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Health Scope
Article DOI: 10.5812/jhealthscope.107390
Published in: Health Scope: 9(4); e107390

Peer Review Report for "Soda Bread Making Process Decreases Protein Efficacy Ratio and Causes Debilitation of Hematological Parameters in Male Rats"

Author(s): Hamed Fanaei, Tahereh Eghbali, Abdurrashid Khazaei Feizabad, Alireza Dashipour

Review Timeline:

Submit Date: 18 Jul 2020
Revised Date: 9 Sep 2020
Accepted Date: 5 Oct 2020

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | **Revision** (0) — 3 Aug 2020

Dear editor
Thanks to the authors of this article, the following comments are suggested.
1-The subject is valuable and I think it can be accepted.
2-But some corrections should be done. The authors need to explain the meaning of letters (a, b, c) that is used in tables.
Thanks for your attention

REFEREE: Reviewer | **Revision** (0) — 4 Aug 2020

Dear Editor-In-Chief:
I read the article carefully. It is to be noted that the study needs to be reviewed due to the topic.
1.In sum, Cereals are commonly known as an energy source, not a protein source. Therefore, the author should consider this in the introduction of the manuscript.

2.The beginning of introduction has been begun with a shortage of iron. While the subject is discussed, this section is reviewed based on the purpose of the study
3.According to the methodology and the results of the study, there is no problem.
4. In this discussion, the results were reasonable based on the objectives of this study.
5. If possible, the references should be changed into a newer form.

REFEREE: Reviewer | **Revision** (0) 11 Aug 2020

The subject and statistical analyses are excellent, but this paper was used for the use of SPSS in statistical analysis and English editing is actually necessary for this paper.

REFEREE: Reviewer | **Revision** (0) 29 Aug 2020

Bread baking methods and their effects on PER and statues of some iron-related blood factors were investigated in the present study. The topic is very interesting and it's important in some countries. The English language must be edited . The data volume is considered as a short communication rather than a original article.

REFEREE: Associate Editor | **Revision** (0) 1 Sep 2020

Please see Reviewer Comments:
Reviewer 1:
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Reviewer 2:
The subject and statistical analyses are excellent, but this paper was used for the use of SPSS in statistical analysis and English editing is actually necessary for this paper.

Reviewer 3:
Thanks to the authors of this article, the following comments are suggested.
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Reviewer 4:
Bread baking methods and their effects on PER and statues of some iron-related blood factors were investigated in the present study. The topic is very interesting and it's important in some countries. The English language must be edited . The data volume is considered as a short communication rather than a original article.

REFEREE: EIC | **Revision** (0) 2 Sep 2020

Dear author

According to reviewer comments, the manuscript needs "Minor Revision".

Best Regards

OPEN PEER REVIEW

Revision (1)

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Dear Reviewer:

Thank you for your precious time and the generous efforts you have made to improve this manuscript. We have tried to change the manuscript based on our valuable comments and hope, we have done your request

Reviewers' Comments:

Reviewer 1:
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1. In sum, Cereals are commonly known as an energy source, not a protein source. Therefore, the author should consider this in the introduction of the manuscript.

Thank you for your attention and kindness, your comments were done and marked by yellow color

2. The beginning of introduction has been begun with a shortage of iron. While the subject is discussed, this section is reviewed based on the purpose of the study

Thank you for your kindness, your comments were made and marked with yellow color.

3. .According to the methodology and the results of the study, there is no problem.

4. In this discussion, the results were reasonable based on the objectives of this study.

5. If possible, the references should be changed into a newer form.

Thank you for your attention and kindness, your comments were done and marked with yellow color

Reviewer 2:
Dear editor

Thanks to the authors of this article, the following comments are suggested.

1-The subject is valuable and I think it can be accepted.

2-But some corrections should be done. The authors need to explain the meaning of letters (a, b, c) that is used in tables.Thanks for your attention.

Reviewer 3:
Bread baking methods and their effects on PER and statues of some iron-related blood factors were investigated in the present study. The topic is very interesting and it's important in some countries. The English language must be edited. The data volume is considered as a short communication rather than a original article.

Thank you for your comments, they were made about the English editing and summarizing.

Reviewer 4:
The subject and statistical analyses are excellent, but this paper was used for the use of SPSS in statistical analysis and English editing is actually necessary for this paper.

English editing and requested items were made.

Article title:

Soda bread making process decrease protein efficacy ratio and cause debilitation of hematological parameters in male rats

Abstract

Aim: Bread has long been one of the most popular foods and is the major source to supply energy, protein, minerals and vitamins. Kind of bread making process may affect its nutritional value. This study aimed to investigate~~s the~~ bread baking methods ~~in preparation~~ and their effects in protein efficacy ratio (PER) and statu~~s~~es of some iron-related blood parameters in rats. ~~that are related to Iron.~~

Methods: Four diets were used in this study including: 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 as mean ± standard deviation (SD) and analytical statistics include: One-way ANOVA and Tukey post Hoc-test were used. P< 0.05 was considered as statistically significantce difference.

Results: The wheat flour Fe was 18.68 mg/kg**.** The PER value was negative in the group that received soda bread and the weight gain in the soda bread group was the lowest. 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.

conclusion: Although bread can be used as a major source to supply energy protein and other nutritional values, 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.

Key words: Bread, Fermentation, Baking Soda, PER, Fe

Introduction:

Bread making methods are can have an important role in decreasing or increasing availability and levels of bioactive compounds [1-3]. Bread makes is made up of flour, water, yeast or leavening agent and salt[4-6]. It provides

energy and 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 ~~and~~ in the process of making bread ~~making~~ or other food products [12, 13]. PA reduces bioavailability of nutrients such as protein, carbohydrate and minerals because it has high chelating activity [9, 14].

There are three main methods ~~in~~ of bread making. In straight dough method, all ingredients are mixed in one single step. ~~T~~In this method ~~need to~~ some equipment is needed and it is different ~~according to~~depending on the manufacturer's equipment and ingredients. Sponge and dough method is the second one. There are many ways to make sponge, such as using leavening agent, yeast and certain chemical substances. These substances are mixed with flour, water and other ingredients ~~based~~depending on the required product and the mixture is left to develop for few hours. In the third method, ~~that named~~ Chorleywood method, all ~~the~~ ingredients are mixed in an ultrahigh mixer for a few minutes[15].

Iron deficiency is the most common and widespread nutrient deficiency ~~and it deficiency~~ which has serious negative public health effects, especially in children and women [16]. Iron food fortification, supplementation, dietary diversification 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. ~~B~~Using baking soda ~~using,~~ in sponge and dough bread ~~method~~, is an illegal method. Studies in Iran have shown that baking soda in bread has ranges from 2% to 47% [19]. Due to its alkaline and chemical properties, it causes some disorders such as

gastrointestinal disorders, dyspepsia, disruption in digestion and absorption~~b~~ of protein and minerals and also increases ~~of~~ 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 ~~the~~ bread making methods may reveal more information such as bread quality, macronutrient and micronutrient bioavailability, protein efficiency, effective fortification in public health strategies, ~~and~~ etc.

Therefore, the present study was designed aiming at ~~ed to~~ examining~~e~~ bread baking methods and their effects on protein efficiency ratio (PER) and ~~statues~~ status of some iron-related blood factors.

Material and Methods:

Research protocol ethics was approved by 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 ~~maid~~ made based on Pourafshar *et.al* methods with some modifications[21], including fermentation method~~:~~ in which ~~O~~one portion of iron-fortified wheat flour, 2% of S*accharomyces cerevisiae* (Dez-Maye, Iran)

and 1% of salt (Reifan, Iran) were mixed ~~with~~ in proper portion of drinking water (with 120 ppm total hardness) and the ~~peppered~~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 ~~combined~~ mixed with %2 baking soda (sodium bicarbonate) and 1% salt; 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 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 proper amount of water and pressed ~~to~~ into ~~produce~~ cube shapes ~~form~~ 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 baking time was 9-10 min. Commercial wheat flour with 85% extraction rate was prepared from Flour Factory of Yaghobi Zahedan, Iran.

Animals

~~A~~ The study was conducted on ~~number of~~ 40 male rats ~~with~~ aged 21days ~~age were used in this study~~. The animals were put in ~~F~~four groups and each group ~~containing~~with 10 rats. ~~A~~ The animals were purchased from Laboratory Animal Research Center of Zahedan University of Medical Sciences. They were adapted to the environment one week before the experiment by ~~kept~~ 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.

RThe rats were allocated toin four groups were put into stainless steel cages (25×22×20 cm). For adaption, after weaning (21days after born), the male rats received standard lab chow for one weak 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 in put in the gage cage – rest weight of food left in the gage – weight of food fallen ing in the bottom of the gagecage) and weight gain were determined for calculating PER via the following equation:

PER = increase in weight of the rats /weight of protein consumed. [22].

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

Chemical analysis:

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

Moisture content: gravimetric methods of AOAC (1995) was used to assess of moisture content of the samples. Briefly, 5g of the test sample was weighed into metal container (these containers were dried in 70-80°C for 2h then were cooled in desiccator and weighed). An Ooven with air circulation was used. The

samples were dried at 105°C for 4 hours, then were cooled in desiccator and weighted. Drying and cooling was 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

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

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

Protein content: Kjeldahl method with some modification as reported by Onwuka (2005) was used to determine crude protein content determination[23]. In the Ddigestion stage, 2 g of the test samples was added into a micro-kjeldahl flask containing a metallic catalyst and 5 ml of concentrated H_2SO_4. Digestion continued at red hot temperature for 2 h and the digested sample was transferred into a volumetric flask. Afterwards, the Ddigested sample was diluted to 50 ml

~~with~~ in distilled water. In the ~~D~~distillation stage, 10 ml of each dilution was transferred into Kejeldahl apparatus, ~~and added~~ 10 ml of NaOH (40%) was added, and it was distilled. In the ~~T~~titration stage, 10ml of Boric acid (4%) solution containing 3 drops indicator in 50 ml of distillate from each duplicate was used to make titration with H2SO4 (0.02N) to achieve a pink color. The above stages were done for blank sample. Percentage of nitrogen multiplied by 6.25 as a correction factor was used to calculate protein percentage ~~calculation~~.

~~Below~~ The following formula can be used to determine nitrogen ~~determination~~ 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)

Hematological assessment: The hematological parameters such as: hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC) and platelet (Plt) counts, hematocrit (Hct%), 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 duration (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.

Statistical Analyses:

Descriptive statistics include: mean ± standard deviation (SD) and analytical statistics include: One-way ANOVA and Tukey post Hoc-test ~~were used~~. 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.

Results:

Table 1 shows the chemical and PER parameters among groups. Fe wheat flour was 18.68 ± 2.1 mg/kg**.** Diet protein content was different among groups and it was lower in the fermented and non-fermented bread and 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 among groups. After 3 and 8 weeks of treatment, the weight showed significant difference among 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 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 lowest BUN was seen in the rats which received soda bread.

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

Table 1. Chemicals and biological status parameters in different dietary groups

	Fermented bread mean± (SD)	soda bread mean± (SD)	non fermented bread mean± (SD)	control (animal chow) mean± (SD)	p. value
Ash(%)	7.26 ± (.15)a	7.43 ± (.20) a	9.16 ± (.35)b	9.06 ± (.86)b	.002
Moisture (%)	6.90 ± (.26)a	6.88 ± (.12)a	6.50 ± (.26)a	9.63 ± (.47)b	<.001
protein (%)	10.66 ± (.26)a	11.47 ± (.28)b	10.58 ± (.31)b	20.12 ± (.56)d	<.001
PER	1.03 ± (.23)a	- .12 ± (.33)b	.54 ± (.15)c	.95 ± (.27)a	<.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	120.8 ± (22.66)	83.61 ±	107.60 ± (21.82)a	125.6 ± (14.19)a	<

the end(g)	a	(4.87)b			0.001

a Data reported as mean ± standard deviations. Different 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 mean± (SD)	soda bread mean± (SD)	non fermented bread mean± (SD)	control (animal chow) mean± (SD)	p. value
Ferritin (ng/ml)	7.90± (.46) a	6.22 ± (.28) b	7.40 ± (.34) ab	7.10± (.46) ab	.04
RBC(10^6/µL)	7.32 ± (.11) a	6.34± (.14) b	7.31± (.06) a	7.66 ± (.05) a	<.001
Hb(g/dL)	14.12± (.095) a	12.99± (.13) b	13.51 ± (.24) b	15.11± (.06) c	<.001
Hct (%)	39.41± (.54) a	31.71 ± (1.68) b	38.01± (.54) a	40.52± (.36) a	<.001
MCV (fL)	2.21± (.69) a	4.56 (1.52) b	2.38± (.75) ab	1.73± (.55) a	.004
MCH(Pg)	19.12± (.34)	20.02± (.56)	18.97± (.04)	19.75± (.11)	.08
MCHC(g/dL)	35.15 ± (.89) a	39.87 ± (1.20) b	35.87± (.56) a	37.22± (.82) ab	.004
BUN(mmol/L)	14.70± (1.83) ab	9.78 (1.27) ac	15.30± (2.53) a	18.50±1(.48) b	.02
Creatinin(µmol/L	.28± (.04)	.38±	.43± (.03)	.39± (.05) a	.09

)	a	(.05) a	a		
WBC (10^3/µL)	5.06± (.21) ab	3.90± (.38) a	5.32± (.36) b	9.66± (.29) c	<.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	.01

Data reported as mean ± standard deviations. Different letters (a, b, c) in each row shows that the values are significantly different ($P < 0.05$).

Discussion:

The results of protein efficiency ratio index (PER) in diets showed that soda bread had a negative (-.12) PER. In others groups it did not show a significant difference. PER method is based on assessment of animal or human growth. It shows nitrogen balance, digestibility and absorption 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 others 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 ~~early~~ 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 absorption of protein [32].

Chemical parameters analyses of bread prepared by three methods showed some difference in the ash, protein and moisture contents. This may be explained by difference in moisture content. The flour iron content in our study was higher than that in studies by Rudrigo and et,al. and Silvian et.al ~~studies that~~who used whole meal bread[33, 34](18.68 mg/kg against, 2.4- 3.4 mg/100). This is due to using 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 others groups; hemoglobin (Hb) content was non-significant between soda bread and non-fermented bread group and finally hematocrit (Hct) percentage was significantly different between soda bread and others 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 which received soda bread. Iron absorption from the diet is a multi-factorial process. Achlorhydria or neutralization of gastric acid can decrease Fe absorption. In soda bread and non-fermented bread, pH is higher than fermented bread.

Rodriguez ~~and~~ 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 decomposition of fiber[33]. The strain of *Saccharomyces cerevisiae* is important because it has the ability~~le~~ to grow~~th~~ in iron fortified wheat flour. Zhang et.al (2016) study findings showed a wild-type strain no. YM1504 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 hydrolysed. 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, denature and digestion of proteins and increased concentration of small peptides and free amino acids in the gut and minerals to dissociate from complexes with proteins and other ligands to be absorbed [38]. Hence, the decrease of such parameters that reflect the iron absorption statuse 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 decrease by the increase of flour extraction. Although we used fortified flour with iron and folate, blood parameters related to folate and vitamin B12 statuse (MCV, MCH and MCHC) showed variation.

MCV and MCHC increased in the soda bread group and showed a significant difference compared to ~~the~~ other groups. Ingestion of alkaline substance reduce folate absorption [39] and although cereals are not a good source of vitamin B12 ~~vitamin~~, use of long-term acid suppression substances increase 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 retention of vitamins in the baking process[41] . Batifoulier *et.al* (2005) reported that white bread classical fermentation process (short fermentation) leads to 48% loss of thiamine. Longer fermentation time increased thiamine concentrations. ~~l~~Long

fermentations process with yeast, in whole wheat bread, increased the contents of riboflavin. They did not observe 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 lower level of BUN in the soda bread group was significantly different from other groups. Increased blood urea nitrogen (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 in the non-fermented bread group was relatively higher, ~~there~~ it is not ~~a~~ significantly differen~~tce~~ 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 control group. Although significant differences were not observed between soda bread group against fermented and non-fermented

bread groups, its content was lower. 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 low protein diet lead to loss of body mass, muscle function and immune response in elderly women[47]. Ekis *et.al* (2005) studied the effect of iron deficiency anemia (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 ~~iron~~ body iron status in the immunogenic mechanisms[48].

In this study, Platelets (Plt) was significantly higher in soda bread and non-fermented bread compared to fermented bread and control diet groups. Platelets count is important for blood clotting and increase of platelets may be due to reactive thrombocytosis. The results showed an inverse relationship between Plt and ferritin, RBC, Hb, Htc contents. These finding are in line with the findings by Kadikoylu *et.al* (2006) ~~that~~who ~~showed~~ reported that ~~P~~platelet count~~s~~ increased when serum iron, iron saturation, ferritin and mean platelet volume decreased [49] . Andrewe *et.al* (2018) found a significance difference in Plt

among Vistar rats fed on diet containing calcium carbide ripened mango fruits; moreover, in groups with lower RBC, Hb and HCT content, Plt value was higher [50].

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, kind of processing may decrease or increase the levels of the bioactive compounds. Bread soda 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~~ed~~ that Soda not be used in the bread processing process.

Acknowledgments:

The authors express their gratitude to staff of Zahedan University of Medical Sciences for their kind collaboration. This research is based on the general doctoral thesis of Dr. Tahereh Eghbali (No. 1670).

Conflict of interest:

The authors declare no conflict of interest.

References:

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This is a good article and can be published.

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Dear Editor-In-Chief

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