EFFECT OF NAS/NRC RECOMMENDED MICRONUTRIENTS ON WHEAT FLOUR, DOUGH AND BREAD QUALITIES

by

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Dedicated to my parents

and

The Bread Baking Industry

UNENRICHED | REGULAR | NRC

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INTRODUCTION

Enrichment is the addition of nutrients to foods for public health objectives, and is effective for correcting some nutrient deficiencies in certain foods. Through this process consumer responsibility for making wise food choices is minimized. This also eliminates the long and expensive efforts that otherwise would be required for changing food habits through education.

This report discusses the effects of certain micronutrients on flour, dough, and bread. Specifically it deals with the effects of the micronutrients recommended for inclusion in cereal products by the Food and Nutrition Board of the National Research Council, National Academy of Sciences of the U.S.A. (referred further as NAS/NRC) (1).

Flour and bread enrichments has been practiced in the U.S.A. since the mid-1940's, and most of the white flours and breads produced since in this country have been enriched with thiamin, riboflavin, niacin and iron. In general the enrichment program has been one of the great successes in applied nutrition, and has played a large role in greatly reducing the incidence of nutrient deficiency diseases in humans (1).

Over the years, NAS/NRC has continued to monitor the nutritional status within the U.S. and to review the enrichment program, because of the dynamic nature of the population and changing dietary patterns. The continuing NAS/NRC study, eventually led to an important report issued in 1974 (1). This report, briefly, concludes that certain segments of the U.S. population face a potential risk of deficiency in a number of vitamins and minerals. To combat this risk, the NAS/NRC recommended that a far-reaching enrichment program be instituted, whereby these critical vitamins and minerals would be added to foods consumed by a wide majority of the population. These foods, specifically, comprised of all the cereal based products.

This investigation deals with one limited aspect of the NAS/NRC recommendations, namely the technical implementation of expanded enrichment applied to bread making. NAS/NRC fully realized when they made their recommendations that much work had to be done to determine what possible effects the expanded enrichment might have on the foods to which they are added. The objectives of this investigation were to determine the technological feasibility of the 1974 NAS/NRC recommendations, with respect to bread making. The three major objectives of this investigation were:

1. Study of Flour Properties.

   To determine whether the presence of nutrients altered the physical and chemical properties of bread flour.

2. Study of Bread Making Properties.

   To determine whether the nutrients affected the quality of bread (physical and organoleptic properties of bread).

3. Storage Study.

   To determine the stability of nutrients and their effects on storage properties of wheat flour.

REVIEW OF LITERATURE

IMPORTANCE OF CEREALS

Cereals are inexpensive sources of energy and protein. They are an important factor in the total production and consumption of foods. Being high in carbohydrate content (60-80%), cereals are sources of concentrated energy accounting for over one half of the total world food energy supplies with as much as 2/3 in the developing countries of the Near and Far East, Vogel 1978 (2). Recent studies (3) indicate that the share of world average per capita caloric supply provided by cereals has remained constant at the 50% mark for the past decade or longer. The cereal contribution ranges from a low of 26% of the caloric intake in the developed countries to a high of 65 and 66% in China and M.S.A. (most seriously affected) countries. On the other hand, the average usage of cereal grains as feed has risen from 37% in 1961-63 to around 43%, currently ranging from a low of 2% in the developing countries of the Far East to a high of 88% in North America and 72% for all developed countries.

Besides providing calories, because of their predominant carbohydrate content, cereal grains also contribute to the needs for protein, fat, vitamins and minerals. They provide considerable proportion of proteins needed in the diet. Cereal grains represent 50% of the total protein supply, which is nearly four times that supplied by either the pulses, oilseeds and nuts. This constitutes 13% of the total human intake (Table 1, FAO 1964 [4]).

Cereals constitute the main protein source (65-70%) in the diet of developing nations, because of the scarcity and expense of animal protein, Borlaug 1971 (5). The protein content of cereal grains vary, but in general, they are less than 20%. All essential amino acids are present in cereal proteins, although the level of certain amino acids such as lysine, methionine and tryptophan tend to be low (6). Cereals are not generally considered to be good sources of

Table 1. Present Sources of Protein in the Human Diet[a]

From Animal Sources		-29%
Meat and poultry	-13%	
Dairy products	-11%	
Eggs	- 2%	
Fish	- 3%	
From Vegetable Sources		-71%
Grains	-50%	
Pulses, oilseeds and nuts	-13%	
Vegetable and fruits	- 3%	
Starchy roots	- 5%	

[a]The percentage represents world-wide protein consumption.

protein because of low levels of these amino acids. However, anyone living on a cereal-based diet who eats sufficient to satisfy his energy needs will satisfy his gross protein needs (7).

Cereal grains are low in fats (0.5-8%) and thus cereals by themselves are not particularly fattening. They are good sources of the essential fatty acids. The favorable phosphorus/sulphur ratio of the oils of cereal grains are satisfactory to maintain normal blood cholesterol. In addition to supplying calories, the carbohydrate moiety of cereal grain makes a major contribution to the dietary fibre intake. Cereal grains are important sources of vitamins and minerals - B vitamins: thiamin, riboflavin, niacin, Vitamin B_6, folic acid, pantothenic acid and biotin, Vitamin B_{12}, as well as fat soluble vitamins, Vitamin E; and minerals Fe, Ln, Mg, P, K, S. Cereals meet important post harvest requirements, of easy storage and transport, retaining their quality over long periods. In addition to their indigenous nutritive properties, cereal grains have additionally contributed by functioning as carriers for fortification (6).

Cereal grains being high energy foods and low in cost provide a major nutrition source for low-income families or for large populated areas with limited arable land. They have made a substantial contribution to the food supply of the population of developed countries, all over the world. Following are the statistical data of the total cereals produced in 1976 compared to that produced in 1961-65 (8). Table 2 indicates a positive production trend.

The world's two most important cereal crops are wheat and rice. Wheat, like other cereal grains, has many advantages as food. It is nutritious and easily processed to give highly refined foods. Wheat provides 20% of the total calories for the people of the world. Wheat products are consumed by more people than any other cereal product in the world's population. It is the main

Table 2. Cereals Total

Regions	Area Harvested/ 1000 HA		Yield Kg/HA		Production/ 1000 MT.	
	1961-65	1976	1961-65	1976	1961-65	1976
Developed Economies	147002	162943	2342	2993	344265	487759
Developing Economies	266211	314021	1087	1378	289302	432706
Centrally Planned Economies	263463	282458	1344	1972	354191	556883
World	676676	759422	1460	1945	987758	1477348

staple for about 35% of the world's population (9). Following is the statistical summary of the global production of wheat (Table 3), (8).

The nutrient substances found in wheat help to make wheat one of the most important cereal grains in the food industry. Wheat protein is a satisfactory source of protein when balanced by other foods that supply amino acid such as lysine. Lochart and Hesley 1978 (10) report that apparent digestibilities of the protein of oat flour and wheat flour are within 95% of that of casein protein. When whole grain products and enriched flour are used there is a better supply of vitamins and minerals, than when refined products are used (11, 12). The prominence of wheat as a food grain is not only attributable to its nutritive value, but also to its unique protein gluten which enables a leavened dough to rise by forming a structure of minute cells that retains CO_2 produced during fermentation. The greatest portion of wheat produced is made into either leavened bread or unleavened breads such as chappaties or nans etc.

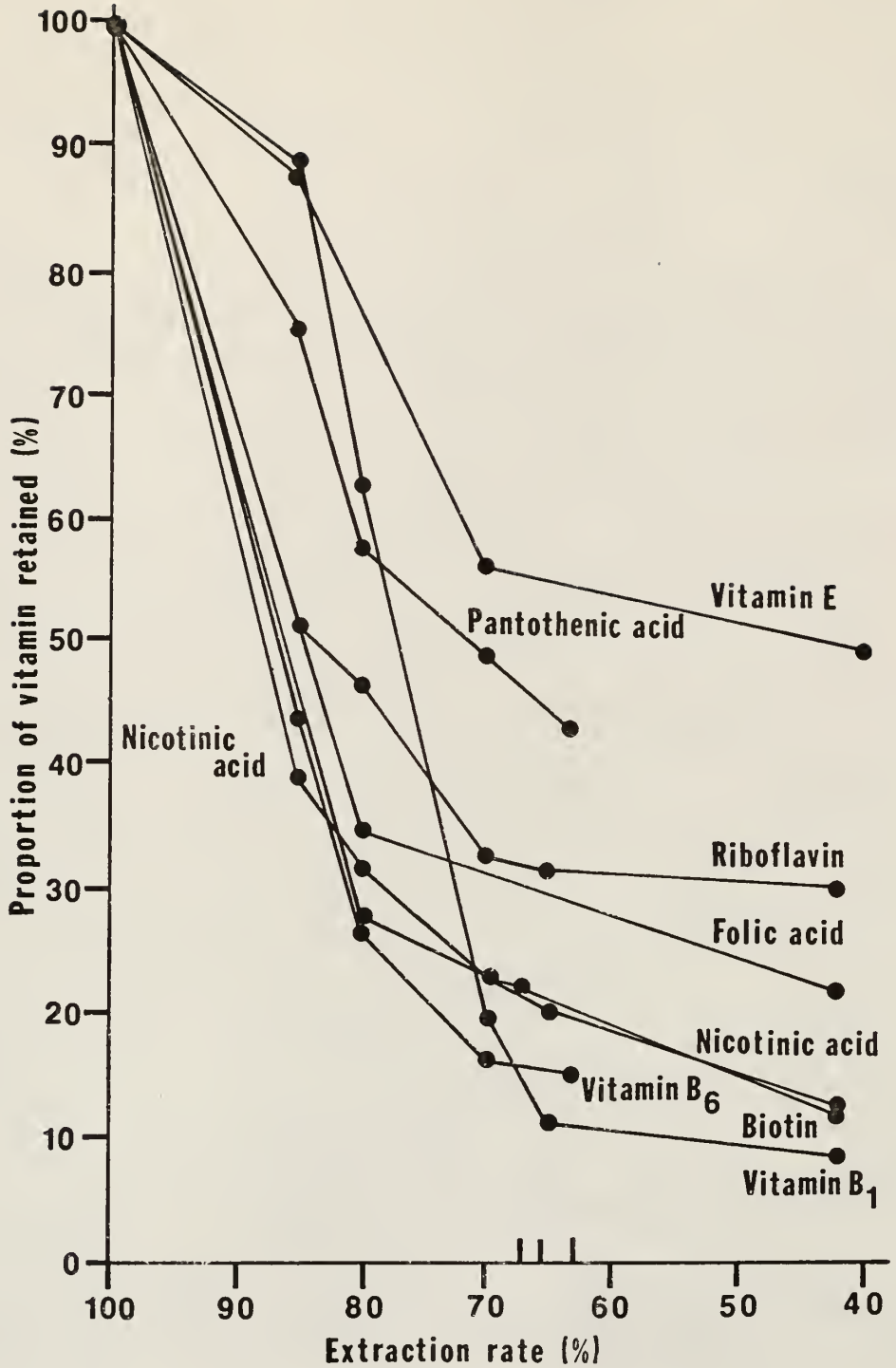
In whole wheat grain, important vitamins and minerals are concentrated in the bran and the germ. During the milling process bran and wheat germ are removed; that consequently influences the nutrient composition in flours of various extraction rate (13) Figure 1. Fairly similar diversion of nutrients has been reported by Teopfer et al 1972 (14), from 70% extraction rate flours. Most wheat used for human consumption in developed countries is milled to produce white flour of approximately 70-75% extraction rate (6).

Populations relying on cereals as a staple diet run the risk of exposure to nutritional problems (vitamins and minerals) especially when a portion of the grain is discarded in favor of grain endosperm as in the case with milling white or refined flour. Thus it becomes imperative to fortify flours so as to counter nutritive inadequacies that may otherwise be manifested in the finished product. The fortification recommendations were thus initiated by NAS/NRC.

Table 3. Wheat Production (8)

Regions	Area Harvested/ 1000 HA		Yield Kg/HA		Production 1000 MT.	
	1961-65	1976	1961-65	1976	1961-65	1976
Developed Economies	59696	68657	1737	2239	103695	153743
Developing Economies	50424	67231	975	1414	49161	95048
Centrally Planned Economies	100267	99414	1013	1697	101570	168687
World	210387	235302	1209	1774	254426	417478

Figure 1. Relation Between Extraction Rate and Proportion of Total Vitamins of the Grain Retained in Flour


Proportion of vitamin retained (%)
100
90
80
70
60
50
40
30
20
10
0
100
90
80
70
60
50
40
Extraction rate (%)
Vitamin E
Pantothenic acid
Nicotinic acid
Riboflavin
Folic acid
Nicotinic acid
Vitamin B6
Biotin
Vitamin B1

NAS/NRC FORTIFICATION: HISTORY AND PROBLEMS

History

Nutrient addition to flour and bread began in the 1940's, after the committee on food and nutrition (now Food and Nutrition Board, established by the National Research Council [NRC]). The American Medical Association (AMA) and Food and Drug Administration (FDA) endorsed a program recommending that flour and bread be enriched with thiamin, niacin, riboflavin and iron (15). Since then such enrichment practiced by the milling and baking industry has resulted in the decrease of deficiency diseases like pellagra (niacin), beriberi (thiamin), ariboflavinosis (riboflavin) and anemia (iron) (16, 17, 18, 19). Thus enrichment has been called a "quiet miracle" (20).

The Food and Nutrition Board of the National Academy of Sciences in 1974 proposed an expanded fortification guide for cereal grain products prepared from wheat, corn and rice (1). The board recommended that a proper enrichment formula (Table 4) for cereal grains should provide niacin, thiamin, riboflavin, vitamin A, vitamin B_6, folic acid, calcium, magnesium, iron and zinc, because a significant proportion of the U.S. population appears to be at a risk of deficiency in one or more of these nutrients.

The fortification standards are based on nutritional studies conducted since the 1950's and were developed because total energy requirements in the U.S. have declined (15), and there has been a general shift from traditional patterns to highly individualized or unstructured modes of eating (21). Vitamin A, vitamin B_6, folic acid, magnesium, zinc and mandatory calcium fortification has been proposed (1). Their incorporation in food industry products needs further evaluation and investigation for possible independent and interaction effects.

Table 4. Proposed Fortification Policy for Cereal Grain Products[a]

Food and Nutrition Board

National Research Council - 1974

Nutrients	mg/lb	mg/100g
Vitamin A[b]	2.2 (7300 IU)[c]	0.29
Thiamin	2.9	0.64
Riboflavin	1.8	0.40
Niacin	24.0	5.29
Vitamin B_6	2.0	0.44
Folic Acid	0.3	0.07
Iron	40.0	8.81
Calcium	900.0	198.20
Magnesium	200.0	44.10
Zinc	10.0	2.20

[a] Wheat flour, corn grits, corn meal, rice and other cereal grains proportion to their cereal grain content.

[b] Retinol equivalent.

[c] The originally proposed level of 2.2 mg/lb (7300 IU) was lowered to 1.3 mg/lb (4300 IU) Hepburn 1976 (3).

Problems

Calcium and Magnesium. Calcium and magnesium nutrients when added according to the NAS/NRC levels have presented some problems (22, 23, 24). Though calcium and magnesium do not influence specific volume, crumb color is dark, grain is poorer and proof time increases (22, 23). Ranhotra et al 1976 (23), however, found that the oxide and carbonate sources of magnesium raised bread pH and exerted a significant deleterious effect on loaf volume and general bread quality including flavor. The off-flavor of bread produced by adding magnesium may be counteracted by adding acetic acid (23) or L-lysine monohydrochloride, Wolf et al 1976 (24). Rubin et al 1977 (22) further indicated that calcium and magnesium may cause some loss of vitamin A and to a lesser extent folic acid at the temperatures and moisture levels in the baking process.

Bioavailability studies (25) indicated that magnesium was equally available from all sources studied. Calcium bioavailability, however, has not been reported in literature. Results of the earlier studies (26, 27, 28, 29) indicated little or no difference in the availability of calcium from various sources, including calcium sulphate and carbonate, but none of these studies involved baked products such as bread. Therefore, before calcium enrichment becomes mandatory, more information is needed to determine which salts should be incorporated. To fully evaluate the possible adverse calcium and magnesium effects without affecting bread quality additional studies are needed (22, 23, 30).

Iron. Iron perhaps is the nutrient which causes many problems for the cereal enrichment program, be they nutritional, functional or regulatory in nature, Ranum et al 1978 (31). Most cereal enrichment is accomplished with four iron sources: ferrous sulphate, reduced iron, ferric orthophosphate and sodium iron pyrophosphate (32). Ferrous sulphate is the most widely used form of iron to the body, because of its high assimilability (36, 37, 38). However, ferrous sulphate is not widely used in flour enrichment because it promotes development

of off-flavors and off-colors in flour going overseas or to grocery stores, Jackel 1975 (20). Millers prefer to use reduced iron for flour enrichment because it is relatively inert, low in cost and avoids flour stability problems (20, 39, 40). Results of bioavailability studies on reduced iron vary widely possibly because of an apparent inverse relationship between availability and particle size -- the finer powdered iron being highly assimilable (41). Other factors affecting the bioavailability of reduced iron are solubility, chemical impurities, surface area, porosity and age, Fritz et al 1975 (42). The question of human bioavailability of iron is broad and complex as pointed out by the adhoc committee on iron enrichment of wheat flour and baked goods (34); it needs resolution by further research. Numerous workers have studied the assimilation of various iron sources and are in general agreement that low-cost ferrous sulphate has high availability, while the availability of ferric orthophosphate is quite low (38, 43, 44). The deleterious oxidation effect can be overcome by using encapsulated ferrous sulphate known as stabilized iron (45, 46) or ferripolyphosphate (47). Nimmo et al 1975 (47) reports that ferripolyphosphate can be used as a source of dietary iron because it possesses excellent storage properties, besides providing a high level of nutritionally available iron. Today millers are reluctant to use more than one type of iron for enrichment and would prefer a standardized reduced iron powder that shall overcome related problems (33).

Iron is presently mandated at 13-16.5 mg/lb of flour or 8.0 to 12.5 mg/lb of baked product according to the NAS/NRC proposed recommendation. The iron level would be increased to 40.0 mg/lb and 25 mg/lb of enriched bread (1). The 25 mg level of iron as ferrous sulphate has been a measure of concern in continuous mix process associated with bread quality (48, 49). The adverse effects were reportedly overcome by increasing oxidizing capacity and properly scheduling bromate and ferrous sulphate addition. The above higher iron level was

recently rejected by FDA (50) because of potential health hazards iron levels might create in persons with iron storage disorders, a condition known as parenchymal iron overload or hemochromatosis (51, 52). Additional research is needed because the storage disease is a rare disorder that could occur regardless of manipulation of dietary intake. Therapy depends on iron removal through phlebotomy or chelate therapy, rather than on decreased intake (53, 54). This debated matter is controversial (55, 56, 57, 58).

In the last 10 years some improvements have been made in the form of iron used in cereal enrichment. Research is still needed to establish better specification on particle size distribution and solubility of reduced iron to insure optimum bioavailability (31).

Calcium and Iron Ratio. Calcium addition to bread and flour at the NAS/NRC proposed levels (1) may affect iron utilization (59, 60, 61, 62, 63, 64, 65) Kletzein, S.W. 1940 (65) reported that addition of 1 and 3% calcium carbonate to a basal diet containing 90% ground whole wheat resulted in lower tissue (liver, blood and carcass) iron values as compared to unsupplemented diet.

Generally large calcium:iron ratios in animal studies have adverse effects on iron utilization, while relatively small calcium:iron ratios have no effect in promoting iron absorption, Chapman and Campbell 1957 (63). When calcium additions are conducted according to the NAS/NRC proposed level (1), the calcium: iron ratio in flour is approximately 70:1. This ratio is considerably lower than the ratio used by Chapman and Campbell 1957 (63). They added calcium carbonate and other salts to bread based diet with calcium to iron ratio ranging from 167:1 to 343:1, and reported that calcium carbonate when added at calcium: iron ratio of 182:1 and 226:1 interferred with iron utilization.

Apte 1964 (60) and Senchak 1973 (66), reported that the prevalence of anemia in populations subsisting on cereals may not be due to high phytate content of the diet, but more to a lower calcium intake or high bulk which impairs iron

absorption or to a failure to adapt to such a diet. Ranhotra et al 1974 (67) support these findings and report that iron availability may not be affected by a somewhat increased consumption of natural phytate in the form of cereals on diet otherwise low in bulk and adequate in calcium, vitamin D and iron. They further inferred that increasing phytate level in the diet did not substantially interfere with iron availability naturally occurring in wheat.

The calcium:iron ratio studies need to be extended to substantiate applications for adequate human utilization.

Folic Acid and Vitamin B_6. Problems are also reported in folic acid and vitamin B_6 microbiological assays. DeRitter (68) reported that the methodology is expensive and difficult, complicated by interference due to conjugated forms of these vitamins.

STORAGE

Storage is a repeated phase in the complex logistics of transporting grain from producer to processor and grain products from processor to consumer. Quality is of concern throughout the whole system, Anderson, J.A. 1973 (69).

The principal variables affecting the storability index of cereal grains in order of importance for conventional storage and handling are:

1. Moisture Content and Equilibrium Relative Humidity.
2. Temperature.
3. Time.

The moisture content and temperature are the critical factors and are dependent on the relative humidity and temperature of the environment, Hall and Dean 1978 (70). Other related factors affecting the keeping quality of flour during storage are particle size (71), hygroscopic nature of the product (72, 73), the type of container (74, 75) and the presence of additives (46).

Moisture Content and ERH

The moisture content gives an estimate of the amount of water in a product, Pixton et al 1971 (76). Cereal grains and their products being hygroscopic absorb moisture from or lose it to the surrounding atmosphere until they establish an equilibrium. The moisture content in a product in equilibrium with its relative humidity (RH) at a constant temperature is referred to as equilibrium relative humidity (ERH) or water activity (Aw), Hunt and Pixton 1971 (72). The relationship between moisture content of different cereal grains and cereal products and their ERH has been studied by many workers (77). Ayerst 1965 (78) and Jones 1969 (79) report that in considering storage potential the ERH of the product is more important than the moisture content. For "safe" storage, the moisture content for products such as grains and oilseeds is usually accepted as that in equilibrium with 70% RH or less. If RH is above 75% moulds develop rapidly during storage and heating of the products occurs with subsequent deterioration and loss (72, 76). The ERH relationship is particularly important for wrapped goods. A wrapper must be somewhat permeable to moisture to allow excess moisture to escape, Jones 1969 (79). Anker et al 1942 (74) report that larger packages do not exhibit more rapid changes in net weight and moisture than smaller ones under the same conditions.

Safe storage of flour may be insured through proper control of its moisture content and RH. The critical water content for fungal growth is approximately 16%. For safety a water content of 15% or less, with storage at relative humidities of not more than 80%, are advisable to eliminate mould growth, Barton-Wright 1940 (80). At high moisture levels lipase activity is increased due to mould growth (81). On the other hand, the moisture must not be reduced too far, far below 5 to 6% (81), since the development of rancidity is accelerated (71, 82 83).

Temperature

Temperature is considered to be a crucial variable for safe and prolonged storage of cereal grains and their products (84). Constant temperature conditions should be maintained during storage. A cycling temperature would not only affect the moisture content of a product, but may initiate the growth of associated microorganisms, Hall et al 1978 (70), Disney 1969 (85). Flour responds more readily to temperature changes than does bulk grain (86) because of its hygroscopic nature (87). It is an established fact that at elevated temperature the moisture content of the flour is reduced and rancidity develops (71, 83) due to hydrolytic and oxidative changes in lipids (88).

Time

Cereal grains and their products may be stored for longer durations if good storage conditions are maintained (89). Generally low temperature and low moisture content favor longer keeping quality, while high temperature and moisture content show that the product stored will remain sound for a much shorter length of time (81, 90).

Wheat can be stored for longer periods under much less exacting conditions than are required for flour storage (91). Under optimum flour storage conditions the baking quality of all flours improves up to a certain point, beyond which deterioration sets in and this continues until all the flour becomes unfit for bread making (92). When flour is aged at the mill, it is recommended that the flour be held at the bakery for a minimum period of a week to permit its temperature equalization, Pyler 1971 (92).

During storage period, the flour components change physically, chemically (83, 92) and biologically (93). These changes are recorded by variations in test indices used to characterize the flour aging phenomena. Test indices used to monitor changes during flour storage include: farinograph (71, 94, 95), limylograph (94, 96, 97, 98, 99), extensograph (71, 95), gassing power (71),

pH (83), flour color (88), free fatty acids (83, 86, 101) and peroxide value. The parameters used to determine changes in baking functionality include: baking absorption (75), loaf volume (95, 102, 103, 104), crumb color (105), crumb firmness (105).

MATERIALS AND METHODS

MATERIALS

Flour

Untreated and chemically improved baker's patent flour (supplied by ADM Milling Company) was used in this study. Chemical analysis of this flour was done in the grain science analytical laboratory. The flour had a protein content of 11.2% and 0.43% ash (on a 14% m.b.) and 12.8% moisture. The farinograph showed a water absorption of 58% (on a 14% m.b.) and 7.5 minutes of mixing time.

Nutrients

The nutrients were added as a vitamin-iron premix in the form of regular enrichment or as a NAS/NRC test blend.

The following nutrients were utilized:

1. Regular Enrichment

   (Supplied by Pennwalt Corporation, Wheat and Flour Services)

   A. Regular Enrichment with Electrolytic Iron

      The addition of 7.875 gms of Type 41 enrichment to 100 pounds of the product adds to each pound the following:

	mg/lb
Thiamine	2.80
Riboflavin	1.75
Niacin	21.0
Iron (Electrolytic)	12.0

   B. Regular Enrichment with Ferrous Sulphate

      The addition of 14.175 gms of the enrichment to 100 pounds of the product adds to each pound the following:

	mg/lb
Thiamine	2.80
Riboflavin	1.75
Niacin	21.0
Iron (Ferrous Sulphate)	14.0

II. Test Enrichment:NAS/NRC Enrichment

(Supplied by Hoffman LaRoche Company, Inc.)

A. Premix I with Electrolytic Iron

Composition	gms
Thiamin Mononitrate	2.57
Riboflavin - Type S	1.80
Niacinamide	21.0
Electrolytic Iron	11.46
Pyridoxine HCl	2.0
Folic Acid	0.26
Vitamin A Palmitate (250 SD)	20.0
Tricalcium PO_4	3.0
Corn Starch Q.S.	100.0

Feed Rate: 10 gms/100 lbs

Electrolytic Iron: Supplied by Glidden and Durkee

B. Premix II with Ferrous Sulphate

Composition	gms
Thiamine Mononitrate	1.71
Riboflavin - Type S	1.20
Niacinamide	14.0
Ferrous Sulphate	29.17
Pyridoxine HCl	1.33
Folic Acid	0.17
Vitamin A Palmitate (250 SD)	13.33
Tricalcium PO_4	3.0
Corn Starch Q.S.	100.0

Feed Rate: 15 gms/100 lbs

Ferrous Sulphate: Supplied by Mallinckrodt Company

C. Minerals

1. Magnesium Oxide (60% Mg). Supplied by Mallinckrodt Company

   Feed Rate: 18 gms/100 lbs

2. Zinc Oxide (80% Zn). Supplied by New Jersey Zinc Company

   Feed Rate: 0.9 gms/100 lbs

3. Calcium Sulphate - anhydrous (29% Ca). Supplied by U.S. Gypsum Company

   Feed Rate: 300 gms/100 lbs

4. Calcium Carbonate - refined (40% Ca). Supplied by Georgia Marble Company

   Feed Rate: 220 gms/100 lbs

   Calcium as sulphate and carbonate were added separately because of their large bulk (107).

For some of the experiments the individual nutrients were added:

1. Thiamine Mononitrate

   Feed Rate: 2.57 mg/lb

2. Riboflavin

   Feed Rate: 1.80 mg/lb

   Both were supplied by Hoffman La Roche Company, Inc.

3. Electrolytic Iron

   Feed Rate: 11.46 mg/lb

   Supplied by Glidden and Durkee

4. Niacin

   Feed Rate: 21.0 mg/lb

5. Niacinamide

   Feed Rate: 21.0 mg/lb

   Both were supplied by Lonza Inc.

METHODS

Physical Dough Tests

Studies on the rheological properties of flour and enriched flour with nutrients were measured using the farinograph, mixograph, extensograph, and viscoamylograph tests according to the Official AACC methods (108). pH and Agtron color measurements were conducted using the corning digital 110 expanded scale pH meter, and Agtron multichromatic abridged relative reflectance spectrophotometer, Model M-500 on the grain mode (546 nm), according to the Official AACC methods (109).

Sponge Dough Formulation and Procedure

The breads were prepared in duplicate by the sponge dough method whose formulation and procedure were based according to the following method (Table 5). Enrichment was added in the form of a prepared flour blend. Each 10 gms of the blend contained all the required micronutrients. This blend was added to the flour in the sponge stage, and replaced 10 gms of the sponge flour. Calcium sulphate was added separately because of its large bulk (107).

Sponge. Mixing for three minutes in a Hobart Model A-200 horizontal mixer.

Fermentation. Four hours - 86°F/86% RH.

Dough. The ingredients were mixed for 30 seconds at Speed 1 in a Hobart mixer. The sponge was then added and mixed for one minute and 15 seconds. The sponge and the dough ingredients were then mixed to optimum dough development for 5½ minutes.

Floor Time. 30 minutes - 86°F/86% RH.

Intermediate Proof. Ten minutes.

After 30 minutes' floor time, the dough was cut into two pieces of 539 gms each, punched on a national roller punch machine first at 5/16" and then 1/4" triple folded, and then given ten minutes intermediate proof time.

Table 5. Sponge Dough Method Formulation and Procedure

Sponge Ingredients	Level %	Dough Ingredients	Level %
Flour	70.0	Flour	30.0
Yeast	2.5	Salt	2.0
Mineral Yeast Food	0.375	Sugar	7.0
Barley Malt	0.05	Shortening	3.0
Water	Variable (66%)[a]	Water	Variable (34%)[b]

Total Water Absorption - 60%.

[a]Based on sponge flour.
[b]Based on dough flour.

Moulding. Moulding was done immediately on the mouline 100 moulder.

Pan Proofing. 1.5 cms above pan at 105°F and 92% RH.

Baking. Breads were baked for 20 minutes at 425°F.

Bread Volume and Weight

The bread volume was measured immediately after baking, by rape seed displacement. Bread weights were taken and specific volume was determined. After an hour of cooling, the breads were bagged and sealed in polyethylene bags and stored at room temperature (approximately 25°C).

Crumb Compressibility Test

Crumb compressibility of the stored breads was measured at 24 and 96 hours with a Bloom gelometer, having a plastic plunger of one inch diameter. A one-inch cut slice of bread was placed on the gelometer platform that could be vertically adjusted, such that the slice just touched the bottom surface of the plunger. Lead shots were then released into the cup that depressed the plunger into the slice. The degree of crumb firmness was expressed in terms of weight of the shot required to compress the slice by 4 mm (110). Care was taken while selecting portions of the loaf for crumb firmness test as more variations could be encountered within loaves as a result of improper sampling than within loaves representing different treatments (111). Ponte and co-workers (111) found that intra-loaf firming variations were attributed to differences in specific volume within loaves.

The loaf was sliced into nine one-inch slices. Two slices from each end of the loaf were discarded. Measurements were taken on six of the slices in the loaf.

After 24 hours, crumb firmness measurements were made on one loaf from each dough, and the loaves were then scored for external and internal characteristics. The other set of loaves from each dough were stored for firming measurements after 96 hours.

Crumb Color

Crumb color was measured by Agtron multichromatic abridged reflectance spectrophotometer Model M-300 with four spectral lines, of which the blue mode (436 nm) was used. The scale was standardized using a standard disc #24 and #75 to read 0.0 and 100.0, respectively. Measurements were taken on all six one-inch thick slices of the loaf. A black paper mask (2 x 2") was placed on the viewer to cut the escaping light at the sides of the slice. The bread slice was placed on the mask and then the disc #24 was placed on top of it. After that the readings were recorded.

pH Measurements on Bread

pH was measured with a corning digital 110 expanded scale pH meter according to hydrogen ion activity (pH), electrometric method, AACC official method (109).

Organoleptic Studies

On days three and six of storage, the breads were sliced one-half inch thick on the commercial slicing machine. From each loaf the two slices from each end were discarded leaving 15 one-half inch thick slices. These 15 slices from each loaf were immediately placed in polyethylene bags and fastened. The taste testers received three slices--two slices of regular enrichment which served as the reference control and one slice of bread which was enriched with the test enrichment (NAS/NRC enrichment). All slices were coded (with different random numbers) and the order of treatments was randomized. One of the slices of the regular enrichment was labelled as "R" meaning the reference, while the other two slices were coded by different numbers. This was done to reorient the panel members to the base line for rating bread identity of enriched breads.

The panel for the taste test consisted of 48-49 members. Each member received three bread slices, a score sheet and water as a mouth rinse between samples. The panel members were required to evaluate the enriched breads as

compared to the control for overall aroma, flavor and mouthfeel, when crumb and crust were eaten together. They were asked to define their response according to five descriptive ratings and give reasons for their preference. The score sheet used for taste evaluation is reproduced on page 28.

Statistical Procedure

The results of the organoleptic evaluations on bread were analyzed by the Wilcoxon Signed Rank Test (112).

Enriched Breads Analyzed for Magnesium

The enriched breads were analyzed for their magnesium content in the grain science analytical laboratory.

NAS/NRC Fortified Bread Flours Prepared for Storage

The storage studies were conducted with flours containing the proposed level of NAS/NRC nutrients (1). Prepared premixes were supplied by Hoffman La Roche Company, Inc.--one containing electrolytic iron and the other ferrous sulphate as iron sources and quantities of minerals to be added viz.: MgO, ZnO and anhydrous $CaSO_4$. These were weighed into polyethylene bags according to their feed rates, for blending with 200 lbs of flour. The premix and three or four lbs of flour were put into large polyethylene bags. The bags were inflated and shook to insure thorough mixing. About 50 lbs of flour and 1/4 of the premix were alternately placed in a double ribbon batch mixer, until the mixer was filled with a total of 200 lbs of flour plus premix.

The components were then mixed for 20 minutes and emptied into fiber drums, from which the flour was placed into eight 25-lb paper sacks. These sacks were sewn and stored at four different temperatures: (i) cool storage; (ii) deep freeze; (iii) room temperature; and (iv) hot storage. Untreated control flours were also stored in the freezer to minimize changes. Paper sacks were chosen for storage at the above-mentioned temperatures so as to stimulate commercial practice.

NAME _____ DATE _____

CEREAL-BASED FOODS TASTE TEST

Please taste the reference sample labeled "R".
Compare "R" with each of the coded samples provided.
Mark the amount of difference detected between the reference and the coded samples by placing an "x" in the appropriate box.

AMOUNT OF DIFFERENCE BETWEEN R AND:

	NONE	SLIGHT	MODERATE	LARGE	EXTREME
721					
537					

Taste test the samples and check how much you like or dislike each one, taking everything about the samples into consideration (flavor, color, texture, etc.). Please give a reason, if possible, for your rating.

	R	721	537
Like very much			
Like moderately			
Like slightly			
Dislike slightly			
Dislike moderately			
Dislike very much			

Reason	Reason	Reason

After three and six months' storage at the four different temperatures, the stored flours were transferred from paper sacks into polyethylene bags to prevent further moisture absorption from the atmosphere. The polyethylene bags were shaken well to insure uniform moisture conditions throughout the sample and analyzed for moisture in the K.S.U. grain science analytical laboratory. Finally these flours were examined for their effect on physical properties of flour and dough (viz.: color and rheological properties) by using Agtron color measurements, the farinograph, viscoamylograph and pH measurements according to the official AACC methods (113), and characteristics such as volume, texture, color, flavor and odor with the help of the bake test, crumb compressibility and crumb color measurements and taste panel evaluations, as discussed on pages 23 to 26.

Odor Test

The NAS/NRC stored flour sample containing the two iron sources, electrolytic iron and ferrous sulphate, were placed in glass jars and labelled with random code numbers. The respective temperatures within each iron treatment were also randomized. The members scored each batch of iron treatment of four temperatures, separately in different sections of the large room. Panel members (staff and students) were required to evaluate the enriched flours as regards overall color, odor and acceptance. The panel score sheet used for evaluation is reproduced on page 30.

Taste Test

Fresh bread baked from three and six months' NAS/NRC stored flour samples was used for taste panel evaluations. The score sheet used for evaluation of both iron treatments is reproduced on page 31.

Name: _____

Date: _____

ODOR TEST

	529	568	503	597
Like very much				
Like moderately				
Like slightly				
Dislike slightly				
Dislike moderately				
Dislike very much				
	Reason	Reason	Reason	Reason

Name: _____

Date: _____

TASTE TEST

	363	379	315	328
Like very much				
Like moderately				
Like slightly				
Dislike slightly				
Dislike moderately				
Dislike very much				
	Reason	Reason	Reason	Reason

Statistical Procedure for Taste Test and Odor Test

The organoleptic evaluations of breads prepared from NAS/NRC fortified flours stored for three and six months and the results of the odor test on these stored fortified flours were analyzed by the Freidman's test (112).

Mycological Examination

When the taste test results showed that the breads baked from flour stored for six months in the deep freeze had poor organoleptic properties, a mycological examination was conducted to determine what caused the poor taste.

A small amount of the NAS/NRC fortified flours stored for six months at each of the four storage conditions was sprinkled on three agar media: malt agar with 4% NaCl, potato dextrose agar and nutrient agar. The agar plates were held at room temperature (approximately 25°C) for one week and examined with a binocular dissecting microscope. Mold colony growth on plates was transferred to differential media for pure culture isolation and identification. Media used included: malt agar with 10% NaCl, Czapek's solution agar, Blakelee's malt agar, Czapek's agar with 20% and 40% sucrose, and malt agar with 20% and 40% sucrose.

Gassing Power

Gas production of the sponges and the doughs was measured by pressuremeters using the official AACC method (114). Seventeen gms of the dough was placed in the pressuremeter. These were tightly closed and placed in the water bath (30°C). Readings were taken every 30 minutes over four hours for the sponges and over three hours for the doughs. Gassing power values were the average of such readings taken on two different days.

Statistical Analysis

The results of this study were analyzed by the analysis of variance procedure. Duncan multiple range test was used for making multiple comparisons.

RESULTS AND DISCUSSION

EFFECT OF MICRONUTRIENTS ON PHYSICAL AND CHEMICAL PROPERTIES OF FLOUR, DOUGH AND BREAD

This study was conducted to test the effect of the NAS/NRC micronutrients and their possible interaction on bread baking properties.

Farinograph and Mixograph Dough Properties

Acidic and basic salts have a marked effect on the physical dough properties and affect gluten consistency (115, 116, 117, 118). The general effect of acids is to decrease both mixing time or dough development time and stability (117, 118, 119, 120), whereas salts increase the mixing time and stability (117, 118, 121, 122, 123, 124, 125). Incorporation of acids and salts further increase mixing time and stability (117, 118, 120).

The independent and interaction roles of the NAS/NRC nutrients, consisting of a combination of both weak acidic ($CaSO_4$) and basic salts (MgO, $CaCO_3$, and ZnO), were examined with the help of the farinograph and mixograph (Table 6). Both instruments are useful in predicating absorption and mixing time of flours.

The farinograph data (Table 6) shows that the regular enrichment had little effect on either the mixing time or tolerance of the control flour. The NAS/NRC enrichment containing $CaSO_4$ or $CaCO_3$ increased the mixing time (7.5 to 9.0 and 9.25 mins) and tolerance (12.5 to 15.0 and 17.0 mins), respectively. The strengthening effect is largely due to MgO, since when the premix and the flour were fortified with MgO increased mixing time and tolerance were observed (Table 6). The dough strengthening effect was pronounced when MgO and $CaSO_4$ were added together in the premix and the flour. Here the mixing time increased from 7.5 to 9.0 and 10.5 mins, and tolerance time extended from 12.5 to 16.0 and 17.0 mins. Of the two calcium sources, the weak acidic sulphate seems to have a larger effect on the peak time, than its weak basic carbonate form (Table 6).

Table 6. Effect of Micronutrients on Farinograph and Mixograph Dough Properties

Treatments	Farinograph				Mixograph
	Mixing Time mins	Arrival Time mins	Departure Time mins	Tolerance mins	Mixing Time mins
Unenriched	7.5	1.5	14.0	12.50	4.0
Regular Enrichment with Fe*	7.5	1.75	15.0	13.25	4.0
Regular Enrichment with $FeSO_4$	6.75	1.25	14.5	13.25	4.25
NRC** with Fe* ($CaSO_4$)	9.0	2.0	17.0	15.0	4.50
NRC with $FeSO_4$ ($CaSO_4$)	8.5	1.5	17.0	15.5	4.50
NRC with Fe* ($CaCO_3$)	9.25	2.0	19.0	17.0	4.50
NRC with $FeSO_4$($CaCO_3$)	-	-	-	-	4.50
Premix 1 with Fe*	7.0	1.25	14.5	13.25	4.0
Premix 2 with $FeSO_4$	7.0	1.5	14.5	13.0	4.0
Premix 1 & ZnO	8.0	1.5	15.0	13.5	4.25
Premix 1 & $CaSO_4$	8.25	2.5	15.0	12.5	4.25
Premix 1 & $CaCO_3$	8.0	3.0	15.0	12.0	4.25
Premix 1 & MgO	8.5	1.3	17.5	16.2	4.50
Premix 1 & MgO & $CaSO_4$	9.0	2.5	18.5	16.0	4.50
Premix 1 & MgO & $CaCO_3$	-	-	-	-	4.50
Unenriched & ZnO	8.0	2.0	16.0	14.0	4.25
Unenriched & $CaSO_4$	8.75	1.5	14.5	13.0	-
Unenriched & $CaCO_3$	7.5	2.0	17.5	15.5	4.25
Unenriched & MgO	9.25	2.0	19.0	17.0	4.25
Unenriched & MgO & $CaSO_4$	10.5	1.5	18.5	17.0	4.50

Fe* = Electrolytic Iron
NRC** = NAS/NRC Enrichment

Though calcium salts caused dough strengthening effects, the effects were more pronounced in association with MgO.

The mixograph mixing time results show a similar effect as observed in the farinograph, but differences were smaller. The NAS/NRC enrichment containing either $CaSO_4$ or $CaCO_3$ increased the mixograph mixing time from 4.0 to 4.5 mins. MgO again seemed to be responsible for the increase in dough strength. The additive effect of MgO and $CaSO_4$ also was observed (Table 6).

Similar increases in dough development time and stability due to Ca^{++} and Mg^{++} ions have been reported. Ca^{++} ions had a toughening effect on gluten, suggestive that these ions perhaps decreased the water-holding capacity (126). The use of dough improvers which contain calcium peroxide as an active component have been commonly used in baking industry to produce less sticky, drier-appearing doughs even with somewhat increased absorption. Such doughs possess improved machinability properties; this may be desirable in overcoming the effects of mechanical handling on doughs made with low tolerance flours (127). The decrease in gluten consistency could be counteracted by the addition of magnesium salts such as $MgSO_4$ (128), as a consequence of the salting-out effect of these ions on hydrated gluten protein. $CaSO_4$ caused a dough strengthening effect in association with MgO salt, perhaps because $CaSO_4$ is an acidic salt while MgO is a basic salt; therefore, the additive effect of acid plus salt on dough provides a strengthening effect (118).

Viscoamylograph Peak Consistency and Pasting Temperature

An important bread making flour property is its enzymatic activity which determines its bread baking performance. In the U.S. enzymatic activity is often measured by the amylograph. The instrument is well-adapted for investigating the effects of inorganic salts on gelatinization temperature, and paste viscosity. It also determines the effect of granulation and heat processing on wheat flour viscosity studies (129).

The amylograph (Table 7) shows a control flour viscosity reading of 649 B.U. Regular enrichment addition had little effect. The NAS/NRC enrichment with $CaSO_4$ lowered the peak viscosity to 614 and 608 B.U., while $CaCO_3$ increased the viscosity to 756.6 and 772 B.U. These variations were significant ($p > 0.05$), Table 7. MgO and $CaSO_4$ together are responsible for the decrease in amylograph viscosity, while MgO and $CaCO_3$ seem to increase the amylograph values. This was true when both the electrolytes were added together in the flour, or in the premix. Mineral interations between MgO, $CaSO_4$ and $CaCO_3$ with flour components are presumably the electrolytic factors responsible for this variation. A slight increase (0.5 units) in pasting temperature is observed in flours fortified with $CaSO_4$. Pasting temperature increases have been reported to occur due to presence of sulphate (So_4^{--}), flouride (F^-), and chloride (Cl^-) ions (130, 131, 132). Thus the calcium source can appreciably affect the enzymatic activity index.

The peak consistency and pasting temperature for amylograph depends on: (i) the enzymatic activity--the heat inactivation of α-amylase; (ii) starch swelling due to electrolytes; (iii) the interaction of the electrolytes with flour components; and (iv) the combined effects these factors may have on the pH optimum for α-amylase activity.

Ash

Mineral (ash) content is an important measure of flour quality (133). For the control flour the typical ash value was 0.43% (Table 7). Addition of regular or present-day enrichment had little effect. As expected, addition of the NAS/NRC enrichment containing $CaSO_4$ and $CaCO_3$ increased the value to 1.05 and 0.75%, respectively. The principal contributors for the increase in ash values were the calcium salts, as MgO increased the ash value slightly, Table 7.

Table 7. Effect of Micronutrients on Physical and Chemical Properties of Flour

Treatments	Viscoamylograph				
	Viscosity B.U.	Pasting Temp (mins)	Agtron - Color	Ash %	pH
Unenriched	649.0 d,e	20.93 b,c	55.5 c,d	0.43	5.87 l
Regular Enrichment with Fe*	635.7 d,e,f	21.07 b,c	55.77 b,c,d	0.45	5.86 l
Regular Enrichment with $FeSO_4$	635.7 d,e,f	20.87 b,c	51.87 e,f,g	0.44	5.86 l
NRC** with Fe* ($CaSO_4$)	614.0 e,f,g	21.10 b,c	55.33 c,d	1.09	6.07 i
NRC with $FeSO_4$ ($CaSO_4$)	608.3 f,h	21.07 b,c	49.70 i	1.09	6.39 d
NRC with Fe* ($CaCO_3$)	756.6 b	20.64 b,c	55.33 c,d	0.75	6.64 b
NRC with $FeSO_4$ ($CaCO_3$)	772.3 b	20.57 b,c	51.10 g,h	0.75	6.62 b
Premix 1 with Fe*	636.7 d,e,f	20.63 b,c	54.83 d	0.45	5.88 l
Premix 2 with $FeSO_4$	627.7 e,f,g	21.13 b,c	52.07 e,f,g	0.45	5.86 l
Premix 1 & ZnO	628.3 e,f,g	21.07 b,c	-	-	5.92 k
Premix 1 & $CaSO_4$	705.0 c	21.30 b	-	-	5.71 m
Premix 1 & $CaCO_3$	633.7 d,e,f	21.13 b,c	56.77 a	-	6.24 g
Premix 1 & MgO	647.3 d,e	20.87 b,c	55.73 b,c,d	-	6.29 f
Premix 1 & MgO & $CaSO_4$	632.3 d,e,f	20.93 b,c	56.17 b,c	-	6.03 j
Premix 1 & MgO & $CaCO_3$	864.0 a	21.17 b,c	56.5 a,b	-	6.59 c
Unenriched & ZnO	637.7 d,e,f	20.63 b,c	-	0.43	5.91 k
Unenriched & $CaSO_4$	710.3 c	21.13 b,c	-	0.97	5.71 m
Unenriched & $CaCO_3$	640.0 d,e,f	20.67 b,c	-	0.73	6.19 h
Unenriched & MgO	664.3 d	21.03 b,c	-	0.48	6.33 e
Unenriched & MgO & $CaSO_4$	620.0 e,f,g	22.20 a	-	-	6.06 i,j
Unenriched & MgO & $CaCO_3$	768.0 b	20.40 c,d	-	-	6.70 a
Premix 2 & ZnO			52.27 e,f		
Premix 2 & $CaSO_4$			51.33 f,g		
Premix 2 & $CaCO_3$			52.77 e		
Premix 2 & MgO			50.17 i		
Premix 2 & MgO & $CaSO_4$			50.00 i		
Premix 2 & MgO & $CaCO_3$			51.70 f,g		

Means sharing different letters are significantly different at $p \geq 0.05$.
Data are means of three observations.
Fe* = Electrolytic Iron NRC** = NAS/NRC Enrichment

If fortification is carried out at the mill as favored by the National Research Council, ash values will not have much meaning as a bakery flour purchasing control factor, since ash contents do not indicate absolute flour or end product quality (134).

Flour Color

Although flour color has been important throughout the history of the milling industry, considerably more interest has been shown recently in Agtron flour color readings rather than the ash content for quality control of wheat milling and flour specifications (134, 135). Since the consumer is more concerned with color of the crumb of the loaf, Watson and Shuey 1977 (134) suggested using flour color instead of ash content as criterion for flour specifications.

The control flour gave an Agtron value of 56.0 (Table 7). Both regular and NAS/NRC enrichment with electrolytic iron gave a value similar to the control. Regular and NAS/NRC enrichment with $FeSO_4$ as the iron source gave darker readings of 51.87 (regular enrichment), 49.70 (NAS/NRC + $CaSO_4$), 51.1 (NAS/NRC + $CaCO_3$), respectively (Table 7). The increase was at about the same level as regular enrichment and ferrous sulphate appeared to be the principal contributor to flour color.

Flour pH

Flour is a dough ingredient that exerts the most significant effect on final bread. This is in part attributable to the buffering action of its protein which contains both ionizable acid (carboxyl) and alkaline (amino) groups (124).

The control flour had a pH of 5.87 (Table 7), consistent with the value reported in literature (124, 136). Addition of regular enrichment had no effect on pH. However, the pH of the flour increased with NAS/NRC enrichment

containing $CaSO_4$ and $CaCO_3$ to 6.07 - 6.39 and 6.62 - 6.64, respectively. Individual studies of the nutrients with flour (Table 7) suggest that MgO particularly contributes to this pH increase (Table 7) (23, 137). Further, $CaCO_3$ also appears to increase the pH, while $CaSO_4$ has no effect. When both the basic salts MgO and $CaCO_3$ were added together in the flour or in the premix, the pH increased to 6.7 and 6.59, respectively, indicating their additive effect.

Extensograph

The extensograph measures structural parameters of the dough, which the farinograph does not pick up, namely the ratio between dough extensibility and dough resistance (138).

The extensograph allows an opportunity to observe some changes in dough properties which may occur during fermentation (139).

The extensograph measurements (Table 8) show that the control flour after 45 minutes had a resistance to extension of 707.3 B.U. and extensibility of 175 mm. The addition of regular enrichment had little effect. However, NAS/NRC enrichment containing $CaSO_4$ and $CaCO_3$ slightly lowered the resistance to extension (by 33.3 and 52.3 B.U., respectively), but reduced the extensibility (by 29.75 and 26.62 mm, respectively). MgO and $CaCO_3$ appear to be largely responsible for reducing the extensibility of the doughs (Table 8). For when both MgO and $CaSO_4$ or $CaCO_3$ are added together in the premix the resistance to extension is slightly lowered (by 36.80 and 41.80 B.U.) while extensibility is reduced (by 29.75 and 53.25 mm, respectively). Here the additive effects of both basic salts (MgO and $CaCO_3$) in reducing extensibility appears to be more pronounced.

The extensograph values are influenced by effects of salts and acids on gluten proteins. Salts usually have a strengthening and tightening effect on dough gluten as they influence the dough hydration capacity, additionally having an inhibitory effect on proteolytic enzymes (124). Thus the effect of salts in the extensograph test is to increase both resistance to extension and

Table 8. Effect of Micronutrient on Extensograph Properties of Dough

Treatments	Time mins	Extensograph Properties	
		Resistance to Extension B.U.	Extensibility mm
Unenriched	45	707.3 c,d,e,f	175.0 a
	90	896.8 g,h	136.8 a,b
	135	925.3 d,e	121.5 a,b,c
Regular Enrichment with Fe*	45	751.8 b,c,d	165.3 a,b,c,d
	90	937.0 e,f,g	136.0 a,b,c
	135	915.5 e	131.8 a
Regular Enrichment with $FeSO_4$	45	737.3 c,d,e	171.0 a,b
	90	854.8 h	141.0 a
	135	831.3 f	123.3 a,b
NRC** with Fe* ($CaSO_4$)	45	674.3 e,f,g	146.3 a,b,c,d,e,f,g
	90	995.5 a,b,c,d,e	104.8 c,d,e
	135	958.8 c,d,e	94.5 b,c,d,e
NRC with $FeSo_4$ ($CaSO_4$)	45	688.0 d,e,f,g	146.3 a,b,c,d,e,f,g
	90	990.0 a,b,c,d,e,f	106.0 b,c,d,e
	135	1001.3 a,b,c,d	96.3 b,c,d,e
NRC with Fe* ($CaCO_3$)	45	655.5 f,g	149.4 a,b,c,d,e,f
	90	1026.5 a,b,c	102.1 d,e
	135	1055.6 a,b	92.5 b,c,d,e
NRC with $FeSo_4$ ($CaCO_3$)	45	749.9 b,c,d	140.6 b,c,d,e,f,g
	90	1026.5 a,b,c	108.3 b,c,d,e
	135	1059.1 a,b	100.3 a,b,c,d,e

Table 8. Effect of Micronutrient on Extensograph Properties of Dough (cont'd)

Treatments	Time mins	Extensograph Properties	
		Resistance to Extension B.U.	Extensibility mm
Premix 1 with Fe*	45	752.7 b,c,d	169.5 a,b,c
	90	921.0 e,f,g,h	123.8 a,b,c,d
	135	983.3 c,d,e	115.2 a,b,c,d
Premix 1 and ZnO	45	817.5 b	138.8 d,e,f,g
	90	1040.0 a,b	104.8 c,d,e
	135	1011.3 a,b,c	99.5 b,c,d,e
Premix 1 and $CaSO_4$	45	720.0 c,d,e,f	157.5 a,b,c,d,e
	90	963.8 c,d,e,f,g	104.5 d,e
	135	837.8 f	102.8 a,b,c,d,e
Premix 1 and $CaCO_3$	45	774.0 b,c	148.5 a,b,c,d,e,f,g
	90	1020.0 a,b,c,d	107.3 b,c,d,e
	135	987.0 a,c,d,e	104.0 a,b,c,d,e
Premix 1 and MgO	45	612.3 g	147.8 a,b,c,d,e,f,g
	90	989.0 a,b,c,d,e,f	102.3 d,e
	135	1062.5 a	94.0 b,c,d,e
Premix 1 and MgO and $CaSO_4$	45	660.5 e,f,g	145.3 a,b,c,d,e,f,g
	90	958.5 d,e,f,g	106.8 b,c,d,e
	135	958.8 c,d,e	102.5 a,b,c,d,e
Premix 1 and MgO and $CaCO_3$	45	868.8 a	121.8 g
	90	1060.0 a	94.0 e
	135	1015.0 a,b,c	85.8 e

Means sharing different letters are significantly different at $p \geq 0.05$.
Data are means of at least duplicate determination. Fe* = Electrolytic Iron. NRC** = NAS/NRC Enrichment

extensibility (117, 140, 141). Acids reduce the resistance to extension and extensibility at low pH (117, 120, 142). When both acids and salts are present, the resistance to extension is increased, but extensibility shows a marked decrease, due to slight denaturation of gluten proteins or due to difficulties of sulphydryl disulphide exchange reaction (117). Other factors which influence gluten properties (dough hydration capacity) are pH and enzymatic action (125).

The pH values (Table 7), acidity and bascity of salts does not explain the present data (Table 8). Though speculating, it may be said that MgO and calcium salts link with protein by virtue of their divalent ion, and hence slightly lower the resistance to extension and reduce the extensibility.

EFFECT OF REGULAR AND NAS/NRC ENRICHMENT ON BREAD BAKING PROPERTIES

The baking results (Table 9) indicate that regular or NAS/NRC enriched breads, whether prepared with elemental iron or ferrous sulphate, had little specific volume differences as compared to unenriched control breads. The NAS/NRC enriched breads had somewhat poor grain, texture and longer proof time (55.0 mins). Proof times were 52.0 minutes for control and 49.0 minutes for breads treated with regular enrichment (Table 9).

Rubin et al 1977 (22) reported a similar specific volume trend of the control or the enriched breads (regular or NAS/NRC enriched). Poor grain and texture and longer proof times of the NAS/NRC breads were also observed. Suggestions were made that calcium and magnesium affected proof time and grain of the NAS/NRC enriched breads. Similar results on the specific volume, proof time and internal crumb characteristics were obtained as in Table 10.

EFFECT OF REGULAR AND NAS/NRC ENRICHMENT ON CRUMB pH, COLOR AND FIRMNESS

Crumb pH

pH plays a highly-significant role in bread baking. In bread production it exerts its principal effect during fermentation, controlling yeast activity,

Table 9. Effect of Regular and NAS/NRC Enrichment on Bread Quality

Treatments	Specific Loaf Volume cc/g	Proof Time mins	Crust* Color	Break* & Shred	Symmetry*	Texture*	Grain*
Unenriched	6.68 [a,b]	52.0 [b]	7.7 [b,c]	7.6 [a]	7.85 [a]	7.75 [b,c]	7.90 [a]
Regular Enrichment with Fe**	6.74 [a]	48.0 [c]	7.85 [a,b]	7.95 [a]	7.80 [a]	8.10 [a]	7.90 [a]
Regular Enrichment with $FeSO_4$	6.62 [b,c]	49.0 [c]	7.6 [c]	7.95 [a]	7.85 [a]	8.05 [a,b]	7.85 [a]
NRC with Fe**	6.67 [a,b,c]	55.0 [a]	7.95 [a]	7.85 [a]	7.95 [a]	7.65 [c]	7.6 [b]
NRC with $FeSO_4$	6.55 [c]	55.0 [a]	7.55 [c]	7.65 [a]	7.75 [a]	7.55 [c]	7.6 [b]

Means sharing different letters are significantly different at $p \geq 0.05$.
Data are means of duplicate doughs.
*Bread score based on 1-10 scale.
Fe** = Electrolytic Iron.

Table 10. Effect of Regular and NAS/NRC Enrichment on Bread Quality.

Treatments	Specific Loaf Volume cc/g	Proof Time mins	Crust* Color	Break* & Shred	Symmetry*	Texture*	Grain*	Crumb Firmness g 24 hrs	72 hrs	144 hrs
Regular Enrichment with Fe**	6.59 [a]	49.0 [b]	7.78 [a]	7.65 [a]	7.73 [a]	7.95 [a]	7.95 [a]	112.3 [a]	181.4 [b]	224.1 [b]
NRC with Fe**	6.53 [a]	54.0 [a]	7.80 [a]	7.78 [a]	7.78 [a]	7.63 [b]	7.68 [b]	116.8 [a]	214.4 [a]	259.1 [a]

Means sharing different letters are significantly different at $p \geq 0.05$.
Data are means of duplicate doughs.
*Bread score based on 1-10 scale.
Fe** = Electrolytic Iron.

amylolytic action and gluten behavior (124). Bread produced with correct pH and T.T.A. is softer, with a longer shelf life and maximum flavor (136). The optimum pH value for white bread and rolls is 5.2 (143).

The crumb pH data (Table 11) shows that the pH of the control breads was 5.13. Regular enrichment had no effect on pH, while the addition of NAS/NRC enrichment increased crumb pH (5.49). MgO appears to be the potent contributor for the increase in pH. Increases in pH due to MgO have been reported (23, 137).

Crumb Color

Crumb color is an important property of bread (144), since brightness of the crumb of the loaf is one way consumers judge bread quality (145).

Bread enriched with regular and NAS/NRC enrichment with elemental iron as an iron source caused a lowering of crumb color (46.1 and 46.4, respectively) compared to control (47.9), (Table 11). Lowest Agtron readings were observed with ferrous sulphate in both regular and NAS/NRC enriched breads (44.7 and 42.0, respectively). Ferrous sulphate appears to be the principal contributor of crumb color as was previously shown in flour color studies (Table 7).

Crumb Firmness

Staling is a complex phenomena (110, 111, 146, 147). Crumb firmness is one of the indices of staling and is an important factor in the baking industry (148, 149, 150).

Breads enriched with regular enrichment using elemental iron or ferrous sulphate were softer (168.8g and 173.4g) than the control breads (187.1g) after 24 and 96 hours. The NAS/NRC enriched breads (Table 11), however, were firmer (201.2 g and 207.3g) with the differences being significant $p \geq 0.05$. Similar trends were obtained on crumb firmness of regular and NAS/NRC enriched breads after 24, 72 and 144 hours (Table 10).

Table 11. Effect of Regular and NAS/NRC Enrichment on Crumb pH, Color and Firmness

Treatments	pH	Crumb Color	Crumb Firmness g 24 hrs	Crumb Firmness g 96 hrs
Unenriched	5.13 [b]	47.9 [a]	109.8 [a,b]	187.1 [b]
Regular Enrichment with Fe*	5.10 [b,c]	46.1 [b]	97.3 [c]	168.8 [c]
Regular Enrichment with $FeSO_4$	5.08 [c]	44.7 [c]	101.4 [b,c]	173.4 [c]
NRC with Fe*	5.49 [a]	46.4 [b]	113.6 [a]	201.2 [a]
NRC with $FeSO_4$	5.49 [a]	42.0 [d]	116.2 [a]	207.3 [a]

Means sharing different letters are significantly different at $p \geq 0.05$.
Data are means of duplicate doughs.
Fe* = Electrolytic Iron.

ORGANOLEPTIC EVALUATIONS ON BREAD PREPARED WITH REGULAR AND NAS/NRC ENRICHMENT

Statistical Test

The breads fortified with regular enrichment (the standard or control product) and NAS/NRC enrichment (the experimental product) after three and six days of storage at room temperature were compared by the Wilcoxon Signed Rank Test (112). The preferences as outlined on the taste panel sheet were described previously (page 28). For each individual the degree of difference between the standard and fortified product was analyzed. For example, if an individual had rated the standard as like moderately (+1) and the fortified product as dislike slightly (-1), the difference was recorded as di=(1) - (-1) = 2. Similarly if another individual rated the standard product as like moderately (+1) and the experimental product as like very much (+3), the difference di= (+1) - (+3), di = -2 was recorded.

The results of the difference in the taste of the enriched breads after three and six days of storage at room temperature were recorded by the taste panel members. An example of the Wilcoxon Signed Rank Test with 48 panel members is shown in Table 12. This table shows that the panel members were unable to detect any significant difference in the overall flavor, aroma or mouthfeel of the NAS/NRC or the regular enriched breads, whether they were three or six days old. The results were significant ($p \geq 0.05\%$). This is somewhat at variance with the work of previous workers who have reported ill effects on flavor due to some of these micronutrients (22, 23).

A slight off-flavor in breads fortified with NAS/NRC enrichment after a week storage statistically significant at the 5% level was reported by Rubin et al.(22). They attributed the off-flavor in the fortified breads due to magnesium. Also, Ranhotra et al 1976 (23) reported that magnesium causes off-taste in breads when used at the NAS/NRC proposed level of 200 mg/lb or 44.1 mg/lb.

Table 12. Organoleptic Studies on Breads Prepared with Regular and NAS/NRC Enrichment

Statistical Test for Fortified Breads. Wilcoxon Signed Rank Test.

Rating Differences	-4	-3	-2	- 1	0	+1	+2	+3
Frequency	1	1	1	12	26	3	3	1

N = 48

Ha = standard product was preferred to fortified product.

Ho = no preference for the standard and fortified product.

$\|Ws^*\|$	0	1	2	3	4
Frequency	26	15	4	2	1
Midrank Scores	13.5	34.0	43.5	46.5	48.0
# pos	0	3	3	1	0

$|Ws^*|$ = Absolute value of Wilcoxon Score.
pos = # of positive scores.
$Vs^* = 279$
$EH(Vs^*) = 412.5$ $\quad V(Vs^*) = 7884.4$
$Pr[Vs \leq 279] = \Phi = \left(\frac{279 - 412.5}{\sqrt{7884.4}} \right)$
$\Phi = (-1.50) = 0.066$
The test failed to reject Ho at p = 0.05%.

PHYSICAL DOUGH TESTING OF NAS/NRC FLOURS AFTER THREE AND SIX MONTHS OF STORAGE AT FOUR DIFFERENT TEMPERATURES

Moisture

Unfortified flours at zero time had 12.8% moisture (Table 13). The NAS/NRC fortified flours stored at four different temperatures increased in their moisture content after three and six months of storage (Table 13). The moisture increase of the flours stored at cold and room temperature was slight, whereas the deep freeze samples reflected a 3.0 to 3.2% increase (Table 13). However, the samples stored at hot temperature lost 5.0% moisture at the end of three months. Their moisture was down to 7.4%. At the end of six months they further lost 1.0% more moisture (Table 13).

Farinograph Data

The farinograph data of unfortified flours at zero time indicated a farinograph absorption of 58.6%, peak time of 7.5 minutes and a tolerance time of 12.5 minutes. The NAS/NRC electrolytic iron and ferrous sulphate fortified flours after three and six months of storage, showed an increase in most of the farinograph parameters with increase in time and temperature of storage (Table 13).

The farinograph absorption of the NAS/NRC fortified flours at the end of three months increased by about 7.8% compared to the control. However, minor variations were observed, among these fortified flours stored, for three months at four different temperatures. At the end of six months the fortified flours showed a definite trend of an increase in farinograph absorption with increase in storage temperature (Table 13). After three and six months of storage: the peak time of the fortified flours increased to 10.0 and 11.0 minutes, respectively, in flours stored at -17.8 to -20.6^{o}C; the peak time increased to about 13.0 minutes in flours stored at 40 to 43^{o}C. The tolerance of the flours stored at 18 to 35^{o}C increased to 19.0 and 19.25 minutes, respectively. Ferrous

Table 13. Physical Dough Testing of NAS/NRC Flours After 3 and 6 Months of Storage at Four Different Temperatures

	Farinograph Data											
Treatments	Moisture %	Farinograph Absorption %	Peak Time (mins)	Arrival Time (mins)	Departure Time (mins)	Tolerance (mins)	Moisture %	Farinograph Absorption %	Peak Time (mins)	Arrival Time (mins)	Departure Time (mins)	Tolerance (mins)
Control	12.8	58.6	7.5	1.5	14.0	12.5						
Duration Time	Three Months						Six Months					
						Electrolytic Iron						
-17.8 to -20.6°C	15.8	66.4	11.0	7.0	18.5	11.50	16.0	57.2	10.0	4.0	21.0	17.0
1.7 to 2.2°C	13.6	65.0	10.0	4.5	19.0	14.5	13.4	59.6	9.5	4.5	17.75	13.25
18 to 35°C	13.2	67.4	14.0	5.5	24.75	19.25	13.3	60.6	11.0	6.0	25.5	19.5
40 to 43°C	7.4	65.6	13.25	3.75	22.25	18.50	6.4	69.2	12.5	8.5	21.5	13.0
						Ferrous Sulphate						
-17.8 to -20.6°C	16.1	66.4	12.0	6.25	19.25	13.0	16.0	57.0	10.0	6.0	19.0	13.0
1.7 to 2.2°C	14.0	65.0	10.0	2.25	20.0	17.75	13.8	59.0	9.5	2.25	18.0	15.75
18 to 35°C	13.2	67.0	15.0	6.0	25.0	19.0	13.0	60.8	11.25	6.25	25.5	19.25
40 to 43°C	7.4	66.0	13.25	6.0	22.5	16.5	6.5	69.4	13.0	8.0	23.0	15.0

sulphate treated flours stored at 1.7 to 2.2°C had greater tolerance--17.75 and 15.75 minutes, respectively, because of early arrival time (2.25 minutes) than the electrolytic iron treated flours at the end of three and six months of storage (Table 13).

At -17.8 to -20.6°C the flours treated with electrolytic iron and ferrous sulphate stored for three months and also the six months stored flours at -17.8 to -20.6°C treated with ferrous sulphate had low tolerance (11.50 and 13.0 minutes) because of late arrival time (7.0 and 6.25 minutes). This was not true, however, for the six months stored flours treated with electrolytic iron at -17.8 to -20.6°C (Table 13). At the end of six months the electrolytic iron and the ferrous sulphate fortified flours stored at 40 to 43°C had a late arrival time (8.0 minutes) and henceforth showed low tolerance values. It may be noted that the changes in the farinograph parameters of the fortified flour after six months of storage appear more pronounced than observed at the end of three months.

The changes observed in farinograph parameters with flours stored under different storage conditions are expected. Gracza, R., (83) reported that test indices used to characterize flour aging, change with storage; as during storage flour undergoes certain physical, chemical, and as reported by Clark, R.J., 1955 (93) certain biological changes. Marked increase in farinograph absorption, dough development time and improvement in mixing tolerance with stored flours has been reported by Shellenberger et al 1958 (95). Shellenberger et al (95) stored flours in glass containers for one year at temperatures of 4°C and 38°C. He observed that changes in flour characteristics at 38°C were greater than at 4°C. This appears to be in agreement with this study's farinograph data on the flours stored for six months (Table 13).

Viscoamylograph

Flour at zero time had a peak viscosity of 649 B.U. and a pasting temperature of 20.93 minutes (Table 14). The NAS/NRC flours fortified with electrolytic iron and ferrous sulphate after three and six months of storage gave high peak viscosity with increase in storage time (Table 14). The three months stored NAS/NRC fortified flours had higher values of peak viscosity than the control, but only minor variations were observed in the three months stored flours at four different temperatures (Table 14). However, at the end of six months the fortified flours showed more pronounced changes in their amylograph values. A definite regular trend of an increase in hot paste viscosity with increase in time and temperature of storage was observed in these samples (Table 14).

After three and six months of storage the flours stored at 40 to 43°C decreased in their pasting temperature by about 1.0 minute (Table 14). The -17.8 to -20.6°C flour samples had higher peak viscosity than the 1.7 to 2.2°C stored flour samples after both three and six months of storage. The six months flours stored at 40 to 43°C had the highest hot paste viscosity values. This was, however, not true for the flours stored at 40 to 43°C for three months which had lower value of peak viscosity than the 18 to 35°C stored flour samples (Table 14).

An increase in hot paste viscosity during flour storage has been earlier reported (94, 96, 97, 98, 99). Pixton et al 1975 (96) studied the amylograph maximum viscosity on various flours during the first ten years of storage. The amylograph maximum viscosity particularly for wheat flour showed a tendency to increase with time at ambient temperature. They state that they do not know if amylase activity actually decreases or if during storage the starch becomes less susceptible to amylase attack. Pixton et al 1967 (97) earlier reported on the

Table 14. Physical and Chemical Properties of NAS/NRC Flours After 3 and 6 Months of Storage at Four Different Temperatures

Treatments	Viscoamylograph				Agtron Color		pH
	Viscosity in B.U.	Pasting Temp. mins	Viscosity in B.U.	Pasting Temp. mins			
Control	649.0	20.9	-	-	55.5		Fe* / 6.07
Duration Time	Three Months		Six Months		Three Months	Six Months	Six Months
Flour Storage/Temp.							
				Electrolytic Fe*			
-17.8 to -20.6°C	891.3	21.13	663.0	21.67	53.0	53.0	6.08
1.7 to 2.2°C	704.7	21.13	596.0	21.93	54.3	54.0	6.08
18 to 35°C	933.7	21.6	820.7	21.13	51.4	52.2	6.07
40 to 43°C	900.0	20.13	1005.7	20.60	45.6	43.0	6.03
				Ferrous Sulphate			
-17.8 to -20.6°C	891.0	20.97	665.7	21.97	46.9	46.0	6.03
1.7 to 2.2°C	695.3	21.07	591.7	21.90	46.4	47.3	6.03
18 to 35°C	920.0	21.9	796.7	21.13	46.2	45.4	6.00
40 to 43°C	880.0	20.07	1007.0	20.53	44.8	36.8	5.98

increase in amylograph values from low temperatures to high temperatures. They studied the amylograph maximum viscosity of flours stored for ten years, and reported that storage at low temperature appeared to reduce the change of amylograph viscosity. Hesseltine (98) found that storage at higher temperature (38-60^{o}C) resulted in increased paste viscosity, presumably because of enzyme destruction during storage, as no starch damage was observed.

Agtron - Flour Color

The unfortified flours at zero time gave an agtron value of 55.50. The NAS/NRC fortified flours stored for three and six months at four different temperatures gave lower agtron readings with increase in time and temperature of storage (Table 14). Lower agtron readings observed during storage result from certain chemical interactions between aldehyde and amino groups of sugars and proteins referred to as nonenzymatic or Maillard browning reaction (100). The effect of temperature on flour color appears more pronounced for flours fortified with electrolytic iron and ferrous sulphate at 40 to 43^{o}C after both three and six months of storage. After three months of storage the agtron readings in the hot storage samples fortified with electrolytic iron and ferrous sulphate increased by 9.90 and 10.70, respectively, and for flours stored for six months by 12.50 and 18.70, respectively. The -17.8 to -20.6^{o}C samples gave slightly darker agtron readings after three and six months of storage than samples stored at 1.7 to 2.2^{o}C, (Table 14).

All ferrous sulphate fortified flours stored at -17.8 to -20.6^{o}C and 40 to 43^{o}C increased in flour color after three months of storage by 8.60 and 10.70, respectively. On the other hand, after six months the flours increased in flour color by 9.50 (-17.8 to -20.6^{o}C) and 18.70 (40 to 43^{o}C).

These results confirm this study's earlier findings that ferrous sulphate is the principal contributor of flour color (Table 7).

At elevated temperatures (40 to 43°C) after three and six months lower agtron readings were obtained. Potter 1973 (100) reports that Maillard type browning like other chemical reactions is favored by temperature and by high concentration of reactive groups in the presence of some water. Further Maillard browning generally proceeds most rapidly during drying when the moisture content is decreased. The moisture in fortified flours stored at 40 to 43°C decreased from 12.8% in control flour to 7.4 and 6.4% after three and six months of storage periods, respectively (Table 13). Hence darker agtron readings were obtained with flours stored at elevated temperatures.

pH

Bread flour pH increased from 5.87 in control flours to 6.08 and 6.03 in the six months stored NAS/NRC flours fortified with electrolytic iron and ferrous sulphate (Table 14). The results appear to be in agreement with the earlier flour pH studies (Table 7). Both the electrolytic iron and ferrous sulphate fortified flour show a slight decrease in pH with increase in storage temperature. Slightly lower pH values were obtained for the flours stored at 40 to 43°C fortified with the two sources of iron (Table 14). The pH of wheat flour has been reported to decrease with age, as acidity level in the flour changes (83).

BAKING PROPERTIES OF NAS/NRC FLOURS STORED FOR THREE MONTHS AT FOUR DIFFERENT TEMPERATURES

The baking data indicates that the breads prepared from flour at zero time had a baking absorption of 60% and a farinograph absorption of 58.6% (Table 15). The breads prepared from the NAS/NRC electrolytic iron and ferrous sulphate fortified flours stored for three months at four different temperatures had higher baking absorption, as these flours also possessed higher farinograph absorption. The baking absorption increased by about 2% from the farinograph absorption,

Table 15. Baking Properties of NAS/NRC Flours Stored for 3 Months at Four Different Temperatures

Treatments	Moisture %	Farinograph Absorption %	Baking Absorption %	Specific Loaf Volume cc/g	Proof Time mins	Crust* Color	Break* & Shred	Symmetry*	Texture*	Grain*	Crumb Color	Crumb Firmness g 24 hrs	Crumb Firmness g 96 hrs
Control	12.8	58.6	60.0	6.68	52.0	7.7	7.6	7.85	7.75	7.9	47.92	109.8	187.1
						Electrolytic Iron							
-17.8 to -20.6°C	15.8	66.4	66.4	6.37	53.0	7.75	7.65	7.8	7.7	7.75	48.0	111.4	193.1
1.7 to 2.2°C	13.6	65.0	67.0	6.41	56.0	7.65	7.85	7.9	7.85	7.8	49.0	109.3	188.8
18 to 35°C	13.2	67.4	69.4	6.40	51.0	7.7	7.75	7.75	7.7	7.8	47.0	113.0	197.0
40 to 43°C	7.4	65.6	67.6	6.27	48.0	7.5	7.65	7.65	7.6	7.6	45.0	115.9	213.9
						Ferrous Sulphate							
-17.8 to -20.6°C	16.1	66.4	66.4	6.35	53.0	7.5	7.75	7.7	7.75	7.8	45.0	110.4	190.4
1.7 to 2.2°C	14.0	65.0	67.0	6.39	55.0	7.8	7.85	7.7	7.75	7.75	45.0	108.5	184.9
18 to 35°C	13.2	67.0	69.0	6.37	51.0	7.65	7.7	7.9	7.75	7.8	44.0	112.6	195.0
40 to 43°C	7.4	66.0	68.0	6.23	48.0	7.45	7.65	7.7	7.6	7.55	42.0	114.0	208.5

*Bread score based on 1-10 scale.

with the exception of the breads prepared from flours stored at 1.7 to 2.2°C which had a baking absorption equal to the farinograph absorption (Table 15).

The specific volumes of the breads prepared from fortified flours were lower (6.41 - 6.23) than the breads prepared from the flours at zero time (6.68). But small differences in specific volume were observed in the breads prepared from flours stored at -17.8 to -20.6°C, 1.7 to 2.2°C, and 18 to 35°C, fortified either with electrolytic iron or ferrous sulphate. However, the electrolytic iron or ferrous sulphate fortified flours at 40 to 43°C had the lowest specific volume (6.27 and 6.23, respectively) and also a low score of grain and texture. Breads prepared from flour at zero time had a proof time similar to the electrolytic iron and ferrous sulphate fortified flours stored at -17.8 to -20.6°C (proof time = 52.0 minutes). Somewhat longer proof times of 53.0 and 55.0 minutes were obtained for the breads prepared from flours stored at 1.7 to 2.2°C than the breads prepared from -17.8 to -20.6°C or from control flour. With increase of storage temperature lower proof times were obtained for the breads prepared from NAS/NRC electrolytic iron and ferrous sulphate fortified flours stored at 18 to 35°C and 40 to 43°C (51.0 and 48.0 minutes).

Crumb Color

The crumb color data of breads prepared with unfortified control flour and fortified flours indicates that the breads prepared with control flour had an agtron value of 47.92 (Table 15). The breads prepared with flours stored at 40 to 43°C fortified both with electrolytic iron and ferrous sulphate gave lower agtron readings of 45.0 and 42.0, respectively, than the breads prepared with flours stored at -17.8 to -20.6°C. The latter breads had crumb color readings of 48.0 and 45.0, respectively (Table 15). The breads prepared with all $FeSO_4$ fortified NAS/NRC flours stored for three months gave lower agtron readings than the reduced iron fortified flours (Table 15).

The breads prepared from flours stored at elevated temperatures (40 to 43°C) gave lower crumb color readings (Table 15). Stenberg et al 1949 as reported by Pomeranz 1960 (105) found that on storage at elevated temperature a browning reaction takes place. For under normal conditions changes in crumb color of wheat bread are negligible. This browning reaction is favored by high temperature and high concentration of reactive groups and is referred to as nonenzymatic or Maillard type of browning (100).

Crumb Firmness

Crumb firming measurements (Table 15) on the breads prepared with control flours and with NAS/NRC fortified flours stored for three months show only minor variations in crumb firming values after 24 hours. Breads prepared with control flour had a crumb firming value of 187.1 after 96 hours. The fortified breads prepared with electrolytic iron and ferrous sulphate NAS/NRC fortified flours at 18 to 35°C and 40 to 43°C had slightly higher crumb firming values (Table 15).

BAKING PROPERTIES OF NAS/NRC FLOURS STORED FOR SIX MONTHS AT FOUR DIFFERENT TEMPERATURES

The baking data of the breads prepared with control flours indicates that these breads had a baking absorption of 60% and a farinograph absorption of 58.6% (Table 16). All fortified breads prepared from NAS/NRC electrolytic iron and ferrous sulphate fortified flours stored for six months show a linear increase in baking absorption with increase of temperature. Breads prepared from both iron fortified flours stored at 40 to 43°C had the highest values of baking absorption (73.2 and 73.4%, respectively) (Table 16).

The specific volume of breads prepared with both electrolytic iron and ferrous sulphate fortified flours stored at -17.8 to -20.6°C, 1.7 to 2.2°C, and 18 to 35°C were similiar to the unfortified breads prepared from the control flour (Table 16). However, the breads prepared from the fortified flours stored at 40 to 43°C had low specific volumes (6.34 and 6.29, respectively) and a low

Table 16. Baking Properties of NAS/NRC Flours Stored for 6 Months at Four Different Temperatures

Treatments	Moisture %	Farinograph Absorption %	Baking Absorption %	Specific Loaf Volume cc/g	Proof Time mins	Crust* Color	Break* & Shred	Symmetry*	Texture*	Grain*
Control	12.8	58.6	60.0	6.68	52.0	7.7	7.6	7.85	7.75	7.9
					Electrolytic Iron					
-17.8 to -20.6°C	16.0	57.2	57.2	6.55	52.0	7.8	7.7	7.8	7.8	7.7
1.7 to 2.2°C	13.4	59.6	61.6	6.65	54.0	7.8	7.9	8.0	7.8	7.9
18 to 35°C	13.3	60.6	62.6	6.65	51.0	7.9	7.8	7.8	7.9	8.0
40 to 43°C	6.4	69.2	73.2	6.34	48.0	7.8	7.8	7.8	7.6	7.5
					Ferrous Sulphate					
-17.8 to -20.6°C	16.0	57.0	57.0	6.51	52.0	7.8	7.8	8.0	7.7	7.8
1.7 to 2.2°C	13.8	59.0	61.0	6.59	54.0	7.9	7.8	8.2	7.8	8.0
18 to 35°C	13.0	60.8	62.8	6.61	51.0	7.8	7.8	8.0	7.9	7.8
40 to 43°C	6.5	69.4	73.4	6.29	48.0	7.7	7.8	7.8	7.5	7.6

*Bread score based on 1-10 scale.

grain and texture score. The proof times of the unfortified breads prepared from control flour were similar to the breads prepared with flours stored at -17.8 to -20.6°C (52.0 minutes), whereas the breads prepared with flours stored at 1.7 to 2.2°C had two minutes longer proof time than the breads prepared from the flour stored at the above conditions. Lower proof times of 48.0 minutes were obtained for NAS/NRC electrolytic iron and ferrous sulphate fortified flours stored at 40 to 43°C.

BAKING PROPERTIES OF NAS/NRC FLOURS STORED FOR THREE AND SIX MONTHS AT FOUR DIFFERENT TEMPERATURES

All NAS/NRC fortified flours after three and six months of storage increased in their baking absorption (Table 15, 16). The increase in baking absorption of breads prepared with stored flours has been reported by many investigators. Cathcart, W.H., 1939 (75) reported that Thiessen in 1933 studied the effect of aging on Wyoming hard wheat flours stored in cotton sacks for 12 months and in tightly-closed cans for 21 months. He found that throughout the storage period the water absorption of the flour increased and was most rapid when the flour was stored in sacks. The breads prepared from flours stored at 40 to 43°C after three and six months of storage had low specific volumes, poor grain and texture, low proof times and had higher crumb firming rates (Table 15, 16). Mangels 1924 (151) gave evidence supporting this observation that warm storage of flour was more detrimental than cold storage. Loaf volume was a sensitive index of physicochemical changes in proteins, Pence et al 1953 (102), and was directly proportional to the concentration of undenatured proteins (103). Further, the viscoamylograph data shows that the flours stored at 40 to 43°C, especially after six months, had high hot paste viscosity values (Table 14), probably because of a decrease in amylase activity during storage, Pixton et al 1975 (96). Also other enzymes present in flour systems may have lost their

activity at elevated temperatures during storage, due to moisture loss (82). Breads prepared with fortified flours stored at 40 to 43°C had lower proof time after both three and six months of storage, probably due to the fact that the proteins in the doughs of these breads could not withstand proofing, because of the above physicochemical changes in flour during storage.

ORGANOLEPTIC EVALUATIONS OF BREADS PREPARED WITH NAS/NRC FORTIFIED FLOURS STORED FOR THREE MONTHS AT FOUR DIFFERENT TEMPERATURES

Breads prepared with NAS/NRC fortified flours stored for three months were presented for taste testing, after one and four days of storage at room temperature (18 to 35°C) as indicated by Tables 17 and 18. On both these days the panel members, (10 on day 1) and (13 on day 4) were unable to detect any significant differences in the breads prepared with either electrolytic iron or ferrous sulphate fortified flours stored at -17.8 to -20.6°C, 1.7 to 2.2°C, 18 to 35°C and 40 to 43°C. However, on day four the Freidman's test statistics for the breads prepared with electrolytic iron was significant at $p \geq 0.05$ (Table 18), but differences within a comparing group were non-significant.

ODOR TEST ON THE NAS/NRC FLOURS STORED FOR THREE MONTHS AT THREE STORAGE TEMPERATURES

The odor test results on the NAS/NRC fortified flours stored for three months (Table 19) show that 33 panel members were unable to detect any difference in the electrolytic iron fortified flours stored at four different temperatures. On the other hand, the panel members were able to detect significant differences in the odor from flours fortified with ferrous sulphate (Table 19). The order of preference and numerical ranking of these flours significant at $p \geq 0.01\%$ is shown in Table 19. The odor of the ferrous sulphate fortified flours stored at room temperature (18 to 35°C) was slightly fruity, while the flour stored at 40 to 43°C was considered to have a stale odor.

Table 17. Organoleptic Evaluations of Breads Prepared with NAS/NRC Fortified Flours Stored for Three Months at Four Different Temperatures

Statistical Test for Fortified Breads.* Freidman's Test.

One Day Old Bread

Electrolytic Iron Fortified Breads				
1.7 to 2.2°C	-17.8 to -20.6°C	18 to 35°C	40 to 43°C	N.S. Freidman's Test Statistics Q* = 5.23
MIDRANK SCORES				
21.0 [a]	21.5 [a]	28.0 [a]	29.5 [a]	

Ferrous Sulphate Fortified Breads				
-17.8 to -20.6°C	18 to 35°C	1.7 to 2.2°C	40 to 43°C	N.S. Freidman's Test Statistics Q* = 6.79
MIDRANK SCORES				
19.0 [a]	23.5 [a]	26.0 [a]	31.5 [a]	

* 10 panel members

Ho = no treatment (temperature) differences.

We fail to reject Ho.

Means sharing the same letters within a comparison group were non-significant at $p \geq 0.05$.

Table 18. Organoleptic Evaluations of Breads Prepared with NAS/NRC Fortified Flours Stored for Three Months at Four Different Temperatures

Statistical Test for Fortified Breads.* Freidman's Test.

Four Day Old Bread

Electrolytic Iron Fortified Breads				
18 to 35°C	-17.8 to -20.6°C	1.7 to 2.2°C	40 to 43°C	S^{++} Freidman's Test Statistics Q = 8.52
MIDRANK SCORES				
27.5 ^{o}	29.5 ^{a}	30.0 ^{a}	43.0 ^{a}	

Ferrous Sulphate Fortified Breads				
1.7 to 2.2°C	18 to 35°C	-17.8 to -20.6°C	40 to 43°C	N.S. Freidman's Test Statistics Q = 4.69
MIDRANK SCORES				
27.5 ^{a}	30.0 ^{a}	33.0 ^{a}	39.5 ^{a}	

* 13 panel members

Ho = no treatment (temperature) differences.

S^{++} = We reject Ho.

Means sharing the same letters within a comparison group were non-significant at $p \geq 0.05$.

Table 19. Odor Test on NAS/NRC Flours Stored for Three Months at Four Different Temperatures

Statistical Test for Fortified Flours.* Freidman's Test.

Electrolytic Iron Fortified Flours				
18 to 35°C	1.7 to 2.2°C	-17.8 to -20.6°C	40 to 43°C	N.S. Freidman's Test Statistics Q = 2.72
MIDRANK SCORES				
75.0 [a]	82.0 [a]	86.0 [a]	87.0 [a]	

Ferrous Sulphate Fortified Flours				
-17.8 to -20.6°C	1.7 to 2.2°C	18 to 35°C	40 to 43°C	S^{++} Freidman's Test Statistics Q = 14.18
MIDRANK SCORES				
63.0 [a]	81.0 [a,b]	91.5 [b]	94.5 [b]	

* 33 panel members

Ho = no treatment (temperature) differences.

S^{++} = We reject Ho.

Means sharing the different alphabets within a comparison group were significant at $p \geq 0.05$.

However, when the 40 panel members were asked to compare differences in the odor of the electrolytic iron and ferrous sulphate fortified flours stored at 1.7 to 2.2°C, 18 to 35°C, and 40 to 43°C for the same time period (Table 20) the members were unable to detect significant differences in the odor of these fortified flours. The order of preference is shown in the Table.

ORGANOLEPTIC EVALUATIONS ON BREADS PREPARED WITH NAS/NRC FORTIFIED FLOURS STORED FOR SIX MONTHS

The taste test results (Table 21) on the breads prepared with NAS/NRC electrolytic iron and ferrous sulphate fortified flours stored for six months at four storage temperatures indicates that 39 panel members were able to detect significant differences ($p \geq 0.01$) in the breads prepared with -17.8 to -20.6°C and 40 to 43°C stored flours. The breads prepared with ferrous sulphate fortified flours stored at 40 to 43°C were significantly different, from other sample breads. The taste panelists described the breads prepared with 40 to 43°C stored flours as being off-flavor with a sour, rancid stale odor with a bitter taste, while the breads prepared from flour stored at -17.8 to -20.6°C were considered to have a mouldy, yeasty unpleasant odor and a sour, beany taste. However, the panel members were unable to detect significant differences in the breads prepared from flours stored at 1.7 to 2.2°C or 40 to 43°C (Table 21).

On the other hand, when 32 panel members were required to compare differences in the electrolytic iron and ferrous sulphate fortified flours stored for six months at 1.7 to 2.2°C, 18 to 35°C, and 40 to 43°C (Table 22), the members were able to detect significant differences between the breads prepared from flour stored at 1.7 to 2.2°C and 40 to 43°C. Freidman's statistics as well as the differences within a comparing group were significant at $p \geq 0.01$. The breads prepared with the flours stored at 40 to 43°C were described as having very poor organoleptic properties. The taste testers, however, were unable to detect significant differences in the breads prepared from flours stored at

Table 20. Odor Test on the NAS/NRC Fortified Flours Stored for Three Months at Three Storage Temperatures

Statistical Test for Fortified Flours.* Freidman's Test.

Comparing Electrolytic Iron (Fe^+) and Ferrous Sulphate ($FeSO_4$)

Fortified Flours Stored at Cold, Room, and Hot Storage Temperatures

1.7 to 2.2°C Fe^+	18 to 35°C Fe^+	18 to 35°C $FeSO_4$	1.7 to 2.2°C $FeSO_4$	N.S. Freidman's Test Statistics Q = 4.26
MIDRANK SCORES				
93.5 [a]	94.0 [a]	101.0 [a]	111.5 [a]	

1.7 to 2.2°C $FeSO_4$	1.7 to 2.2°C Fe^+	40 to 43°C $FeSO_4$	40 to 43°C Fe^+	N.S. Freidman's Test Statistics Q = 5.57
MIDRANK SCORES				
87.0 [a]	101.0 [a]	105.0 [a]	107.0 [a]	

* 40 panel members

Ho = no treatment (temperature) differences.

We reject Ho.

Means sharing same alphabets within a comparison group were non-significant at $p \geq 0.05$.

Table 21. Organoleptic Evaluations on Breads Prepared with NAS/NRC Fortified Flours Stored for Six Months at Four Storage Temperatures

Statistical Test for Fortified Breads.* Freidman's Test.

Electrolytic Iron Fortified Breads				
18 to 35°C	1.7 to 2.2°C	40 to 43°C	-17.8 to -20.6°C	S^{++} Freidman's Test Statistics Q = 42.62
MIDRANK SCORES				
71.5 [a]	75.0 [a]	121.5 [b]	122.0 [b]	

Ferrous Sulphate Fortified Breads				
1.7 to 2.2°C	18 to 35°C	-17.8 to -20.6°C	40 to 43°C	S^{++} Freidman's Test Statistics Q = 34.40
MIDRANK SCORES				
75.5 [a]	78.5 [a,b]	107.0 [b,c]	129.0 [c]	

* 39 panel members

Ho = no treatment (temperature) differences.

S^{++} = We reject Ho.

Means sharing different alphabets within a comparison group were significant at $p \geq 0.05$.

Table 22. Organoleptic Evaluations on Breads Prepared with NAS/NRC Fortified Flours Stored for Six Months at Three Storage Temperatures

Statistical Test for Fortified Breads.* Freidman's Test.

Comparing Breads Prepared from Electrolytic Iron (Fe^+) and Ferrous Sulphate ($FeSO_4$) Fortified Flours Stored at Cold, Room, and Hot Storage Temperatures

18 to 35°C Fe^+	1.7 to 2.2°C Fe^+	1.7 to 2.2°C $FeSO_4$	18 to 35°C $FeSO_4$	S^{++} Freidman's Test Statistics Q = 8.30
MIDRANK SCORES				
73.5 [a]	74.5 [a]	76.5 [a]	95.5 [a]	

1.7 to 2.2°C $FeSO_4$	1.7 to 2.2°C Fe^+	40 to 43°C Fe^+	40 to 43°C $FeSO_4$	S^{++} Freidman's Test Statistics Q = 59.15
MIDRANK SCORES				
53.5 [a]	55.0 [a]	102.0 [b]	109.5 [b]	

* 32 panel members

Ho = no treatment (temperature) differences.

We reject Ho.

Means sharing different alphabets within a comparison group were significant at $p \geq 0.05$.

1.7 to 2.2°C or 18 to 35°C. Here the differences among the bread samples were not significant, but Freidman's test statistics were significant at $p \geq 0.05$. The source of iron had no effect.

ODOR TEST ON THE NAS/NRC FORTIFIED FLOURS STORED FOR SIX MONTHS

The odor test results of the NAS/NRC electrolytic iron and ferrous sulphate fortified flours stored for six months at four storage temperatures (Table 23) show that 40 panel members were able to detect significant differences in the odor of the flours stored at -17.8 to -20.6°C and 40 to 43°C ($p \geq 0.01$). However, they were unable to detect any significant difference between the flours stored at 1.7 to 2.2°C or 18 to 35°C. The iron source had no effect on flour odor (Table 23). The flours stored at -17.8 to -20.6°C were considered mouldy with musty unpleasant odor, whereas the flours stored at 40 to 43°C were described as having a strong rancid off-odor with a stale and a sour smell.

Fortified flours stored at three storage temperatures were presented to a panel of 38 members to distinguish differences in the odor of the flours stored for the same time period (six months) (Table 24). The panel members were able to detect significant differences between the flours stored at 1.7 to 2.2°C and 40 to 43°C. They were, however, unable to detect any significant differences in the flours stored at 1.7 to 2.2°C or 18 to 35°C. This was true for both electrolytic iron and ferrous sulphate fortified flours (Table 24). The order of preferences and numerical ranking of these flours is given in the Table.

CONCLUSION ON THE ORGANOLEPTIC EVALUATIONS AND ODOR TEST ON THE NAS/NRC FORTIFIED FLOURS STORED FOR THREE AND SIX MONTHS

Cort et al 1976 (152) conducted storage studies with flours fortified with vitamins, iron (electrolytic iron) and mineral premix, according to the NAS/NRC recommendations. They stored flours in sealed glass jars for four months at room temperature and prepared breads from these flours according to a

Table 23. Odor Test on the NAS/NRC Fortified Flours Stored for Six Months at Four Different Temperatures

Statistical Test for Fortified Flours.* Freidman's Test.

Electrolytic Iron Fortified Flours				
18 to 35°C	1.7 to 2.2°C	40 to 43°C	-17.8 to -20.6°C	S^{++} Freidman's Test Statistics Q = 39.32
MIDRANK SCORES				
76.5 [a]	77.5 [a]	114.5 [b]	131.5 [b]	

Ferrous Sulphate Fortified Breads				
18 to 35°C	1.7 to 2.2°C	40 to 43°C	-17.8 to -20.6°C	S^{++} Freidman's Test Statistics Q = 37.0
MIDRANK SCORES				
77.0 [a]	77.5 [a]	118.0 [b]	127.5 [b]	

* 40 panel members

Ho = no treatment (temperature) differences.

S^{++} = We reject Ho.

Means sharing different alphabets within a comparison group were significant at $p \geq 0.05$.

Table 24. Odor Test on the Flours Fortified With NAS/NRC Flours Stored for Six Months at Three Storage Temperatures

Statistical Test for Fortified Flours.* Freidman's Test.

Comparing Electrolytic Iron (Fe^{+}) and Ferrous Sulphate ($FeSO_4$)

Fortified Flours Stored at Cold, Room, and Hot Storage Temperatures

1.7 to 2.2°C Fe^{+}	1.7 to 2.2°C $FeSO_4$	18 to 35°C Fe^{+}	18 to 35°C $FeSO_4$	N.S. Freidman's Test Statistics Q = 5.50
MIDRANK SCORES				
85.0 [a]	93.0 [a]	94.0 [a]	108.0 [a]	

1.7 to 2.2°C Fe^{+}	1.7 to 2.2°C $FeSO_4$	40 to 43°C Fe^{+}	40 to 43°C $FeSO_4$	S^{++} Freidman's Test Statistics Q = 36.37
MIDRANK SCORES				
65.5 [a]	88.5 [a]	100.5 [b]	125.5 [b]	

* 38 panel members

Ho = no treatment (temperature) differences.

S^{++} = We reject Ho.

Means sharing different alphabets within a comparison group are significant at $p \geq 0.05$.

school lunch program. These workers reported that panel members could not detect any off-flavor and, in fact, preferred breads with the mineral premix. Other workers have conducted storage studies with different iron sources using higher levels of iron--100-308 mg/Kg of flour (42, 46). Harrison et al 1976 (46) stored flours in glass jars in a lab oven at 50°C and at room temperature in plastic bags. No serious rancidity was detected in enriched flour samples stored for two years. However, rancidity by smell was detected in samples enriched with ferrous sulphate after four days at 50°C and after 11-28 days in samples with electrolytic iron. These workers report that ferrous sulphate appears to show some potential rancidity effects when stored at such elevated temperatures for an extended period. They suggest that ferrous sulphate can be used as an alternate to electrolytic iron if the enriched product is kept at constant temperature of no more than 26°C. But this is often impractical, Ranum et al (31).

The results on the organoleptic properties of the breads prepared from NAS/NRC fortified flours stored for three months at four storage temperatures in paper sacks and the results of the odor tests on these fortified flours show that the untrained panelist could not detect any difference in the organoleptic properties of breads prepared from flours stored for three months. However, the panel members were able to detect significant differences in the odor of the flours fortified with ferrous sulphate. The flours stored at 18 to 35°C and 40 to 43°C had off-odor. Differences were significant at $p \geq 0.01$. Ferrous sulphate has been reported to catalyze the development of oxidative reactions which result in off-flavors, colors or odors (33, 153). The three months stored flours were allowed to equilibrate to room temperature after they were taken out from their respective storage conditions. The evaluations by panel members were held immediately. Hence, the results of these tests are conclusive.

At the end of six months the taste panel evaluations on the breads prepared from NAS/NRC fortified flours showed that the panel members were able to detect significant differences in the overall flavor of the flours stored at -17.8 to -20.6°C and 40 to 43°C ($p \geq 0.01$). The taste panelists described the breads prepared from flours stored at -17.8 to -20.6°C as being mouldy, yeasty, with an unpleasant odor and sour beany taste, whereas the breads prepared from flours stored at 40 to 43°C were considered off-flavor with a sour, rancid stale odor with bitter taste and aftertaste. Also the odor test results showed that the panelists could detect significant differences in the aroma of the flours stored at -17.8 to -20.6°C and 40 to 43°C. Both taste panel evaluations and odor test results indicated that the source of iron, whether using electrolytic or ferrous sulphate, had little effect.

All the six months stored fortified flours were kept at room temperature in plastic bags, closed in tins for seven weeks before breads were prepared from these flours for taste panel evaluations. Rancidity detected in flours stored at elevated temperatures for six months would be expected due to fat oxidation (47) and moisture loss (81). The flours at elevated temperatures lost about 6.4% moisture (Table 13) at the end of six months. Further flour contains some residual fat and this fat may become rancid during storage period, especially when ferrous sulphate is used as a source of iron (46).

The poor organoleptic properties and off-odor detected in flours stored at -17.8 to -20.6°C was unexpected. These flours, as was determined later by mycological examinations, got musty and mouldy due to mould growth. The -17.8 to -20.6°C fortified flours which had already absorbed 16% moisture (Table 13) during storage were kept at room temperature in plastic bags closed in tins. Therefore, the high moisture and high humidity conditions in the tins helped the growth of fungi. Hence the panelists described these flours as having poor flavor and odor.

MYCOLOGICAL EXAMINATIONS OF ELECTROLYTIC IRON AND FERROUS SULPHATE NAS/NRC FORTIFIED FLOURS STORED FOR SIX MONTHS

After sprinkling small quantities of the stored flours on three agar media viz. malt agar with 4% NaCl, potato dextrose agar and nutrient agar, the following organisms were found in all samples: Aspergillus candidus, A. glaucus, A. flavus, Cladosporium sp, Mucor sp, Penicillum oxalicum, P. viridicatum, yeasts and bacterial colonies. Also present in one or more flour samples were: A. versicolor, A. niger, A. ochraceus, A. terrus, Fusarium roseum and P. cyclopium. These moulds were present in low numbers with not more than three or four colonies of any species on any one plate. Plates of malt agar with 4% NaCl which were inoculated with both -17.8 to -20.6°C stored flours had large numbers of poorly developed colonies. On high osmotic tension media (malt agar with 10% NaCl, Czapeks solution agar with 20% and 40% sucrose and malt agar with 20% and 40% sucrose) Aspergillus restritus, A. gracilus (154) and two Penicillum species were isolated. Identification of the Penicillum species has not been completed; both are mono-verticulate, and one has characters which show relationship to the members of P. javanicum series.

The moulds common to most of the flour samples were of species frequently isolated from wheat flour (155). Fortified flours stored at -17.8 to -20.6°C, 1.7 to 2.2°C, 18 to 35°C, and 40 to 43°C had moisture contents of 16.0, 13.4 - 13.8, 13.3 - 13.0, and 6.4 - 6.5%, respectively, at the end of six months storage (Table 13). These flours were kept for about seven weeks at room temperature before taste panel evaluations. Aspergillus restritus species which were isolated from fortified flours attaining 16% moisture content are known to grow during storage at -17.8 to -20.6°C at moisture contents as low as 13.5%, Christensen and Kaufmann 1974 (156). Less is known about Penicillum on low moisture substrates, but isolates in the mono-verticilate group have been shown to grow well on high asmotic tension media, Raper et al 1949 (157).

Barton-Wright and Tomkins 1940 (80) reported that the critical water content for fungal growth to take place in flour was approximately 16%. This moisture level would be near the critical point in commercial practice. A water content of 15% or less is advisable to eliminate mould growth. The fungi which grew in the flours stored at -17.8 to -20.6^{o}C, while being held at 16% m.c. for seven weeks, probably produced the unacceptable organoleptic properties.

BREAD BAKING PROPERTIES AS AFFECTED BY REGULAR ENRICHMENT AND ITS COMPONENTS

Regular and NAS/NRC bake test results (Tables 10 and 11) revealed that regular enrichment, whether using electrolytic iron or ferrous sulphate as an iron source, lowered crumb firming values. Further were investigated the effects of the components of regular enrichment (viz. thiamin, riboflavin, niacin or electrolytic iron) on bread softening (Table 25).

The baking data of Table 25 represents the mean of 28 test doughs or eight loaves per variable baked on two different days. Regular enriched breads with electrolytic iron (Pennwalt mix and lab mix) as well as the niacin enriched breads had slightly higher specific volume, good grain and texture and lower proof time (50.0 mins) (Table 25). These differences were significant at $p \geq 0.05$. The unenriched breads and breads enriched with electrolytic iron had poor grain and texture. Their specific volumes were similar to the riboflavin and thiamin enriched breads, but had higher proof time (60.0 mins) as compared to the riboflavin (53.8 mins) and thiamin (57.8 mins) enriched breads.

Crumb Color

The thiamin, niacin and electrolytic iron enriched breads gave similar crumb color values as the control (Table 26). Breads with regular enrichment and riboflavin enriched breads had lower crumb color, 44.8 and 45.2, respectively (Table 26). The lowering of crumb color due to regular enrichment agrees with the earlier findings (Table 11) and riboflavin seems to be the principal influencing component.

Table 25. Bread Baking Properties as Affected by Regular Enrichment and Its Components

Treatments	Specific Loaf Volume cc/g	Proof Time mins	Crust* Color	Break* & Shred	Symmetry*	Texture*	Grain*
Unenriched	6.28 [c]	60.0 [a]	7.83 [a,b]	7.75 [a]	7.88 [a]	7.65 [c,d]	7.75 [b]
Regular Enrichment with Fe** (Pennwalt Mix)	6.41 [a]	50.3 [d]	7.83 [a,b]	7.73 [a]	7.85 [a]	7.85 [a,b]	7.90 [a]
Regular Enrichment with Fe** (Lab Mix)	6.43 [a]	50.0 [d]	7.93 [a]	7.90 [a]	7.90 [a]	7.95 [a]	7.95 [a]
Riboflavin	6.31 [b,c]	53.8 [c]	7.85 [a,b]	7.88 [a]	7.88 [a]	7.70 [c,d]	7.88 [a]
Thiamin	6.32 [b]	57.8 [b]	7.75 [b]	7.73 [a]	7.88 [a]	7.75 [b,c]	7.88 [a]
Niacin	6.43 [a]	50.4 [d]	7.85 [a,b]	7.88 [a]	7.90 [a]	7.95 [a]	7.95 [a]
Fe**	6.31 [b,c]	60.0 [a]	7.80 [a,b]	7.70 [a]	7.88 [a]	7.58 [d]	7.73 [b]

*Bread score based on 1-10 scale.
Fe** = Electrolytic Iron.
Means sharing different letters are significantly different at $p \geq 0.05$.

Table 26. Effect of Regular Enrichment and Its Compoments on Crumb Color and Crumb Firmness

Treatments	Crumb Color	Crumb Firmness 24 hrs	Crumb Firmness 96 hrs
Unenriched	49.2 [a]	110.7 [a,b]	184.3 [a]
Regular Enrichment with Fe* (Pennwalt Mix)	44.8 [b]	107.9 [b]	157.0 [c]
Regular Enrichment with Fe* (Lab Mix)	44.9 [b]	108.0 [b]	156.6 [c]
Riboflavin	45.2 [b]	109.2 [b]	171.0 [b]
Thiamin	50.3 [a]	112.0 [a,b]	170.9 [b]
Niacin	50.3 [a]	99.7 [c]	152.0 [d]
Fe*	49.6 [a]	113.4 [a]	185.6 [a]

Fe* = Electrolytic Iron
Means sharing different letters are significantly different at $p \geq 0.05$.

Crumb Firmness

Niacin enriched breads were softer with lower crumb firming values than the unenriched control breads. The latter were firmer after 24 and 96 hours (Table 26). The two regular enriched breads prepared with Pennwalt mix and lab mix had lower values of crumb firmness after 96 hours. These differences were significant ($p \geq 0.05$). Riboflavin and thiamin enriched breads had slightly lower crumb firming values (171.0 g) after 96 hours, compared to unenriched and electrolytic iron enriched breads that had higher crumb firmness values of 184.3 and 185.6 grams, respectively (Table 26).

Thus niacin appears to be the principal contributor of crumb softness, with riboflavin and thiamin having smaller effects in lowering the crumb firming values. This data supports this study's earlier findings that regular enrichment when added at the bakery level made breads softer (Table 11).

EFFECTS OF DIFFERENT LEVELS OF NIACIN AND NIACINAMIDE

Bake test results (Table 26) with regular enrichment and its individual components (viz. thiamin, riboflavin, niacin and electrolytic iron) suggested that niacin lowered crumb firming values. A bake test was then conducted in triplicate to investigate the effects of the two forms of vitamins (niacin and niacinamide) on bread quality, and to determine the level at which these vitamins were more active in lowering crumb firming values as compared to SSL treated doughs, used as an index for comparing the softening effects.

Breads enriched with 5x (105 mg/lb) the niacin and niacinamide level and with 0.5% SSL had slightly higher specific volume ($\sim$ 6.40), good grain and texture and lower proof time of 45.0 and 43.0 minutes (Table 27). The control breads had a specific volume of 6.22, a lower grain and texture score, with a longer proof time (50.0 mins). On the other hand, the breads enriched with 1/2 (10.5 mg/lb), normal level 1x (21 mg/lb) and 2x (42.0 mg/lb) the niacin and

Table 27. Effect of Different Levels of Niacin and Niacinamide on Bread Baking Characteristics

Treatments	Specific Loaf Volume cc/g	Proof Time mins	Crust* Color	Break* & Shred	Symmetry*	Texture*	Grain*	Crumb Firmness g 24 hrs	Crumb Firmness g 96 hrs
Control	6.22 [d]	50.0 [a]	7.67 [a]	7.67 [a]	7.77 [a]	7.63 [c]	7.70 [a]	118.2 [a]	207.0 [a]
Niacin 1/2x	6.32 [c]	48.0 [a,b]	7.77 [a]	7.70 [a]	7.87 [a]	7.77 [b,c]	7.87 [a]	112.7 [a]	175.0 [d,e,f]
Niacinamide 1/2 x	6.32 [c]	47.7 [b,c]	7.77 [a]	7.90 [a]	7.67	7.83 [a,b,c]	7.77 [a]	114.8 [a]	185.2 [b,c,d,e]
Niacin 1x**	6.36 [a,b,c]	47.0 [b,c]	7.73 [a]	7.83 [a]	7.83 [a]	7.73 [b,c]	7.87 [a]	113.9 [a]	183.7 [c,d,e]
Niacinamide 1x**	6.32 [c]	46.20 [c,d]	7.80 [a]	7.70 [a]	7.83 [a]	7.90 [a,b]	7.77 [a]	107.3 [a]	172.0 [e,f]
Niacin 2x	6.37 [a,b,c]	45.0 [d]	7.77 [a]	7.70 [a]	7.73 [a]	7.80 [b,c]	7.73 [a]	110.2 [a]	190.0 [b,c,d]
Niacinamide 2x	6.34 [b,c]	45.0 [d]	7.80 [a]	7.70 [a]	7.77 [a]	7.90 [a,b]	7.73 [a]	112.2 [a]	201.5 [a,b]
Niacin 5x	6.39 [a,b]	44.67 [d]	7.73 [a]	7.67 [a]	7.83 [a]	7.83 [a,b,c]	7.90 [a]	109.2 [a]	192.2 [a,b,c]
Niacinamide 5x	6.41 [a]	45.0 [d]	7.83 [a]	7.77 [a]	7.83 [a]	7.90 [a,b]	7.83 [a]	107.0 [a]	193.4 [a,b,c]
Control with 0.5% SSL	6.40 [a,b]	42.67 [e]	7.93	7.87	7.90	8.03 [a]	7.97	107.0 [a]	161.92 [f]

*Bread score based on 1-10 scale.
1x** = Normal addition of Niacin or Niacinamide 21 mg/lb.
Means sharing different letters are significantly different at $p \geq 0.05$.

niacinamide level had slightly higher proof time (48.0, 47.0 and 45.0 minutes, respectively) than breads treated with 0.5% SSL (43.0 mins). The proof times of the niacin or niacinamide enriched breads, however, were lower than the control breads (50.0 mins) (Table 27).

Crumb Firmness

No significant differences in the crumb firming values were observed among the various treatments after 24 hours (Table 27), whereas after 96 hours the breads enriched with 1/2 (10.5 mg/lb) and normal level 1x (21.0 mg/lb) of niacin and niacinamide had lower crumb firming values (Table 27) as compared to the control breads that were firmer. Breads treated with 0.5% SSL gave the lowest values of crumb firmness (161.92 g). These differences were significant at $p \geq 0.05$. The control breads and breads enriched with 2x (42.0 mg/lb) and 5x (105 mg/lb) the niacin and niacinamide level had higher crumb firming values (Table 27).

The above data is suggestive of the limiting effect of niacin and niacinamide at higher levels (2x and 5x).

BREAD BAKING CHARACTERISTICS AS AFFECTED BY ADDITION OF NIACIN AND SSL IN SPONGE OR DOUGH STAGES

The bake test with niacin and niacinamide revealed the maximum effect in lowering crumb firming values at 1/2 (10.5 mg/lb) and normal level (21.0 mg/lb). At higher levels the niacin or niacinamide effects (2x and 5x) were limiting. Also the viscoamylograph results with untreated flour and flour enriched with 1/2 (10.5 mg/lb), normal level (21.0 mg/lb), 2x (42.0 mg/lb) and 5x (105.0 mg/lb) the level of niacin showed that differences were minor in the peak viscosity and the curve shape as compared to 0.5% SSL treated flour. This is suggestive that the mechanism by which niacin exerts its effect as a crumb softener is different from the mechanism by which SSL acts as a crumb softener. SSL has

been widely known to exert its effect both as a dough strengthener and crumb softener (110, 147, 158, 159, 160). As a dough strengthener it regulates and improves proofing rate (159). It acts as a crumb softener by forming a complex both with amylose and amylopectin fraction of starch, Destefanis et al 1977 (158).

Breads enriched with niacin in the sponge or the dough stage of the sponge dough process and breads treated with 0.5% SSL had slightly higher specific volume, a good texture and grain rating, and a lower proof time (47.0, 45.0 and 43.0 minutes, respectively) than the control breads (Table 28). The niacin enriched breads, with niacin added in the sponge stage and 0.5% SSL, had good overall baking characteristics (specific volume and external and internal characteristics) and lowest proof time (43.0 mins) as compared to control. The latter had poor grain, texture, and a higher proof time (53.0 mins). The variations were significant $p \geq 0.05$ and are indicative of the additive effect of niacin and SSL.

Crumb Firmness

The control breads after 96 hours had a crumb firming value of 194.8 grams (Table 28). Breads enriched with niacin, whether added in the sponge or dough stage of the sponge dough process, gave lower values of crumb firmness, compared to the control breads. This was particularly true of breads enriched with niacin in the dough stage that had crumb firmness somewhat similar to the breads treated with 0.5% SSL, 174.7 grams and 167.0 grams, respectively (Table 28). The lowest crumb firmness values were obtained with breads enriched with niacin in the sponge stage + 0.5% SSL. These were softer by about 44.9 grams compared to the control breads (Table 28). The difference was significant at $p \geq 0.05$.

Niacin evidently affects crumb softness. Its effect seems to be more pronounced in the dough stage, and it also exerts its additive effect when added with SSL.

Table 28. Bread Baking Characteristics as Affected by Addition of Niacin and SSL in Sponge or Dough Stage

Treatments	Specific Loaf Volume cc/g	Proof Time mins	Crust* Color	Break* & Shred	Symmetry*	Texture*	Grain*	Crumb Firmness g 24 hrs	Crumb Firmness g 96 hrs
Control	6.32 [c]	53.0 [a]	7.65 [a]	7.80 [a]	7.90 [a]	7.60 [b]	7.60 [c]	120.30 [a]	194.8 [a]
Niacin in Sponge 1x**	6.37 [b,c]	47.0 [b]	7.70 [a]	7.75 [a]	7.85 [a]	7.75 [b]	7.75 [b,c]	118.70 [a]	186.6 [a,b]
Niacin in Dough 1x**	6.43 [a,b]	47.0 [b]	7.70 [a]	7.65 [a]	7.80 [a]	7.85 [a,b]	7.95 [a,b]	112.70 [a]	174.7 [b,c]
Control with 0.5% SSL in Sponge	6.42 [a,b]	45.0 [b,c]	7.90 [a]	8.0 [a]	7.85 [a]	8.10 [a]	8.10 [a]	106.1 [a]	167.0 [c]
Niacin in Sponge 1x** + 0.5% SSL	6.48 [a]	43.0 [c]	7.85 [a]	7.85 [a]	7.85 [a]	8.10 [a]	8.05	103.8 [a]	149.9 [d]

*Bread score based on 1-10 scale.
1x** = Normal addition of Niacin 21 mg/lb.
Means sharing different letters are significantly different at $p \geq 0.05$.

GASSING POWER VALUES AS AFFECTED BY ADDITION OF NIACIN AND SSL IN SPONGE OR DOUGH STAGES

The sponges enriched or unenriched with niacin had no significant differences in their gassing power values (Table 29). In the dough stage, gassing power differences were observed in the various treatments after 90, 120, 150 and 180 minutes. The unenriched control and the 0.5% SSL treated control had lower gassing power values in the dough stage (Table 29). Higher gassing power values were obtained when niacin was added in the sponge stage, when it was added with SSL in the sponge stage, and when added alone in the dough stage. The value variations were significant ($p \geq 0.05$).

THE EFFECT OF NIACIN ON BREAD BAKING PROPERTIES

The use of yeast in bread making is based on sound biochemical principles. It is a favorite organism for bread bakers as well as biochemists because of its availability, reproducibility, and enzymatic activity, Pomper 1969 (161). Yeast functions in bread making are (i) it increases dough volume by gas evolution during fermentation of the available carbohydrates in the flour; (ii) modifies protein gluten structure produced by mechanical stretching and expansion due to gas production which helps to develop dough structure and texture; (iii) it has a slackening effect due to enzyme catalyzed reactions involving sulphydryl and disulphide bonds of flour proteins; (iv) imparts a distinctive flavor, and (v) enhances the nutritive value of bread, Burrow 1970 (162).

Vitamins in yeast are important for two practical reasons. To a certain extent the fermentative power of yeast is related to its vitamin content, and the emphasis on the nutritive value of yeast and yeast-containing products is based on their content of vitamins and unidentified growth factors, Othmer 1970 (163). The growth substances generally considered to be essential for optimum growth of strains of bakers yeast are biotin (164), nicotinic acid and its amide, Fries 1965 (165) and Suolamanien 1965 (166), pantothenic acid and inositol (167),

Table 29. Gassing Power* Values as Affected by Addition of Niacin and SSL in Sponge or Dough Stage

Treatments	Time in Minutes													
	SPONGE STAGE								DOUGH STAGE					
	30	60	90	120	150	180	210	240	30	60	90	120	150	180
Control	80.0 [a]	137.5 [a]	221.5 [a]	330.0 [a]	445.0 [a]	497.0 [a]	537.5 [a]	572.5	67.5 [a]	117.5 [a]	189.0 [b]	242.5 [b]	315.0 [c]	380.0 [b]
Niacin in Sponge 1x**	80.0 [a]	135.0 [a]	225.0 [a]	331.5 [a]	450.0 [a]	517.5 [a]	560.0 [a]	602.5 [a]	72.0 [a]	140.0 [a]	205.0 [a]	277.5 [a]	345.0 [a]	412.5 [a]
Niacin in Dough 1x**	85.0 [a]	140.0 [a]	222.5 [a]	340.0 [a]	450.0 [a]	517.5 [a]	555.0 [a]	587.5 [a]	75.0 [a]	140.0 [a]	206.0 [a]	275.0 [a]	342.5 [a,b]	420.0 [a]
Control with 0.5% SSL in Sponge	82.5 [a]	132.5 [a]	232.5 [a]	331.5 [a]	444.0 [a]	505.0 [a]	540.0 [a]	579.0 [a]	65.0 [a]	138.5 [a]	190.0 [b]	250.0 [b]	327.5 [b,c]	397.5 [a,b]
Niacin in Sponge 1x** + 0.5% SSL	85.0 [a]	130.0 [a]	225.0 [a]	332.5 [a]	449.5 [a]	511.0 [a]	555.0 [a]	607.5 [a]	68.0 [a]	132.5 [a]	202.5 [a]	277.5 [a]	355.0 [a]	417.5 [a]

*Gassing power values in mm of Hg.
1x** = Normal addition of Niacin 21 mg/lb.
Means sharing different letters are significantly different at $p \geq 0.05$.

thiamin (168). Nicotinic acid, thiamin and pyridoxine are synthesized by many strains of yeast, but additional exogenous supplies may sometimes be necessary for optimal development of enzyme activities (169).

The fermentative power of yeast is related to its vitamin content because vitamins play a significant role in yeast as coenzymes. Thus such familiar vitamins as niacin, thiamin, riboflavin, and pantothenic acid constitute essential parts of the following coenzymes complexes: coenzyme I (NAD), coenzyme II (NADP), cocarboxylase (thiamin pyrophosphate), flavin mononucleotide and coenzyme A, Pyler 1973 (170).

The bake test data of the unenriched breads and those enriched with niacin (Tables 27 and 28) or with regular enrichment containing niacin (Table 25) indicates that niacin is the principal contributor of crumb softness. Thiamin and riboflavin had smaller effects in lowering crumb firming values. The niacin enriched breads also had good specific volume, a higher grain and texture score, and lower proof time (Tables 25, 27, 28). Nicotinic acid and its amide have been reported as equally active and necessary growth factors for yeast (165, 166). Nicotinamide is a part of hydrogen transferring coenzyme NAD and NADP. This vitamin is important because oxidation reactions in catabolic pathways are typically linked to NAD, while most reductive biosynthetic pathways are specifically or preferentially linked to NADP. Yeast growing anerobically on glucose has considerable $NADH_2$ formation in the glycolytic pathway, while the overall requirement for the building up of new cell material requires large amounts of $NADPH_2$. This contrast suggests the possibility of potential involvement in anerobically growing yeast of certain enzymes or isoenzymes able to catalyze efficiently major biosynthetic reducing reaction with $NADH_2$ as electron donor, Sols et al 1971 (171). The stimulating effect of thiamin on the growth of bakers yeast has been reported by William and Roehm in 1930 (172). These workers found the stimulating effect of flour on yeast fermentation, due to the

presence of so-called "Z" factor called thiamin. Reed and Peppler 1973 (173) report that thiamin is a potent stimulator for the fermentation of doughs and for this reason it is frequently added.

Lower crumb firming rates obtained with breads enriched with niacin may be explained on the basis of the vital role played by nicotinic acid and its amide in the coenzyme system of yeast. Flour originally contained 5.4 mg/lb of niacin (107). The breads were enriched with 21.0 mg/lb of niacin. Suomalainen et al 1965 (166) report that the ability of bakers yeast to synthesize nicotinic acid and its amide under anerobic conditions is limited, and thus it is a necessary growth factor. Hence the addition of more niacin may well be an important factor in exerting a stimulating effect on yeast growth by more vigorous CO_2 production. The increased CO_2 production in turn probably caused a dough slackening effect because of additional enzymatic effects due to yeast reductases acting through such intermediate substances as thioctic acid contained in flour or glutathione contained in yeast. Yeast contains glutathione reductase; it may catalyze the dough slackening reaction of GSH and GSSH, Kuninori et al 1968 (174, 175). Technically, it is well known that reducing compounds have demonstrable slackening effects on doughs, and the effect is related to the cleavage of the internal disulphide bonds on flour proteins. Dahle and Hinz 1966 (176) have suggested that flour strength may be inversely related to the endogenous thioctic acid of flour. Other investigators, Black et al 1960 (177), have shown that yeast contains a thioctic acid reducing enzyme which would continuously replenish the reduced form of thioctic acid, thus catalyzing the dough-slackening reaction.

The effect of niacin on crumb softness (Table 28) and gassing power values (Table 29) was more pronounced when it was added in the dough stage of sponge dough process. This may presumably be due to yeast proteases being effective

on milk protein. Federici and Martini 1975 (178) examined several strains of yeast in order to ascertain their capacity for producing extracellular caseinolytic enzymes. From 128 strains studied, 32 were able to grow on a milk culture, modifying its protein component. In five of these strains a renin-like enzyme was isolated.

SUMMARY

This study included three major areas. Results for each area are as follows:

A. The effects of NAS/NRC micronutrients and regular enrichment on flour, dough and bread.

The NAS/NRC micronutrients increased flour pH, ash and color. $FeSO_4$ alone contributed to color, while magnesium and Ca salts influenced pH and ash. Farinograph peak times, tolerance, mixograph characteristics all increased as a consequence of Mg and Ca salts. $CaSO_4$ decreased amylograph values, while these were increased by $CaCO_3$. The extensograph values showed lower resistance to extension, but reduced extensibility. MgO and $CaCO_3$ appeared to be largely responsible for reducing dough extensibility.

Compared to the regular enriched sponge dough breads, NAS/NRC breads had minor specific volume difference, poor grain, poor texture, longer proof time and increased pH, possibly as a function of MgO. Consistent with the flour color studies, both regular and NAS/NRC enriched breads fortified with $FeSO_4$ gave low agtron readings. Though the NAS/NRC breads were firmer ($p \geq 0.05$) than the regular enriched breads, panel members failed to detect any organoleptic differences between them, whether they were one or six days old.

B. Storage (0, 3, and 6 months) and temperature effects (-17.8 to -20.6°C, 1.7 to 2.2°C, 18 to 35°C, and 40 to 43°C) on (i) the physical and chemical properties of bread flour and (ii) baking functionality of flour.

All NAS/NRC fortified flours after three and six months of storage increased in moisture content with the exception of flours stored at 40 to 43°C. The farinograph parameters had high amylograph peak viscosity and low pasting temperatures. Changes in these physico-chemical parameters were more pronounced after six months than after three months of storage. Increase in time and

temperature gave progressively lower agtron readings of the fortified flour predominantly influenced by $FeSO_4$. All NAS/NRC fortified flours after three and six months of storage had increased baking absorption. Bread flours stored at 40 to 43°C after both three and six months storage had low specific volumes, poor grain, poor texture, low proof times and low agtron readings. The breads prepared from three months stored flour at 18 to 35°C and 40 to 43°C had slightly higher crumb firming values. The six months fortified flours stored at 40 to 43°C had slightly lower pH.

Organoleptic bread properties were not influenced after three months storage at any temperature. However, off-odor was detected in flours fortified with $FeSO_4$ stored at 18 to 35°C and 40 to 43°C for the same duration. Poor organoleptic properties and off-odor detected in flours stored for six months at -17.8 to -20.6°C were attributed to mould growth, and at 40 to 43°C due to fat oxidation.

C. The softening effects of breads enriched with regular enrichment and its components (thiamin, riboflavin, niacin and electrolytic iron) were also investigated.

Niacin was the principal contributor of crumb softness when added at the proposed normal level of 21.0 mg/lb. At higher levels (2x and 5x) niacin or niacinamide effects were limiting. Pronounced niacin effects occurred when it was added along with SSL, and in the dough stage of the sponge dough process. Niacin supplemented dough had higher gassing power values and breads prepared from these doughs had slightly higher specific volume, good grain, texture, and shorter proof times. Thiamin and riboflavin had smaller effects in lowering crumb firming rates. Viscoamylograph results suggest that the crumb softening effect of niacin was different than that of SSL. Riboflavin significantly lowered crumb color readings.

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EFFECT OF NAS/NRC RECOMMENDED MICRONUTRIENTS ON WHEAT FLOUR, DOUGH AND BREAD QUALITIES

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The National Research Council - National Academy of Sciences (NAS/NRC) recommended in 1974 that cereal-based foods be enriched with 10 micronutrients (vitamin A, thiamin, riboflavin, niacin, vitamin B_6, folic acid, iron, calcium, magnesium and zinc). This study determined the effects of these nutrients on the quality of flour, dough and bread. Comparisons were made among (i) NAS/NRC fortified [with electrolytic iron (Fe) and ferrous sulphate ($FeSO_4$)], (ii) regular enrichment (with Fe and $FeSO_4$), (iii) unenriched flours and breads. The NAS/NRC micronutrients increased flour pH, ash, and color. $FeSO_4$ alone contributed to color, while magnesium and calcium salts influenced pH and ash. Farinograph peak times, tolerance and mixograph characteristics increased as a consequence of the addition of magnesium and calcium salts. Calcium sulphate ($CaSO_4$) decreased amylograph values; these were increased by calcium carbonate ($CaCO_3$). The extensograph values showed lower resistance to extension and reduced extensibility due to NAS/NRC enrichment. Specifically magnesium oxide (MgO) and $CaCO_3$ appeared to be largely responsible for reducing dough extensibility.

Sponge dough breads prepared with NAS/NRC micronutrients had minor specific volume differences, but exhibited poor grain, texture, a longer proof time and higher pH than unenriched or control bread. Darker crumb color readings obtained with regular and NAS/NRC enriched breads were due to $FeSO_4$. Crumb firming measurements after 96 hours storage revealed that the NAS/NRC enriched breads were slightly firmer than the regular enriched breads ($p \geq 0.05$). Organoleptic evaluations on the breads indicated no difference due to enrichment, whether the breads were one or six days old.

Storage (0, 3, 6 months) and temperature (-17.8 to -20.6°C, 1.7 to 2.2°C, 18 to 35°C, 40 to 43°C) effects were studied on (i) the physical and chemical properties of bread flour and (ii) baking functionality of flour. Longer

storage duration (6 months) and higher storage temperature (40 to 43°C) adversely affected the physical, chemical, and bread baking properties of flour. Organoleptic bread properties were not influenced after three months storage at any temperature. However, off-odor was detected in $FeSO_4$-fortified flours stored at 18 to 35°C and 40 to 43°C for the same time period. Poor organoleptic properties and off-odor detected in flours stored for six months was attributed to mold growth at -17.8 to -20.6°C and due to fat oxidation at 40 to 43°C.

The softening effect of breads enriched with regular enrichment and its components (thiamin, riboflavin, niacin and electrolytic iron) were also investigated. Niacin was the principal contributor of crumb softness when added at the normal level of 21.0 mg/lb. At higher levels (2x and 5x) niacin or niacinamide, the effect was limiting. Pronounced bread softening effects occurred when niacin and SSL were added in the sponge stage, and also when niacin was added in the dough stage of the sponge-dough process. Niacin supplemented doughs had higher gassing power values, and breads prepared from these doughs had slightly higher specific volume, good grain, texture and shorter proof times. Thiamin and riboflavin had smaller effects in lowering crumb-firming values. Riboflavin significantly lowered crumb color readings.