



Soda Bread Making Process Decreases Protein Efficacy Ratio and Causes Debilitation of Hematological Parameters in Male Rats

Hamed Fanaei ^{1,2}, Tahereh Eghbali ³, Abdurrashid Khazaei Feizabad ⁴ and Alireza Dashipour ^{5,*}

¹Pregnancy Health Research Center, Zahedan University of Medical Sciences, Zahedan, Iran

²Department of Physiology, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

³School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

⁴Genetics of Non-Communicable Disease Research Center, English Department, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

⁵Department of Clinical Biochemistry, Department of Food Sciences and Nutrition, Cellular and Molecular Resarch Center, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

*Corresponding author: Department of Clinical Biochemistry, Department of Food Sciences and Nutrition, Cellular and Molecular Resarch Center, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran. Email: ar_dashipour@yahoo.com

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Abstract

Objectives: Bread has long been one of the most popular foods and is the major source to supply energy, protein, minerals, and vitamins. The type of bread making process may affect its nutritional value. This study aimed to investigate bread baking methods and their effects on protein efficacy ratio (PER) and the status of some iron-related blood parameters in rats.

Methods: Four diets were used in this study, including a control diet (animals chow), fermented bread, non-fermented bread, and soda bread. At first, some chemical parameters of flour and bread were measured, and then PER and some hematological parameters were determined in rats. Descriptive statistics include mean \pm standard deviation (SD), and analytical statistics include one-way ANOVA and Tukey post hoc test. $P < 0.05$ was considered statistically significant.

Results: The wheat flour's Fe was 18.68 mg/kg. The PER value was negative in the group that received soda bread, and the weight gain was the lowest in the soda bread group. Ferritin, RBC, Hematocrit, BUN, MCV, and MCHC mean values were significantly lower in the rats that received soda bread than other groups. There were no differences among groups in MCH.

Conclusions: Although bread can be used as a major source to supply energy protein and other nutritional values, the soda bread making process may decrease protein efficacy ratio and cause debilitation of hematological parameters. These effects of soda can disrupt the body's physiological processes and lead to disease in the long run.

Keywords: Bread, Fermentation, Baking Soda, PER, Fe

1. Background

Bread making methods can have an important role in decreasing or increasing availability and levels of bioactive compounds (1-3). Bread is made up of flour, water, yeast or leavening agent, and salt (4-6). It provides energy and an important amount of most dietary nutrients, including carbohydrate, protein (7, 8), vitamins, particularly thiamine, riboflavin, niacin, and pyridoxine, minerals (9), antioxidants, and phytochemicals (10). Cereals contain phytic acid (PA) or myo-inositol hexakisphosphate (1-4%) (11), which is considered to be an anti-nutritional factor in the process of making bread or other food products (12, 13). Phytic acid reduces the bioavailability of nutrients such as protein, carbohydrate, and minerals because it has high chelating activity (9, 14).

There are three main methods of bread making. In the straight dough method, all ingredients are mixed in one single step. In this method, some equipment is needed, and it is different depending on the manufacturer's equipment and ingredients. The sponge and dough method is the second one. There are many ways to make sponge, such as using a leavening agent, yeast, and certain chemical substances. These substances are mixed with flour, water, and other ingredients depending on the required product, and the mixture is left to develop for a few hours. In the third method, the Chorleywood method, all ingredients are mixed in an ultrahigh mixer for a few minutes (15).

Iron deficiency is the most common and widespread nutrient deficiency, which has serious negative public health effects, especially in children and women (16). Iron food fortification, supplementation, dietary diversifica-

tion, and other public health programs are used to counteract iron deficiency (17). In Iran, flour has been chosen as the food vehicle for iron fortification (18). Bioavailability of fortification is important. Using baking soda in sponge and dough bread is an illegal method. Studies in Iran have shown that baking soda in bread ranges from 2% to 47% (19). Owing to its alkaline and chemical properties, it causes some disorders such as gastrointestinal disorders, dyspepsia, disruption in digestion, and absorption of protein and minerals, as well as increases heavy metals absorption (e.g., lead and mercury) (20).

Given the disadvantages of using baking soda in bread processing, knowing the harm of baking soda is an important priority in health care. Since bread is a popular food in Iran, using soda in making bread can cause a variety of illnesses in the long run (19). Hence, investigation into bread making methods may reveal more information such as bread quality, macronutrient, and micronutrient bioavailability, protein efficiency, effective fortification in public health strategies, etc.

2. Objectives

The present study aimed to examine bread baking methods and their effects on protein efficiency ratio (PER) and the status of some iron-related blood factors.

3. Methods

The research protocol was approved by the Faculty of Medicine Ethics Committee for Animal Research of Zahedan University of Medical Sciences, (ethical code: IR.ZAUMS.REC.1394.286).

Four types of diet were used in this study. Control diet was animals chow, and three other diets were made based on Pourafshar et.al. methods with some modifications (21), including fermentation method in which one portion of iron-fortified wheat flour, 2% of *Saccharomyces cerevisiae* (Dez-Maye, Iran) and 1% of salt (Reifan, Iran) were mixed in a proper portion of drinking water (with 120 ppm total hardness) and the prepared dough was kept at room temperature (25 - 27°C) for 120 min. Chemical method (soda bread): in this method; iron-fortified wheat flour was mixed with 2% baking soda (sodium bicarbonate) and 1% salt; a proper portion of water was then added, and it was all then mixed. Non-fermentation method: in this process, one percent of salt was added to iron-fortified wheat flour mixed in a proper portion of water. After kneading, the dough was cooked in the oven (300°C). The prepared bread was cooled and dried at room temperature, then finely ground, mixed with a proper amount of water, and pressed into cube shapes, and stored at 4°C in airtight containers.

The flour temperature was 20°C, water temperature was 35°C, mixing time was 2 - 3 min, kneading time was 3 - 5 min, and the baking time was 9 - 10 min. Commercial wheat flour with an 85% extraction rate was prepared from the Flour Factory of Yaghoobi Zahedan, Iran.

3.1. Animals

The study was conducted on 40 male rats aged 21 days. The animals were categorized into four groups, and each group with 10 rats. The animals were purchased from the Laboratory Animal Research Center of Zahedan University of Medical Sciences. They were adapted to the environment one week before the experiment by keeping them under controlled environmental conditions such as free access to water and food, a 12-hour cycle of light-darkness, and 22°C ± 2°C.

The rats allocated to four groups were put into stainless steel cages (25 × 22 × 20 cm). For adaption, after weaning (21 days after born), the male rats received standard lab chow for one week and then were allocated to one of the 4 diets, randomly. Protein efficiency ratio (PER) was determined after 21 days of diet consumption. Food (protein) intake (weight of food consumed = weight of the total food put in the cage - weight of food left in the cage) and weight gain were determined for calculating PER via the following equation (22):

PER = increase in weight of the rats / weight of protein consumed.

The study was continued for one more month to determine the effect of diet on the blood parameters such as ferritin, hemoglobin (Hb), hematocrit (HCT), red blood cells (RBC), total white blood cells (WBC), mean cell volume (MCV), and some other factors.

3.2. Chemical Analysis

3.2.1. Flour Iron

Atomic absorption spectrophotometer (Shimadzo AA-670, Kyoto) was used to measure iron content in the fortified wheat flour.

3.2.2. Moisture Content

Gravimetric methods of AOAC (1995) was used to assess the moisture content of the samples. Briefly, 5 g of the test sample was weighed into metal containers (these containers were dried at 70 - 80°C for 2 h then were cooled in a desiccator and weighed). An oven with air circulation was used. The samples were dried at 105°C for 4 h, then were cooled in a desiccator and weighed. Drying and cooling were repeated at 30 min intervals until a constant weight

was obtained. The below formula shows the percentage weight of moisture calculation:

Moisture content (%) = ((weight of metal container + sample before drying) - (weight of metal container + sample after drying)) / (weight of metal container + sample before drying) × 100

3.2.3. Ash Content

An electric furnace at 550°C for 2 h and Onwuka method with some modifications was used to determine ash content (23). Briefly, 2 g of the test sample was weighed into porcelain crucible (dried and weighed previously), and heated in an electric furnace; it was then cooled in desiccators. Heating and cooling were repeated to reach the constant weight. Ash content (%) was calculated as follows:

Ash content (%) = ((porcelain crucible weight + sample weight before turning to ash) - (porcelain crucible weight + sample weight after turning to ash)) / (porcelain crucible weight + sample weight before turning to ash) × 100

3.2.4. Protein Content

Kjeldahl method with some modification as reported by Onwuka (2005) was used to determine crude protein content (23). In the digestion stage, 2 g of the test sample was added to a micro-Kjeldahl flask containing a metallic catalyst and 5 ml of concentrated H₂SO₄. Digestion continued at red hot temperature for 2 h, and the digested sample was transferred into a volumetric flask. Afterward, the digested sample was diluted to 50 ml in distilled water. In the distillation stage, 10 mL of each dilution was transferred into Kjeldahl apparatus, 10 mL of NaOH (40%) was added, and it was distilled. In the titration stage, 10 mL of Boric acid (4%) solution containing three drops of indicator in 50 mL of distillate from each duplicate was used to make titration with H₂SO₄ (0.02 N) to achieve a pink color. The above stages were done for a blank sample. Percentage of nitrogen multiplied by 6.25 as a correction factor was used to calculate protein percentage. The following formula can be used to determine nitrogen percentage:

Percentage of nitrogen in the sample = 1.4V × NW

Where, V = acid used in titration (mL), N = normality of standard acid and W = weight of sample (g).

3.2.5. Hematological Assessment

The hematological parameters such as Hb, RBC, WBC, and platelet (Plt) counts, hematocrit (Hct) percentage, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were assessed by a hematology cell counter apparatus (Sysmex KX-21, Japan).

Serum samples were used for diagnostic tests such as ferritin, blood urea nitrogen (BUN), and creatinine (Cr) using commercial enzymatic kits (Pars Azmun, Theran, Iran), according to the manufacturer's instructions.

At the end of the experiment (day 85), under an overnight fasting (12 - 14 h), the rats were anesthetized by diethyl ether (Merck Germany) and blood samples were collected from cervical vessels, after sacrificing the animals, in two tubes for each of them and the samples were kept on ice and transferred to the laboratory for determination of hematological parameters.

3.3. Statistical Analyses

Descriptive statistics included mean ± standard deviation (SD), and analytical statistics included one-way ANOVA and Tukey post hoc test. The level of significance applied to statistical tests was P < 0.05. SPSS software version 16.0 (SPSS Inc., Chicago, Illinois, USA) was used for statistical analyses.

4. Results

Table 1 shows the chemical and PER parameters among the groups. Fe of wheat flour was 18.68 ± 2.1 mg/kg. Diet protein content was different between the groups and it was lower in the fermented and non-fermented bread and the highest in the control group (animal chow diet). The PER value was negative in the group that received soda bread. The difference in the fermented bread and control groups was non-significant. Body weight at the weaning time (day 21) showed no significant difference between the groups. After 3 and 8 weeks of the treatment, the weight showed a significant difference between the groups. The weight was constant in the soda bread group, while the control group had the highest weight gain.

Table 2 shows some blood parameters. Mean of ferritin, RBC, HCT, MCV, and MCHC were significantly lower in the rats, which received a soda bread diet (P < 0.001). Regarding other parameters such as MCH, significant differences were not observed. Only in the control group, BUN was in the normal range (24), while it was significantly lower in the other groups and the lowest BUN was seen in the rats, which received soda bread.

Although Hb (g/dL) was in the normal range in all groups, in non-fermented bread and soda bread groups, it was the same and lower compared to fermented and control groups.

5. Discussion

The results of protein efficiency ratio index (PER) in diets showed that soda bread had a negative (-0.12) PER. In

Table 1. Chemicals and Biological Status of Parameters in Different Dietary Groups ^{a, b}

	Fermented Bread	Soda Bread	Non-Fermented Bread	Control (Animal Chow)	P-Value
Ash (%)	7.26 ± 0.15 ^A	7.43 ± 0.20 ^A	9.16 ± 0.35 ^B	9.06 ± 0.86 ^B	0.002
Moisture (%)	6.90 ± 0.26 ^A	6.88 ± 0.12 ^A	6.50 ± 0.26 ^A	9.63 ± 0.47 ^B	< 0.001
protein (%)	10.66 ± 0.26 ^A	11.47 ± 0.2 ^A	10.58 ± 0.31 ^B	20.12 ± 0.56 ^D	< 0.001
PER	1.03 ± 0.23 ^A	- 0.12 ± 0.33 ^B	0.54 ± 0.15 ^C	0.95 ± 0.27 ^D	< 0.001
weight at day 21 (g)	61.5 ± 5.46 ^A	62 ± (3.77) ^A	62.3 ± 3.09 ^A	57.60 ± 3.94 ^A	0.06
weight at the end (g)	120.8 ± 22.66 ^A	83.61 ± 4.87 ^B	107.60 ± 21.82 ^A	125.6 ± 14.19 ^A	< 0.001

^aData reported as mean ± standard deviation (SD).

^bDifferent capital letters (A, B, C) in each row shows that the values are significantly different (P < 0.05).

Table 2. Hematological and Iron Status Parameters in Different Dietary Groups

	Fermented Bread	Soda Bread	Non-Fermented Bread	Control (Animal Chow)	P-Value
Ferritin (ng/mL)	7.90 ± 0.46 ^A	6.22 ± 0.28 ^B	7.40 ± 0.34 ^{AB}	7.10 ± 0.46 ^{AB}	.04
RBC (10 ⁶ /μL)	7.32 ± 0.11 ^A	6.34 ± 0.14 ^B	7.31 ± 0.06 ^A	7.66 ± 0.05 ^A	< 0.001
Hb (g/dL)	14.12 ± 0.095 ^A	12.99 ± 0.13 ^B	13.51 ± 0.24 ^B	15.11 ± 0.06 ^c	< 0.001
Hct (%)	39.41 ± 0.54 ^A	31.71 ± 1.68 ^B	38.01 ± 0.54 ^A	40.52 ± .36 ^A	< 0.001
MCV (fL)	2.21 ± 0.69 ^A	4.56.152 ^B	2.38 ± 0.75 ^{AB}	1.73 ± 0.55 ^A	0.004
MCH (Pg)	19.12 ± 0.34	20.02 ± 0.56	18.97 ± 0.04	19.75 ± 0.11	0.08
MCHC (g/dL)	35.15 ± 0.89 ^A	39.87 ± 1.20 ^B	35.87 ± 0.56 ^A	37.22 ± .82 ^{AB}	0.004
BUN (mmol/L)	14.70 ± 1.83 ^{AB}	9.78 ± 1.27 ^{AC}	15.30 ± 2.53 ^A	18.50 ± 1.48 ^B	0.02
Creatinine (μmol/L)	0.28 ± 0.04 ^A	0.38 ± 0.05 ^A	0.43 ± .03 ^A	0.39 ± 0.05 ^A	0.09
WBC (10 ³ /μL)	5.06 ± 0.21 ^{AB}	3.90 ± 0.38 ^A	5.32 ± 0.36 ^B	9.66 ± 0.29 ^C	< 0.001
Plt (X100)(10 ³ /μL)	551.10 ± 15.59 ^A	770 ± 74.35 ^B	598.70 ± 60.27 ^{AB}	685.30 ± 10.48 ^A	0.01

^aData reported as mean ± standard deviation (SD).

^bDifferent capital letters (A, B, C) in each row shows that the values are significantly different (P < 0.05).

other groups, it did not show a significant difference. PER method is based on the assessment of animal or human growth. It shows nitrogen balance, digestibility, and absorption of amino acids in ingested food (25). Several factors, such as starch and protein digestibility, type of dietary fiber, mineral absorption, fermentation, and toxic substance, may explain the differences between soda bread and other groups (26-28). Multiple factors influence protein quality, such as basic amino acids or carbonyl-amine reaction (29), baking temperature, crump, limited amino acids, pH, and Maillard browning reaction that decrease lysine and methionine (30). Alkali treatments cause racemization in amino acids. This phenomena has been known since the first half of the 20th century (31) and some D-amino acids, such as D-tyrosine and D-lysine, may be deleterious and inhibit the growth of rats. Moreover, gastric juice neutralized by NaOH and also digestion of protein can disrupt and prevent the absorption of protein (32).

Chemical parameters analyses of bread prepared by three methods showed some differences in the ash, pro-

tein and moisture contents. This may be explained by a difference in moisture content. The flour iron content in our study was higher than that in studies by Rodriguez-Ramiro et al. and Bruggraber et al., who used whole meal bread (33, 34) (18.68 mg/kg against, 2.4 - 3.4 mg/100). This is due to the use of fortified wheat flour.

Serum ferritin content showed a significant difference between fermented bread and soda bread groups; RBC content was significantly different between soda bread and other groups; Hb content was not significant between soda bread and non-fermented bread group, and finally, Hct percentage was significantly different between soda bread and other groups. Ferritin is a major iron storage protein, and there is a positive correlation with body iron storage (35). Hemoglobin and hematocrit are parameters that show Fe status in the body and can be used in iron deficiency anemia (IDA) detection. In this study, they decreased in the rats that received soda bread. Iron absorption from the diet is a multi-factorial process. Achlorhydria or neutralization of gastric acid can decrease Fe ab-

sorption. In soda bread and non-fermented bread, pH is higher than fermented bread.

Rodriguez et al. (2017) showed that the sourdough bread process increased the bioaccessible endogenous iron compared to the conventional yeast and Chorleywood bread making process. They mentioned that the results were due to the decomposition of fiber (33). The strain of *Saccharomyces cerevisiae* is important because it has the ability to grow in iron-fortified wheat flour. Zhang et al. (2016) study showed a wild-type strain no. YMI504 had an effective restorative function by returning the blood Fe parameters (36). Although phytases during bread making could decrease from 13% to 100%, the method of dough processing is very important. In bread processed by bicarbonate of soda, very little or no phytate was hydrolyzed. In fact, phytic acid content reduced 52% in bread fermented by baker's yeast and 71% in sourdough bread (37).

Low-pH of gastric juice is needed for the activation of gastric enzymes, denaturation and digestion of proteins and increased concentration of small peptides and free amino acids in the gut and minerals to dissociate complexes with proteins and other ligands to be absorbed (38). Hence, the decrease of such parameters that reflect the iron absorption status can be explained in the soda bread.

Cereals have been known as a good source of B vitamins such as thiamine and folate. These vitamins are decreased by the increase of flour extraction. Although we used fortified flour with iron and folate, blood parameters related to folate and vitamin B12 status (MCV, MCH, and MCHC) showed variation.

Moreover, MCV and MCHC increased in the soda bread group and showed a significant difference compared to the other groups. Ingestion of alkaline substances reduces folate absorption (39), and although cereals are not a good source of vitamin B12, the use of long-term acid suppression substances increases its deficiency (40). Yeast fermentation, lactic acid bacteria, and sourdough bacteria, however, have different abilities to increase the folate content in the baking process. The minimal effect of the fermentation is the retention of vitamins in the baking process (41). Batifoulouier et al. (2005) reported that the white bread classical fermentation process (short fermentation) leads to a 48% loss of thiamine. Longer fermentation time increased thiamine concentrations. Long fermentation process with yeast, in whole wheat bread, increased the contents of riboflavin. They did not observe a synergistic effect by using mixed fermentation of yeast plus sourdough on vitamin B levels (42). Blood Urea Nitrogen (BUN) test and Cr are markers that show kidney function, consumption of protein, muscle function, muscle composition, activity, and health status. The present study showed that the lower level of BUN in the soda bread group was sig-

nificantly different from the other groups. Increased BUN seems to be associated with kidney disease or failure, dehydration, fever, shock and bleeding in the digestive tract. Low levels are also seen in trauma, surgery, opioids, malnutrition, and anabolic steroid use. Creatinine blood levels rise up when kidney filtration is abnormal (43). In this study, lower BUN can be explained by malnutrition (low weight gain) and decrease of PER, indicating that although soda bread has protein like other kinds of bread, its quality is low. This finding is in line with Nawagwu et al. (2000) study that showed maternal low protein diet lead to progressive deterioration of renal function in rats (44). In the present study, although creatinine blood level is relatively higher in the non-fermented bread group, it is not significantly different from other groups, which indicates that the kidney was not yet adversely affected by bread processing.

Moreover, in this study, WBC content in the groups that used bread was significantly lower than the control group. Although significant differences were not observed between the soda bread group against fermented and non-fermented bread groups, its content was lower. In fact, WBC plays a major role in the defense mechanism in organisms. WBC content and its subgroups are affected by many factors such as diet, energy, protein, essential fatty acids, minerals and vitamins, etc. (45). Prestes-Carneiro et al. (2006) showed that long term maternal protein malnutrition retarded growth curve, macrophage function, body composition, and hematological status (46). Castaneda et al. (1995) showed that a low protein diet leads to loss of body mass, muscle function, and immune response in elderly women (47). Ekis et al. (2005) studied the effect of IDA on immunity in children. They found that there was no difference in the distribution of T lymphocyte subgroups, but the percentage of monocytes with phagocytic activity or oxidative burst activity and percentage of neutrophils monocytes with phagocytic activity or oxidative burst activity were lower in IDA. These findings emphasize the importance of the body iron status in the immunogenic mechanisms (48).

In this study, platelets (Plt) were significantly higher in soda bread and non-fermented bread compared to fermented bread and control diet groups. Platelet count is important for blood clotting, and the increase of platelets may be due to reactive thrombocytosis. The results showed an inverse relationship between Plt and ferritin, RBC, Hb, and Htc contents. These findings are in line with the findings by Kadikoylu et al. (2006) who reported that platelet count increased when serum iron, iron saturation, ferritin and mean platelet volume decreased (49). Andrewe et al. (2018) found a significant difference in Plt among Vistar rats fed on a diet containing calcium carbide ripened

mango fruits; moreover, Plt value was higher in the groups with lower RBC, Hb, and HCT content (50).

5.1. Conclusion

Although the cereal group provides important amounts of most nutrients and bread can be used as a major source supply of energy, protein and other nutritional elements, the type of processing may decrease or increase the levels of the bioactive compounds. Soda bread making process has had a dramatically adverse effect on nutritional value of bread. Based on our results, soda bread making process can decrease protein efficacy ratio and cause debilitation of hematological parameters. These effects of soda can disrupt the body's physiological processes and lead to disease in the long run. If in some regions, soda is used routinely or traditionally in the bread making process, the assessment of blood parameters in people can be considered.

Finally, it is necessary to familiarize bread producers and consumers with the disadvantages of soda and recommend that soda not be used in the bread processing process.

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Footnotes

Authors' Contribution: Study concept and design: A. Dashi-pour, H. Fanaie, and T. Eghbali.; analysis and interpretation of data: A. Dashi-pour and H. Fanaie; drafting the manuscript: A. Dashi-pour, H. Fanaie and A.Khazaie; critical revision of the manuscript for important intellectual content: A. Dashi-pour, H. Fanaie; statistical analysis: A. Dashi-pour.

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