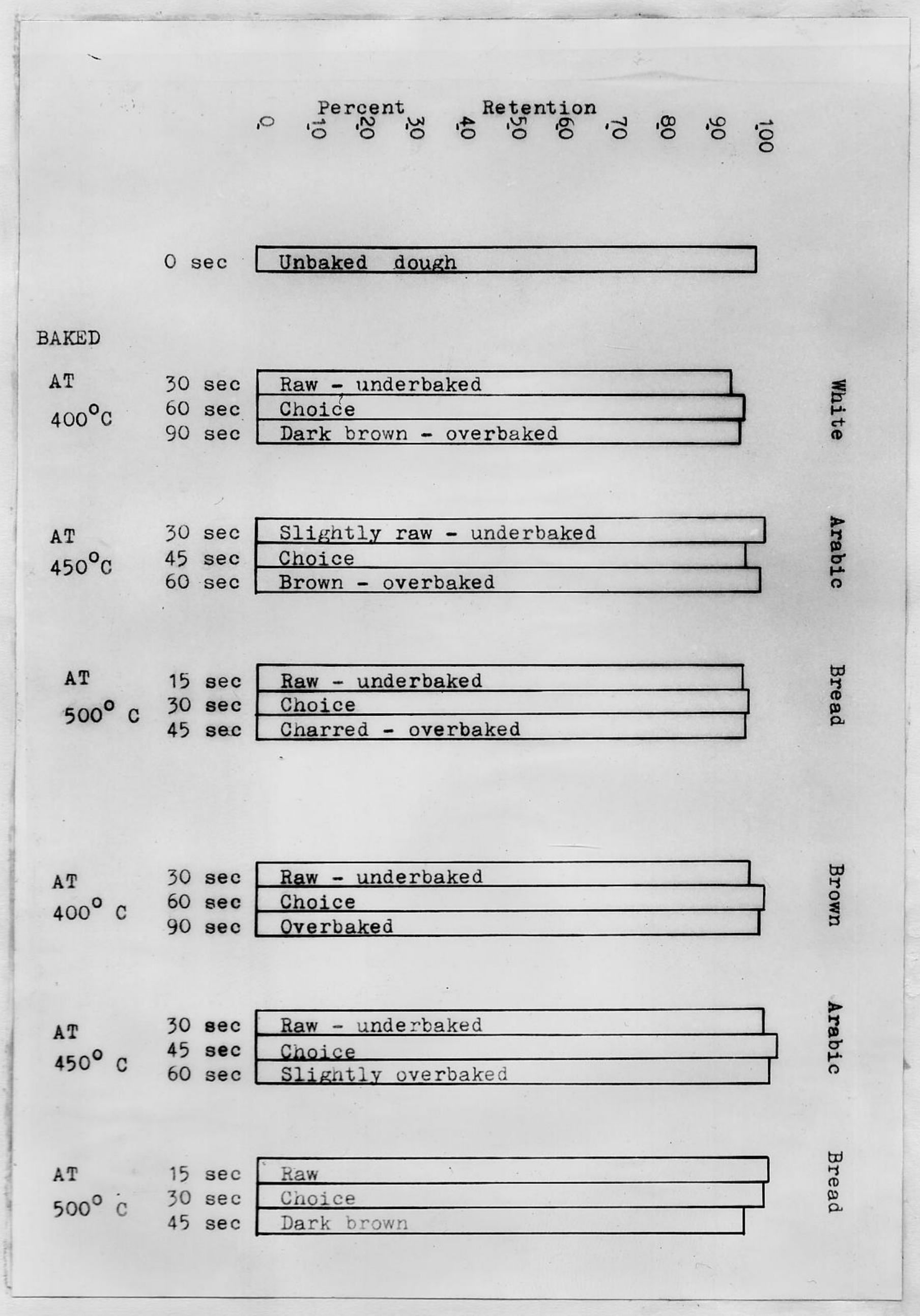

Fig 3. The effect of baking on niacin retention in Arabic bread.

Table 8. The effect of baking on the retention of thiamine, riboflavin and niacin in enriched white Arabic bread.

Vitamin	Level & Enrichment	Bread		Dough	
		Vitamin Content mcg/gr dry matter	% Retention	Vitamin Content mcg/gr dry matter	% Retention
Thiamine	0	1.62	75.57	2.14	100.00
	2	4.06	82.37	4.94	100.00
	5	8.79	92.29	9.53	100.00
	10	13.38	92.50	14.47	100.00
Riboflavin	0	0.46	81.30	0.56	100.00
	1	1.38	84.55	1.63	100.00
	3	2.89	86.50	3.34	100.00
	6	6.50	91.17	7.12	100.00
Niacin	0	30.74	97.77	31.38	100.00
	10	41.90	103.87	40.34	100.00
	40	70.32	98.57	71.34	100.00
	60	85.58	99.71	85.83	100.00

thriftier yeast growth in dough in which thiamine was abundant. More recently, Thorn (47) reported 35% yeast growth in straight doughs fermented for 3.0-3.5 hours.

In flour enriched with synthetic riboflavin the percent retention of the vitamin during baking was better than that in plain flour, especially when a high level of enrichment was adopted. For example, when 6 mcg of riboflavin were added per gr flour, there was 91% retention compared to 81% in plain flour. These results are in agreement with Loy et al. (26) who found that riboflavin in enriched flour was stable even in artificial light and sunlight.

The data in Table 8 shows that negligible losses of niacin occurred in the enriched bread; thus it is evident that supplementary niacin is equally stable to the baking conditions of Arabic bread.

V. SUMMARY AND CONCLUSION

Arabic bread was prepared from white and brown flour and was baked under conditions of varying time and temperature; samples of these breads were analyzed with their respective doughs for thiamine, riboflavin and niacin. In addition, white Arabic bread, enriched with different levels of these vitamins was baked under optimum baking conditions and analyzed for the same vitamins.

Thiamine destruction seemed to be directly related to the intensity of heat applied; the more severe the baking conditions were, the greater was the destruction of the vitamin. In brown Arabic bread, the percent loss of thiamine was greater compared to white Arabic bread prepared under identical conditions.

In both brown and white Arabic bread there was a uniform loss of riboflavin; however the percent retention was greater in the white than in the brown. Negligible losses of niacin occurred throughout all baking treatments of both white and brown Arabic breads.

Addition to the bread of levels higher than 5 mcg thiamine per gr flour resulted in an increase of this vitamin during fermentation. The addition of synthetic riboflavin resulted in higher retention values during the experimental baking. The niacin added was not affected by baking and was completely retained. The addition of 5 mcg thiamine per

gr flour along with 1 mcg of riboflavin and 30 mcg niacin per gr flour was sufficient to increase the vitamin content of white flour of 65% extraction to that of brown flour of 85% extraction.

VI. LITERATURE CITED

1. Andrews, J.S., H.M. Boyd, and D.F. Terry. The riboflavin content of cereal grains and bread and its distribution in products of wheat milling. Cereal Chem. 19, 55, 1942.

2. Anonymous. The Hashemite Kingdom of Jordan. Nutrition Survey, April-June, 1962. Interdepartmental Committee on Nutrition for National Defense. Washington. 1963.

3. Anonymous. Republic of Lebanon. Nutrition Survey, February-April, 1961. Interdepartmental Committee on Nutrition for National Defense. Washington. 1962.

4. Arny, E.B., and F. Hanning. Thiamine retention in the baking of muffins and biscuits. J. Am. Diet. Assoc. 23, 690, 1947.

5. Auerman, L.J., V.N. Bukin, Z.I. Zajceva, L.S. Kuceva, V.E. Pasovkin, and Scerbatenka. The preservation and content of vitamin B_1, B_2 and PP in bread from different sorts of flour. Biohim. Lerna. 2, 193, 1954. Abstracted in Nutrition Abstracts and Reviews (1743). Vol.27, 1957.

6. Barton - Wright, E.C. The microbiological assay of nicotinic acid in cereals and other products. Biochem. J. 38, 314, 1944.

7. Beadle, B.W., D.A. Greenwood, and H.R. Kraghill. Stability of thiamine to heat. I: Effect of pH and buffer salts in aqueous solutions. J. Biol. Chem. 149, 339, 1943.

8. Birdsall, J.J., and L.J. Teply. Effects of packaging on partially baked rolls: observations on the retention of reboflavin, moisture and flavour. Food Tech. 11, 608, 1957.

9. Bottomley, R.A., and S. Nobile. The thiamine content of flour and white bread in Sydney, New South Wales. J. Sci. Food Agric. 13, 550, 1962.

10. Brackman, R.A. Thiamine retention in self-rising flour biscuits. Cereal Chem. 19, 121, 1942.

11. Brenner, S., G.S. Dunlop, and O.V. Wodicha. Effect of fortification of canned bread on stability. Cereal Chem. 25, 367, 1948.

12. Chaudhuri, D.K., and E. Kodicek. The availability of bound nicotinic acid to the rat. Brit. J. Nutrition 14, 35, 1960.

13. Clegg, K.M. Bound nicotinic acid in dietary wheaten products. Brit. J. Nutrition. 17, 325, 1963.

14. Coppock, J.B., B.R. Carpenter, and R.A. Knight. Cereal products fortification: the B Vitamins, with special reference to thiamine losses in baked products. J. Sc. Food Agric. 7, 457, 1956.

15. Coppock, J.B., B.R. Carpenter, and R. A. Knight. Thiamine losses in bread baking. Chem. and Ind., 23, 735, 1957. Abstracted in Food Science Abstracts. (2599). Vol. 29, 1957.

16. Dawson, E.R., and G.W. Martin, Vitamin-B estimation in yeast and bread and stability during breadmaking. J. Soc. Chem. Indust. 60, 241, 1941. Abstracted in Nutrition Abstracts and Reviews (2929). Vol. 11, 1941-42.

17. Downs, D.E., and R.B. Meckel. Thiamine losses in toasting bread. Cereal Chem. 20, 352, 1943.

18. Free, A.H., Increase of Vitamin B_1 intake by the use of special high vitamin B_1 bread. Cereal Chem. 17, 725, 1940.

19. Golberg, L., and J.M. Thorp. Loss of thiamine during the baking of bread. Nature. 158, 22, 1946.

20. Guerrant, N.B., and O.B. Fardig. The thiamine and riboflavin content of whole wheat, non enriched and enriched flours and bread made therefrom. J. Nutrition 34, 523, 1942.

21. Hintzer, R.H., Influence of small quantities of thiamine on baking quality of wheat flour. Cereal Chem. 26, 258, 1949.

22. Hoffman, C., T.R. Schweitzer, and G. Dabby. The loss of Thiamine in bread on baking and toasting. Cereal Chem. 17, 737, 1940.

23. Horwitz, W. (Editor). Official Methods of Analysis. Association of Official Agricultural Chemists. Washington, D.C., 9th ed., 1960.

24. Kodicek, E., and P.W. Wilson. The isolation of niacytin, the bound form of nicotinic acid. Biochem. J. 76, 27, 1960.

25. Lincoln, H., L.E. Hove, and G.C. Harrel. The loss of thiamine on cooking breakfast cereals. Cereal Chem. 21, 274, 1944.

26. Loy, H.W. (Jr.), J.F. Haggerty, and E.L. Combs. Light destruction of riboflavin in bakery products. Food Res. 16, 360, 1951.

27. Martin, G.W. Vitamin B_1 content of crust and crumb of white bread. Chem. and Ind., 60, 342, 1941. Abstracted in Nutrition Abstracts and Reviews (1036). Vol. 11, 1941-42.

28. Meckel, B.R., and G. Anderson. Thiamine retention in the commercial production of Zwieback. Cereal Chem. 21, 280, 1944.

29. Meckel, B.R., and G. Anderson. Thiamine retention and composition of U.S. army bread. Cereal Chem. 22, 429, 1945.

30. Melnick, D. Collaborative study of the applicability of microbiological and chemical methods to the determination of niacin in cereal products. Cereal Chem. 19, 553, 1942.

31. Menden, E., and H.D. Cremer. The problem of improving nutritive value with special reference to entichment of food. Food Manuf. 34, 65, 1959.

32. Mickelsen, O. and R.S. Yamamoto. Methods for the determination of thiamine. Methods of Biochemical Analysis. Vol. 6, interscience Publishers, Inc., London. 1958.

33. Mitchell, H.H., T.S. Hamilton, and J.B. Shields. The contribution of non fat milk solids to the nutritive value of wheat breads. J. Nutrition. 25, 585, 1943.

34. Moran, T. Nutritional significance of recent work on wheat, flour and bread. Nutrition Abstracts and Reviews. 29, 1, 1959.

35. Morgan, A.F., and H. Frederick. Vitamin B_1as affected by baking. Cereal Chem. 12, 390, 1935.

36. Morgan, A.F., and M.J. Hunt. The vitamin B_1 and B_2 content of wheat products. Cereal Chem. 12, 411, 1935.

37. Morgareidge, K. The effect of light on vitamin retention in enriched white bread. Cereal Chem. 33, 213, 1956.

38. Pace J. K., and J. Whitcare. Factors affecting retention of B vitamins in corn bread made with enriched meal. I: Relation of pH to retention of thiamine, riboflavin and niacin in corn bread. Food Res. 18, 231, 1953.

39. Pelshenke, P.F. Report to the Government of Lebanon on the Bread Situation. Food and Agricultural Organization of the United Nations. Rome. 1964.

40. Pollock, M.J., and F.W. Geddes. The distribution of thiamine and riboflavin in the wheat kernel at different stages of maturity. Cereal Chem. 28, 289, 1951.

41. Robertson, J.D. White, national or wholemeal bread. Chem. and Ind. 62, 222, 1943. Reviewed in Kent-Jones, Northern Publishing Co., Ltd. Liverpool, 4th ed., 1950.

42. Sakr, A.H. Amino Acid pattern of some wild edible plants growing in Lebanon. M.S. Thesis. American University of Beirut, Beirut, Lebanon. 1961.

43. Schultz, A.S., L. Atkin and C.N. Frey. The vitamin B_1 content of wheat, flour and bread. Cereal Chem. 16, 643, 1939.

44. Sherwood, R.C. Accomplishments in cereal fortification. Am. J. Pub. Health. 3, 526, 1943.

45. Somers, F.G., M.H. Coolidge and K.C. Hammer. The distribution of thiamine and riboflavin in wheat grains. Cereal Chem. 22, 333, 1945.

46. Stephens, L.C., and M.F. Chastoin. Light destruction of riboflavin in partially baked rolls. Food Tech. 13, 527, 1959.

47. Thorn, J.A., and J.W. Ross. Determination of yeast growth in doughs. Cereal Chem. 37, 415, 1960.

48. Westerman, B.B., D.R. Linnn, F. Templeton, and R.I. Wells Improving the nutritive value of flour: the use of enriched flour in diets similar to those consumed by certain low income groups in South Carolina. J. Nutrition. 38, 421, 1949.

49. Williams, R.R. Cereals as a source of vitamin B_1 in human diets. Cereal Chem. 16, 301, 1939.

50. Williams, R.R., and V.H. Cheldelin. Destruction of riboflavin by light. Science.96, 22, 1942.

51. Zaehringer, V.M., and J.C. Personius. Thiamine retention in bread and rolls baked to different degrees of brownness. Cereal Chem. 26, 384, 1949.