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Fortification of Wheat Bread with 3-7% Defatted Soy Flour Improves Formulation, Organoleptic Characteristics, and Rat Growth Rate

Mohammad Reza Mahmoodi, Morteza Mashayekh[1], Mohammad Hasan Entezari[2]

Department of Nutrition and Physiology Research Center, School of Health, Kerman University of Medical Sciences, Kerman, [1]Department of Food Science and Technology, School of Nutrition and Food Technology, Shaheed Beheshti University of Medical Sciences, Tehran, [2]Department of Clinical Nutrition, School of Nutrition and Food Science, Isfahan University of Medical Sciences, Isfahan, Iran

Correspondence to:
Dr. Mohammad Reza Mahmoodi,
Department of Nutrition,
Physiology Research Center, School of Health, Kerman University of Medical Sciences, Haft Bagh-E-Alavi Highway, Kerman - 7635111167, Iran.
E-mail: mr_mahmoodi@kmu.ac.ir

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Original Article

ABSTRACT

Background: The present study designed to test effects of defatted soy-fortified wheat bread on the organoleptic properties as well as influences on rat growth rate.

Methods: Defatted soy flour (DSF) was blended with wheat flour with extraction rate of 82-84% at 3, 7, and 7% levels plus 3% sugar. Bread produced with these blends compared with regular Taftoon bread and was tested for chemical and organoleptic characteristics. The organoleptic characteristics of blends consist of taste and flavor, crust texture, fragrance and aroma, appearance, bendability, and overall acceptability were determined through taste panel by 213 judges. Forty Sprague Dawley rats were randomly given codes and allocated to different groups via tables with random numbers to feed on three DSF-fortified bread blends and control bread for 30 days.

Results: The blending of wheat flour with DSF altered the organoleptic properties of breads. Addition of DSF increased significantly the protein and ash content of the bread ($P < 0.05$). Organoleptic test indicates that the best formulation is between 3 and 7% fortifications of DSF blends. In biological evaluation, rats fed the control diet had the lowest body weight gain and their food efficiency ratio was significantly different ($P < 0.05$) in compare with 7% DSF-fortified blend.

Conclusions: It was concluded that overall acceptability score significantly decreased with increasing DSF substitution level. Rats fed 7% DSF-fortified blend showed privileged food efficiency ratio. Then, the best formulation is between 3 and 7% DSF bread. This formulation can nourish all human at risk of malnutrition.

Keywords: Defatted soy flour, fortified bread, organoleptic properties, rat growth rate

INTRODUCTION

The major component of wheat demand in Iran is for staple food, which constitutes more than 80% of the wheat consumption and is predominantly used for bread and bakery products.[1,2] There are also wastages during consumption. They mainly arise because the bread is often not properly baked and consumers do not conceive all of it as edible. Moreover, many consumers complain that the bread does not keep well, goes stale, and becomes inedible rather quickly. Official reports frequently maintain that the consumers waste about one-third of the bread. This is roughly more than 20% of the wheat production. However, case studies show household waste of bread to vary greatly between places, types, and it may lie between 8 and 33% of the bread brought by consumers.[2]

Soybean has long been recognized as an excellent source of high quality protein. The soybean also contains a wide variety of chemical compounds that have potent bioavailability. Among these compounds are the isoflavones, which may also be of benefit in the prevention of cardiovascular disease,[3,4] cancer,[5-7] and diabetes.[8,9] Incorporation of soy-related ingredients into a staple food such as a bakery product may be a feasible means of increasing daily soy intake in people's diets.[10,11] Soybeans have high protein 38-40% contents; these have great potential in overcoming protein calorie malnutrition. Soy protein is unique among plant proteins because of their high biological value and essential amino acid pattern. They are abundantly rich in lysine, which is deficient in most cereals. Supplementation of soybean, in a suitable form, to cereal foods would not only increase their protein content but also improve the availability of lysine.[12-15] High levels of soy products generally not used in bread because they impaired bread quality (loaf volume, crumb grain, freshness retention, and flavor) and the consumer acceptance of the bread.[16] The increasing importance of various types of bakery products in custom eating habits means that these food products can serve as vehicles for important nutrients while being readily accepted by consumers. Information is scanty on the supplementation of wheat flour with combinations of defatted soy flour (DSF) for bread making.

Therefore, in the present study, we investigated the best formulation of defatted soy-fortified bread on organoleptic properties and moreover, to assess growth rate of rats feed on these formulations compared with control Taftoon bread. The purpose of this study is to supplement wheat flour with DSF to develop a nutritionally rich and acceptable bakery product by consumer in compare with traditional bread without any undesirable effects on it sensory characteristics.

METHODS

Animals, diet, and experimental design

Rat growth assay to determine quality of soy protein products is an excellent and identical marker to determine the quality of soy proteins percentages added to wheat flour bread.[17] A 30-day feeding study was conducted to investigate the effects of soy fortification of wheat flour bread on growth rate of Sprague Dawley rats (weanling 28-days-old) were obtained from the Animal House of the Pasture Research Institute of Iran. Before the study started, they fed commercial rodent diet (food pellet) provided from Pasture Research Institute until they were 35-days-old and tap water was available *ad libitum*. The allocation of animals to different groups of treatment was done randomly. All rats were given codes and allocated to different groups via tables with random numbers. They were divided into four groups of 10 rats each. Rats were housed individually in stainless steel screen bottom cages. We used Resource Equation Method (Mead 1988) for determining sample size because of testing hypotheses is not the main objective.[18,19] Then, degree of freedom for the error term used to test the effect of the variable was chosen 10 for each group. The following equation:[19]

$E = N - B - T$,

Where E is the error degrees of freedom (df) and should be between 10 and 20, N is the total df, B is the blocks df, and T is the treatments df. In a non-blocked design the equation reduces to $E = N - T$ should be 10-20, which is simply: The total number of animals minus the number of treatments should be between 10 and 20. Therefore, we allocated 10 rats for every group. The total number of animals was 40 for our experimental study.

One group was fed the Taftoon bread and the other three groups were fed defatted soy-fortified bread at 3, 7, and 7% plus 3% sugar. All defatted soy-fortified bread blends and control bread dried in incubator and packed in sealed packages. Then, weighing breads was based on dried basis. The bread prepared from the various flour samples was the entire diet of experimental animal groups. The chemical constitutes of all bread were shown in Table 1. The animal house temperature was 25 ± 2°C with alternate 12 h during the experiment. The weight gain or loss of the animals was recorded every other day throughout the duration of the

Table 1: Proximate chemical composition* of wheat bread and different DSF-fortified breads

	Soybean flour	Control (100% wheat)	WF: 97% + DSF: 3%	WF: 93% + DSF: 7%	WF: 93% + DSF: 7%+3% sugar
Moisture	-	25.7±0.3	26.9±0.5	23.7±0.7	23.0±0.3
Ash	6.0±0.1	0.9±0.1	1.1±0.1	1.4±0.1	1.4±0.1
Phytic acid	-	352±6.4	184±2.1	175±3.6	143±1.9
Protein[a]	48.9±1.2	9.9±0.8	11.8±1.0	14.0±1.2	14.2±1.5
Fat	1.0±0.1	1.3±0.1	1.4±0.1	1.3±0.1	1.3±0.1

WF=Wheat flour; DSF=Defatted soy flour. *Values are averages of three repetitions, [a]N×5.7 for wheat bread and all bread blends

experiment. Records of food intakes and left over by the rats were taken.

The criterion used in the assessment of protein quality of the diets was food efficiency ratio. Total food efficiency ratio (FER) was obtained by total increased weight divided by total consumed food of each rat during test period. FER is equal with food conversion ratio (FCR). Generally, the amount of every other day consumed food and body weight gain by each rat was noted and was calculated by the following equation: FER = body weight gain (g)/consumed food (g).

Ethics committee of deputy research of the Isfahan University of Medical Sciences supervised this work.

Formulation of flour blends and breads

The rationale and design have been reported previously.[20] Ground DSF was blended with wheat flour at 3, 7, and 12% levels for 10 min to ensure homogeneity using a mixer. Wheat flour (100%) alone was used as control. Taftoon is sourdough flat bread, round in shape (about 30 cm diameter) with small holes on its surface, which is consumed in Iran. The control bread was bought from the university bakery and the samples with DSF blends were baked in the same bakery with the same procedure.

The bread was prepared by mixing all ingredients such as flour with extraction level of 82-84%, refined salt free iodine (1-2% w/w), water (55-60% w/w), and bakery yeast as leavening agent to optimum. To prepare fermented dough, two consecutive fermentations were carried out using 3% inocula (called sour dough) from a previous fermentation, to start fermentation of each subsequent batch. After fermentation for 90 min, about 450 g balls were made and sheeted in a round shape, and punctured to prevent puffing during oven baking as well as for decorative purposes, stuck to the walls of heated spherical oven and baked for about 2 min at about 300°C.[1,21]

Organoleptic evaluation

The organoleptic characteristics of blends were determined by consumer panelist of 213 judges comprising students, staff, and faculty of Isfahan Medical Science University recruited by advertisements for taste and flavor, crust texture, fragrance and aroma, appearance, bendability (the property of being easily bent without breaking), and overall acceptability. The samples were served in dishes labeled randomly with three digit random numbers and presented in monadic and random order. Each panelist received a rating form scored on a 1-9 hedonic scale (nine being considered excellent; five, acceptable; and 1, extremely poor), as suggested by Austin and Ram.[22] Breads were sliced into small pieces (15 × 15 cm) and were offered in distinct dishes at the same time. Water was provided for rinsing purposes. The tests were conducted in a cafeteria facility where there was no sensory evaluation room and panelists were seated one per table separately and rinsing water were provided for palate cleansing between samples.

Chemical analyses

The proximate chemical compositions of Taftoon bread and defatted soy-fortified bread blends were determined. The methods for sample treatment and analyses were the standard procedures recommended by Association of Official Analytical Chemists.[23] The ash was determined by incineration of known weights of the samples in a muffle furnace. The crude fat was determined by exhaustively extracting of known weight of sample in petroleum ether (boiling point, 40-60°C) in a Soxhlet extractor. The ether was volatilized and the dried, residue quantified gravimetrically, and calculated as percentage of fat.

Protein was determined by the Kjeldahl method. The conversion factors of nitrogen to protein were 5.7 (DSF = 48.9%).

Statistical methods

Data expressed as mean ± standard deviation (SD). Significant differences were determined at the $P < 0.05$ level. Normal distribution of all data (sensory characteristics, initial and final weights, and food efficiency ratio) was tested by Kolmogrov-Smirnov test before using parametric statistics for data analysis. Growth pattern and food efficiency ratio of rats were compared between groups by one-way analysis of variance (ANOVA). When the overall F is significant and more than two groups are being compared, post hoc (Tukey's HSD) tests to determine which pairs of means differ from each other and Statistical Package for Social Sciences (SPSS) software, version 11.0 were used (SPSS Inc., Chicago, IL).

RESULTS

Formulation and chemical composition of bread

Formulations prepared at the beginning of study were traditional Taftoon bread as control and three different DSF blends. The soy-fortified blends were 3, 7, and 12%. After determining organoleptic characteristics of four different breads, 12% DSF gained the lowest score and omitted for the rest of study. Because of we needed another treatment group for substitution, 7% soy plus 3% sugar-fortified blend was substituted. Table 1 shows the proximate composition of DSF, Taftoon and DSF bread at 3, 7, and 7% plus 3% sugar. Addition of DSF to wheat flour increased the protein content from 9.92% for control to 11.82, 13.99, and 14.2%, respectively.

No significant differences in fat content were observed between wheat flour bread and DSF-fortified breads. In addition, the addition of DSF to wheat flour showed an increase in the ash content. The ash content of 3 and 7% soy-fortified wheat breads versus to the control increased 15 and 33%, respectively. Data on the effect of fermentation on phytic acid contents of the control and different soy-fortified wheat breads indicated that, adding sugar to the blend may increase degradation of phytic acid. Adding sugar to bread dough increased yeast enzymes activity and speeded up the rising process. This reduction in phytic acid may be useful in improving nutritional quality of soy with respect to mineral bioavailability.

Organoleptic characteristics

Two hundred and thirteen consumer panelists evaluated samples. Bendability, appearance, flavor and taste, crust texture, fragrance and aroma, and overall acceptability properties were evaluated.

Table 2 was shown that bendability of the bread containing 7% DSF had most satisfactory score. Results indicated that the bendability score decreased significantly for 12% DSF bread.

Appearance score with respect to the control bread decreased significantly upon increasing the blending with DSF. Reduced appearance score of soy-fortified bread is due to crust color changes.

Flavor and taste score decreased with increasing DSF substitution levels. The 12% DSF bread was scored poorest in flavor and taste with significant difference. The flavor of 12% DSF bread might be affected by the bean flavor of soy flour.

The results revealed that crust texture score of 7% DSF bread was highest. However, statistically

Table 2: Effect of blending on sensory characteristics of breads

	Control (100% wheat)	WF: 97% + DSF: 3%	WF: 93% + DSF: 7%	WF: 82% + DSF: 12%	*F* value	*P* value
Bendability*	5.9±1.7	5.9±1.7	6.1±1.6	5.7±1.8	1.941	0.1015
Appearance	6.8±1.5	6.2±1.7	5.6±1.8	5.1±1.9	28.763	0.0001
Flavor and taste	5.7±1.7	5.6±1.7	5.6±1.7	5.0±2.1	6.683	0.0001
Crust texture	5.6±2.0	5.3±1.9	5.6±1.8	5.1±2.0	3.144	0.014
Fragrance and aroma	5.6±1.6	5.5±1.6	5.5±1.7	5.2±1.9	2.798	0.0251
Overall acceptability	6.1±1.6	5.8±1.5	5.9±1.7	5.1±1.8	10.207	0.0001

*The property of being easily bent without breaking. Values are mean±standard deviation of all independent determinations, scored on a 9-point scale. WF=Wheat flour; DSF=Defatted soy flour; SD=Standard deviation significant differences were found between various breads.

Fragrance and aroma score decreased with increasing DSF substitution levels. The 12% DSF bread was rated poorest in fragrance and aroma with significant difference.

Overall acceptability rating was the mean score of all the organoleptic characteristics in the present study. The results showed that the overall acceptability score of control was highest. Overall acceptability score significantly decreased with increasing DSF substitution level. The blending of wheat flour with DSF at different levels altered the organoleptic properties of different blended bread. Since the 12% DSF-fortified bread gained statistically the lowest score in all sensory attributes, it was omitted and instead, 3% sugar was added to 7% DSF for the rest of study.

Biological evaluation of protein quality

DSF-fortified bread at 3%, 7% and 7% plus 3% sugar along with control was subjected to protein quality evaluation through a rat study. Each group of weanling albino Sprague Dawley rats consisted of 10 rats and they were housed in individual cages that allowed for easy measurement of food intake. The mean consumed meal of control, 3%, 7% and 7% soy levels plus 3% sugar were 231.7 ± 55.6, 240.9 ± 46.6, 292.5 ± 43.9 and 247.5 ± 53.5, respectively. Mean food intake was near significant only between control and 7% DSF groups ($P = 0.048$). The results of rats feeding trials, conducted for the determination of protein quality of different DSF-fortified breads in compared with Tafton bread, are presented in Figures 1 and 2 which represent as weight gain and the food efficiency ratio increment trends in groups fed soy-fortified bread. Results of analysis of variance of rats' weights indicated significantly different mean between 7% DSF group and control, 3% DSF, and 7% DSF plus 3% sugar groups by post hoc test (Tukey honestly significant difference (HSD)) ($P = 0.001$) at the end of experimental study. However, there were not any significant differences between the other groups [Figure 1]. These results provide rats fed on control diet had the lowest body weight gain.

Mean food efficiency ratio is obtained by mean increased weight divided by mean consumed food of each group rat during the experimental study. Daily feed intakes were not significantly different among rat groups. In short, results

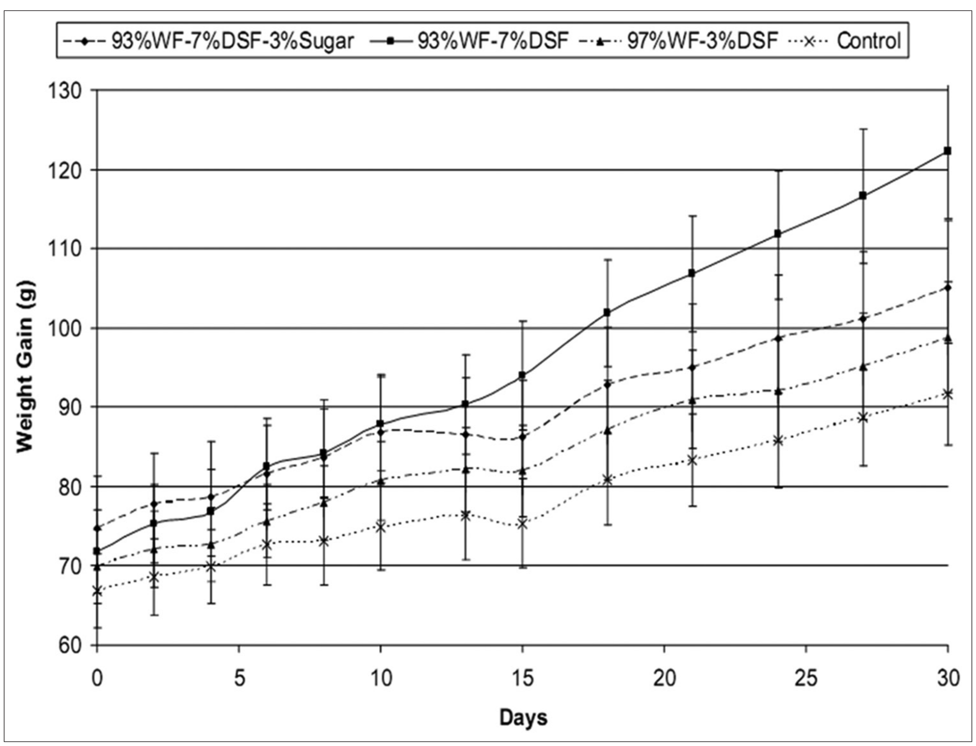

Figure 1: Growth pattern of rats fed defatted soy flour at 3, 7 and 7% level plus 3% sugar breads vs control (100% wheat flour) bread. Ten rats were allocated in each study group

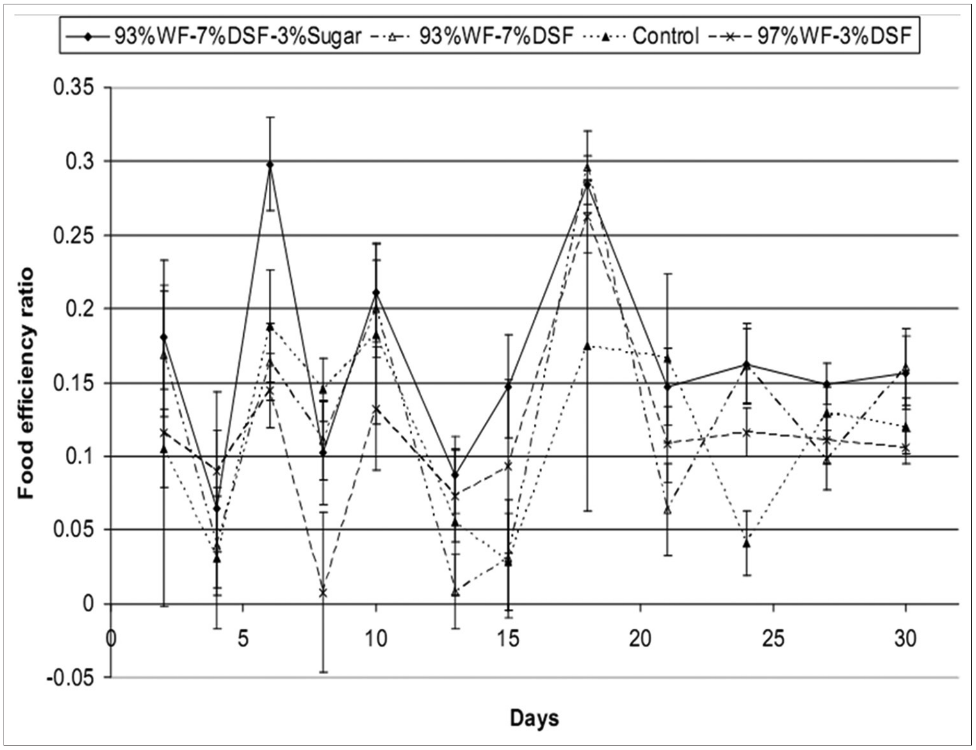

Figure 2: Food efficiency ratio of rats fed defatted soy flour at 3, 7 and 7% level plus 3% sugar breads vs control (100% wheat flour) bread. Ten rats were allocated in each study group

of analysis of variance of rats' food efficiency ratio indicated significantly different mean between 7% DSF group and control, 3% DSF and 7% DSF plus 3% sugar groups by post hoc test (Tukey HSD) (P = 0.001) at the end of experimental study. There were not any significant differences between 3 and 7% DSF plus 3% sugar blends and control [Figure 2]. Result from post hoc test indicated that mean food efficiency ratio of rats fed control diet were poorest and showed significant difference when compared with rats fed 7% DSF-fortified blend ($P = 0.001$). The mean FER of control, 3, 7, and 7% soy levels plus 3% sugar were 0.1017 ± 0.0189, 0.1244 ± 0.0127, 0.1760 ± 0.0140, and 0.1247 ± 0.0218, respectively. The major reason of saw-toothed curve in Figure 2 was FER measurement in every other curve.

The results of present study revealed that the blending of wheat flour with DSF up to 7% DSF, addition to increasing the protein and ash content of bread improved organoleptic characteristic significantly. The results of our previous study revealed that overall acceptability score for 3% DSF-fortified bread and after that 7% DSF-fortified bread were highest. Overall acceptability score significantly decreased with increasing DSF substitution levels.[20] Then, the best formulation is between 3 and 7% fortifications of DSF blends. This defect detected in other studies.[24,25] One study has demonstrated that supplementation of wheat flour with soy flour (full fat and defatted) up to 10% produced breads with good baking and organoleptic characteristics. However, at 15 and 20% levels they were less acceptable.[24]

In another study, addition of 10% soy flour (full fat and defatted), 15% barley plus full fat or DSF to wheat flour produce acceptable bread.[25] Therefore, appearance, bean flavor of soybean flour and crust texture scores decreased with substitution of soy (full fat and defatted) higher than 12%.[11,26] While, our study revealed addition of 3-7% DSF produced the most acceptable organoleptic bread with consumer view.

Results of rat assay revealed that different DSF-fortified breads when compared with Tafton bread represented weight gain and the FER increment trends. Results also indicated that mean FER of rats fed control diet were poorest and showed significantly difference when compared with rats fed 7% DSF-fortified blend. Therefore, confirmation of effectiveness of blending DSF to wheat flour on experimental animal growth was demonstrated. On the other hand, there is a disparity between growth velocity in experimental animals and the human. Hence, inclusion of 3% DSF-fortified bread for improving growth in human may be a controversy. These results agree with the theoretical chemical score of the proteins of these formulations, which indicated that amino acid profile of the mixture could fulfill the amino acid requirements. These results showed that a bread formulation with 7% DSF-fortified bread from a nutritional point of view is an excellent alternative to traditional bread. In another perspective, type of soy product and human or animal sample might alter results of researches. For instance, soy milk replacement with cow's milk in the diet reduced waist circumference in overweight and obese subjects although did not effect on other cardiovascular risk factors and weight gain significantly.[27] Therefore, components of soy product such as phytoestrogen, protein, and fat content may have different effects on animal or human.

The major nutritional problem in most of developing countries is protein-calorie malnutrition. Therefore, looking for inexpensive high protein materials is considered an important task for food scientists in these countries. Such materials will improve and enhance the nutritional quality of staple food like bread and the health of the people thereafter.[26] Soy is a complete, high quality protein, which can be added to a wide variety of products to enhance the texture and nutritional quality of foods such as bread.[28] The US Food and Drug Administration approval of a health claim associated soy protein intake with reduced risk of heart disease in 1999. The claim identifies 25 g/day as the amount needed to derive the claimed health benefit.[29] Bread is a staple food in Iran and its per capita consumption in urbanization, rural, and the entire country has been estimated as 286, 443, and 351 g/day, respectively.[2] With assumption of inclusion 3-7% DSF-fortified bread in Iranian food pattern and mean bread consumption 300 g/day, DSF intake would be 9-21 g/day. In this direction, protein intake from bread would increase from 30 to 35-42 g/day. Intake of 9-21 g/day DSF containing isoflavones is responsible for this claimed health benefit. Nine grams DSF is the minimum requirement that may not meet this benefit in short period. On the other hand, intake of this amount is unknown in long period. We could not measure isoflavones of DSF-fortified breads, which could be a limitation of current study.

Recently, some human studies have revealed the association between dietary soy components with cardiovascular risk biomarkers in metabolic syndrome and diabetes.[3,4,8,9] Some data are available concerning the favorable effects of soy products on inflammatory biomarkers.[9] One study revealed that soy product consumption for 8 weeks could reduce malondialdehyde (MDA) levels in postmenopausal women with the metabolic disorder.[9] In a double-blind randomized clinical trial, 50 g/day soy protein containing 164 mg isoflavones for 10 weeks, reduced the cardiovascular disease risk in hypercholesterolemic postmenopausal women because of both modest reductions in serum lipoproteins and an increase in paraoxonase1 activity.[4] The isoflavones may also be of benefit in the prevention of thromboembolic disorders in diabetic patients[8] and cancer.[5-7] The coagulation cascade much more activates in thromboembolic diseases and soy milk consumption in patients suspected of thrombotic disorders such as diabetic patients, lower d-dimer significantly. It indicates that decreasing this biomarker in plasma rules out thrombosis.[8] Also, the result of the Japan Collaborative Cohort (JACC) Study revealed that intake of soybean curd (tofu) was inversely associated with the risk of ovarian cancer.[5] In another study, decreased risks of breast cancer were found even among postmenopausal women with a moderate intake of soy and isoflavone.[6] A prospective study suggested that men with high consumption of soy milk are at reduced risk of prostate cancer.[7] These results suggested that soy and isoflavone intakes have a protective effect. Although different types of soy products, type of cancer, sample size, and study durations were different; soy ingredients and the effect of food processing on soy products might be important and responsible for these results. Then, the chemical form of isoflavones in soy food products should be taken into consideration. In some studies, isoflavones are not the only factor responsible for protective effects. The findings from a study revealed that isoflavone-free soy protein diet potently inhibited nuclear factor (NF)-κB activation and the subsequent inhibited vascular cell adhesion molecule 1 (VCAM-1), interleukin (IL)-6, IL-8, and monocyte chemotactic protein-1 (MCP-1) protein expression in human vascular endothelial cells *in vitro*. Then anti-inflammatory properties of components of soy protein/peptides may be a possible mechanism for the prevention of chronic inflammatory diseases such as atherosclerosis.[3] Possible associations between soy bean products, isoflavones, and diseases risk in long period should be further investigated.

It is certain that addition of soy flour to bread improves the protein content as well as biological value; however, this must be proven for government to fortify wheat flour with 3-7% DSF in order to increasing protein intake of people in developing countries and improving nutritional status all human at risk of malnutrition.

CONCLUSIONS

It was concluded that overall acceptability score significantly decreased with increasing DSF substitution level. Rats fed 7% DSF-fortified blend showed privileged food efficiency ratio. Then, the best formulation is between 3 and 7% DSF bread. This formulation can nourish all human at risk of malnutrition.

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