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Effect of Soaking and Fermentation of Wheat Bran on Weight Gain, Accumulative Food Intake and Food Efficiency Ratio in Rats

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Abstract

This study was conducted to investigate the effect of different processing treatments in terms of soaking and fermentation of wheat bran on weight gain, accumulative food intake and food efficiency ratio in Sprague-Dawley rats. The experimental diets included casein diet (zero-bran), untreated bran diet, soaked bran diet, fermented bran diet, "soaked and fermented" bran diet and Arabic bread diet. Each group of rats (6/group) was fed one of the six prepared diets for 6 weeks. There was no significant difference ($p > 0.05$) in the body weight gain among all rat groups, although the rats fed soaked diet tended to have the highest weight gain in comparison with other groups. There was no significant difference in FER among all groups, although, there was a difference between groups in the accumulative food intake. Accumulative food intake (AFI) of the rat group fed soaked bran based-diet was the highest (685.6 ± 17.3 gm) among all groups whereas AFI of the rat group fed "soaked and fermented" bran-based diet was the lowest (550.0 ± 19.1 gm). Rat group fed "soaked and fermented" bran-based diet had significantly lower levels ($p < 0.05$) of AFI than those fed untreated bran diet, soaked bran diet and white bread diet (550.0 ± 19.1, 663.4 ± 16.6, 685.6 ± 17.3 and 629.8 ± 28.4 gm respectively). Accordingly, the AFI of the rat group fed soaked bran diet was significantly higher ($p < 0.05$) than those fed casein and fermented diet (685.6 ± 17.3, 598.4 ± 9.2 and 605.8 ± 25.6 gm respectively). It is concluded that preparation of wheat bran foods by soaking or/and fermentation improve some physiological characteristics of insoluble fibers, including the body weight changes, accumulative food intake and food efficiency ratio for 6 weeks.

Keywords: wheat bran, dietary fiber, soaking, fermentation, weight gain, accumulative food intake, food efficiency ratio, sprague-dawley rat

1. Introduction

Carbohydrates, of both insoluble non-starch polysaccharides and soluble NSP, are important contributors to the health benefits of whole grains (Topping, 2007). Dietary fiber is defined as the endogenous components of plant materials in the diet, predominantly non-starch polysaccharides and lignin, which are resistant to digestion by human enzymes (Anderson et al., 1990).Wheat and its products are the major part of the diet for people in many regions of the world, it is second only to rice as the main human food crop (Stevenson et al., 2012; Levrat-Verny et al., 1999).

In Jordan, commercial bread tops the food items that provide dietary fiber with a 38.2% of the 30.9 gm total dietary fiber per capita per day (Takruri & Tukan, 1998). Wheat bran is considered a rich source of insoluble fibers, which constitute more than 95% of extractable fiber (Chen et al., 1998).

It is expected that many factors could alter the physico-chemical properties of dietary fiber (Lopez et al., 1998). Such factors may include soaking and fermentation (Hallberg et al., 1987; Kent & Evers, 1994). Fermentation of wheat bran was reported to change its physio-chemical properties, by degrading the cell wall material, particularly the aleurone layer rather than lignified outer layer (Stevens et al., 1988). It is locally practiced to add soaked and fermented wheat bran to bread in order to improve loaf characteristics.

To the best of our knowledge, no study was conducted about the biochemical implications related to weight gain, accumulative food intake and food efficiency ratio as a result of such practice. Therefore, the objective of this work was to investigate the effect of soaking and fermentation of wheat bran on weight gain, accumulative food intake and food efficiency ratio in Sprague-Dawley rats for 6 weeks.

2. Methods

2.1 Sample Preparation

Hard red winter wheat (Triticum aestivum) bran, obtained from the Modern Flour Mills and Macaroni Factories Co., was used in the study after treatment to get: untreated wheat bran, water-soaked bran, fermented bran and "soaked and fermented" bran. In the soaking process, hot tap water (55 °C) was added to the bran in a ratio of (1:1 w/v bran to water). Rapid mixing was done for 15 minutes and then the mix was left for 120 minutes to be soaked. The wet bran was spread as a thin layer over aluminum trays (1-2 cm thickness) and oven-dried at (82.2 °C) for about 4 hours. The dried bran was milled using a hammer mill. In the fermentation process, hot tap water (55 °C) was added to the bran in a ratio of (1:1 w/v bran to water), and then the yeast was directly added to the mixture (17 g of fresh baker's yeast paste/kg bran). Rapid mixing was done for 15 minutes and the mixture was left at 38 °C for 120 minutes to be fermented. The wet fermented bran was spread as a thin layer over an aluminum tray (1-2 cm thickness) and oven-dried at 82.2 °C for about 4 hours. The dried bran was milled using a hammer mill. In the "soaking and fermentation" process, bran was also prepared as a mixture of (1part bran: 1 part of hot tap water (55 °C), and mixed rapidly for 15 minutes, then left to be soaked for 120 minutes. After soaking 17 g of the fresh bakers-yeast paste/kg bran was added and mixed rapidly for 15 minutes and left to be fermented for 120 minutes. The mixture was then oven-dried at 82.2 °C for about 4 hours. The dried bran was milled using a hammer mill. Arabic bread (white bread) sample was prepared from wheat flour (Triticum aestivum) with an extraction rate between 78-82%, from which the wheat bran was extracted. The other ingredients used in white bread preparation were: sugar (27-30 g/Kg flour), salt (3-5 g/Kg flour), baker's yeast (10 g/Kg flour), and water in a ratio of 2 flour: 1 water. All ingredients were kneaded for 15 minutes in a kneading machine (Bavailler, France). The dough was then transferred to a stainless steel container and left for 20 minutes. This was followed by mechanical cutting into small pieces (200-220 g), which was fermented on a rotary fermenter for another 20 minutes. After flattening the dough into a thickness of (0.5-1.0 cm) and a diameter of (30-35 cm) it was left for another 20 minutes, and then was baked at 350 °C for 40 seconds. The bread after cooling was dried at 82.2 °C for about 4 hours and then milled using a hammer mill.

2.2 Experimental Diets

Experimental diet mixtures were planned to reach an isocaloric, isonitrogenous content. The casein and corn oil were adjusted according to the protein and fat, respectively, provided by wheat bran. The composition of the mineral and vitamin mixtures was added as given by Reeves (1997). The experimental diets included casein diet (zero-bran) as a control, untreated bran diet (16 gm bran), soaked bran diet (16 gm bran), fermented bran diet (16 gm bran), soaked and fermented bran diet and Arabic bread diet. All diets were kept at 4 °C until used for feeding. The ingredients of the diets used in the animal experiment are shown in Table 1.

Table 1. Composition of experimental diets fed to rats [1-4]

Ingredient (gm/100gm)	Type of diet					
	Casein	Untreated bran	Soaked bran	Fermented bran	Soaked & fermented bran	Arabic bread
Test food	-	16	16	16	16	70
Casein [1]	14	11.8	11.8	11.8	11.8	5.6
Starch	69.7	56.7	56.7	56.7	56.7	9.7
Corn oil	9	8.2	8.2	8.2	8.2	7.0
Fat-soluble vitamins[2]	1	1	1	1	1	1
Water-soluble vitamins[3]	2	2	2	2	2	2
Salt mixture[4]	4	4	4	4	4	4
DL-methionine	0.3	0.3	0.3	0.3	0.3	0.3
Total	**100**	**100**	**100**	**100**	**100**	**100**

Note. [1] Casein source: BDH Chemical Ltd. Poole/England; [2] Fat-soluble vitamins mixture: (4000IU vitamin A, 1000 IU vitamin D_2, 10 IU vitamin E) /1 g fat (Reeves, 1997); [3] Water-soluble vitamin mixture: 0.5 g thiamin, 0.4 g riboflavin, 0.4 g pyridoxine, 45.0 g ascorbic acid, 4.0 g pantothenic acid, 4.0 g niacin. 2.5 g chloine, 25 mg inositol, 10 mg paraminobenzoic acid. 0.002 g cyanocobalamine 0.02 g biotin and 0.2 g folic acid, made up to 1 kg with powdered sucrose (Reeves, 1997); [4] Salt mixture: 0.21 g $Al_2(SO_4)_3K_2SO_4{\cdot}24H_2O$, 350g $CaCO_3$, 250 g $KH_2PO_4{\cdot}3H_2O$, 0.26 g $CoCl_2{\cdot}6H_2O$, 0.5 g $CuSO_4{\cdot}5H_2O$, 9.42 g $Fe_2(SO_4)_3{\cdot}7H_2O$, 102.26 g $MgSO_4{\cdot}7H_2O$, 1.22 g $MnSO_4{\cdot}4H_2O$, 0.25 g KI, 135.48 g $K_2HPO_4{\cdot}3H_2O$, 127.58 g NaCl, 63.5 mg NaF, 81.5 mg H_3BO_3, 3.55 g $ZnSO_4{\cdot}7H_2O$, 17.0 mg LiCl, 8.9 mg VCl_3, 0.28 g $CrK(SO_4)_2{\cdot}12H_2O$, 6.01 mg SeO_2, 6.48 mg MoO_3 (Reeves, 1997).

2.3 Animal Experimentation

Thirty six young male adult Albino rats (Sprague-Dawley) were housed individually in plastic cages with wire bottom (North Kent Plastic Cages, England), in an animal room with a 12-hour light: dark cycle at a temperature of 22±1 °C. All animals had free access to tap water and special diets (given *ad libitum*). The animals were randomly divided into six groups of six animals each according to body weights. The difference in mean weight between any two groups did not exceed 1 gm. Each group of rats was fed one of the six prepared diets for 6 weeks.

2.4 Proximate Analysis

Moisture, ash, crude protein, crude fat and crude fiber contents, of bran and bran-based diets, where determined according to AOAC methods (1995). Nitrogen free extract, which represents soluble carbohydrates, was calculated in the sample by difference after subtraction of the crude protein, crude fat, crude fiber, moisture, and ash contents from 100%.

2.4.1 Weight Gain

Weight gain (final weight – initial weight) in rats at the end of the experimental period (6 weeks) is used as an indicator for growth and the performance of the different experimental treatments.

2.4.2 Food Efficiency Ratio (FER)

Food efficiency ratio (FER) was calculated from the weight gain and accumulative food intake data at the end of 6 weeks for the six groups as follows:

$$\text{FER} = \text{Gain in body weight (gm)/food intake (gm)} \times 100\% \quad (1)$$

2.5 Statistical Analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System) package. Analysis of variance (ANOVA) with Duncan's Multiple Range Test (Steel & Torrie, 1980), was used to find the differences among mean values of the following parameters: body weight changes, accumulative food intake and food efficiency ratio. Significance was accepted at ($p < 0.05$).

3. Results

The proximate analysis of the bran and other prepared foods, used to formulate the experimental diets, is presented in Table 2. The percentage composition of crude protein is around 14% for all bran foods while it was lower in Arabic bread (12.4%). Fat percentage ranged from 4.5 to 5.1 in the bran foods, while it was 2.8% for white bread. For crude fiber, untreated bran contained 9.8% (on air-dried basis) while it was lower in other processed bran (soaked 5.6%, fermented 7%, and "soaked and fermented" 6.1%). Bread had lower percentage of crude fiber (0.5%). The proximate analysis of experimental diets, fed to rats for 6 weeks, is presented in Table 3. The results show that all experimental diets tended to be isocaloric and contained similar amounts of protein, fat and carbohydrate (NFE).

Table 2. Proximate analysis of wheat bran and other ingredients used in preparing the experimental diets [1-3]

Composition gm/100gm food		Protein	Fat	N.F.E.	Ash	Crude fiber	Total
Untreated bran	12.1	14.1	4.8	54.9	4.3	9.8	100
Soaked bran	7.3	14.4	5.1	64.2	3.4	5.6	100
Fermented bran	7.7	14.7	4.7	62.0	3.9	7	100
Soaked and fermented bran	8.1	13.8	4.5	63.3	4.2	6.1	100
Arabic bread	11.0	12.4	2.8	71.8	1.5	0.5	100

Note. [1] Mean values of triplicates with C.V. < 5% on air-dried samples; [2] Protein was calculated as (Nx6.3) and (Nx5.7) for bran and white bread respectively (Pellett and Young, 1980); [3] NFE: Nitrogen-free extract was calculated by subtraction of moisture, ash, protein, fat and crude fiber from 100%.

Table 3. Proximate analysis of experimental diets [1-3]

Diet / Composition gm/100 gm food	Casein	Untreated	Soaked	Fermented	Soaked and fermented	Arabic bread
Moisture	5.2	6.0	3.7	3.3	2.8	2.8
Protein	14.2	13.8	13.9	14.3	14.0	15.2
Fat	10.6	9.4	10.7	10.4	9.6	9.9
N.F.E	65.9	66.5	66.6	66.8	68.0	67.0
Ash	4.1	4.0	4.2	4.2	4.6	5.1
Crude fiber	ND	1.4	0.9	1.0	1.0	ND
Energy	416	405	418	418	415	418

Note. [1] Mean values of triplicates with C.V. < 5% on air-dried samples; [2] N.F.E.: Nitrogen-free extract was calculated by subtraction of moisture, ash, protein, fat and crude fiber from 100%; [3] ND means not detected.

3.1 Body Weight Gain

The body weight gain for rats fed the seven experimental diets for 6 weeks is presented in Table 4. Mean ± SD values of weight gain for rats fed casein diet, untreated bran diet, soaked bran diet, fermented bran diet, soaked and fermented bran diet and Arabic bread diet were 97.4 ± 17.9, 91.0 ± 14.2, 118.1 ± 26.1, 100.2 ± 22.6, 99.0 ± 18.6 and 93.3 ± 10.0 gm, respectively. There was no significant difference ($p > 0.05$) in the body weight gain among all rat groups, although the rats fed soaked diet tended to have the highest weight gain in comparison with other groups.

Table 4. Initial and final body weights and weight gain of rats fed the experimental diets for 6 weeks [1-2]

Type of diet	Initial body weight (g)	Final body weight (g)	Weight gain (g)
Casein	$178.4^{a} \pm 20.65$	$275.8^{a} \pm 26.24$	$97.4^{a} \pm 17.87$
Untreated	$178.6^{a} \pm 7.68$	$269.6^{a} \pm 27.40$	$91.0^{a} \pm 14.15$
Soaked	$178.8^{a} \pm 11.98$	$296.9^{a} \pm 32.01$	$118.1^{a} \pm 26.08$
Fermented	$178.5^{a} \pm 18.66$	$278.7^{a} \pm 35.36$	$100.2^{a} \pm 22.62$
Soaked and fermented	$178.7^{a} \pm 9.82$	$277.7^{a} \pm 9.25$	$99.0^{a} \pm 18.62$
White bread	$178.4^{a} \pm 12.87$	$271.7^{a} \pm 19.25$	$93.3^{a} \pm 10.02$

Note. [1] Mean of 6 rats ± SD; [2] Means with similar letters in their superscript within the same column are not significantly different ($p > 0.05$).

3.2 Accumulative Food Intake and Food Efficiency Ratio

Table (5) shows the accumulative food intake (AFI) and food efficiency ratio (FER) of rats fed the six experimental diets for 6 weeks. Mean ± SD values of the food efficiency ratio of rats fed casein diet, untreated bran diet, soaked bran diet, fermented bran diet, soaked and fermented bran diet, and white bread diet were 16.2 ± 1.8, 13.5 ± 1.6, 17.2 ± 2.5, 16.2 ± 0.7, 18.1 ± 2.4 and 14.5 ± 2.4 gm, respectively. There was no significant difference in FER among all groups, although, there was a difference between groups in the accumulative food intake. Accumulative food intake of the rat group fed soaked bran based-diet was the highest among all groups whereas AFI of the rat group fed "soaked and fermented" bran-based diet was the lowest. Rat group fed "soaked and fermented" bran-based diet had significantly lower levels ($p < 0.05$) of AFI than those fed untreated bran diet, soaked bran diet and white bread diet. Accordingly, the AFI of the rat group fed soaked bran diet was significantly higher ($p < 0.05$) than those fed casein and fermented diet.

Table 5. Accumulative food intake and food efficiency ratio of rats fed the experimental diets for 6 weeks [1-3]

Type of diet	Accumulative food intake	Food efficiency ratio
Casein	$598.4^{bc} \pm 9.2$	$16.2^{a} \pm 1.8$
Untreated	$663.4^{ab} \pm 16.6$	$13.5^{a} \pm 1.6$
Soaked	$685.6^{a} \pm 17.3$	$17.2^{a} \pm 2.5$
Fermented	$605.8^{bc} \pm 25.6$	$16.2^{a} \pm 0.7$
Soaked and fermented	$550.0^{c} \pm 19.1$	$18.1^{a} \pm 2.4$
White bread	$629.8^{ab} \pm 28.4$	$14.5^{a} \pm 2.4$

Note. [1] Mean of 6 rats + SD; [2] Food efficiency ratio: grams of body weight gain per 100 gm of diet consumed × 100%; [3] Means with different letters in their superscript within the same column are significantly different ($p < 0.05$).

4. Discussion

This study aimed mainly at identifying the extent to which the processing of wheat bran could alter its effect on on weight gain, accumulative food intake and food efficiency ratio in Sprague-Dawley rats, in comparison with fiber-free diet (casein diet).

Results obtained from this study regarding the proximate analysis were generally in agreement with those reported in the literature. The protein content for wheat bran tended to be similar to that reported by Pomeranz (1988), Holland et al. (1990), and Takruri (2002); while it was slightly higher than what was reported by Kent and Evers (1994), and Williams (1997). The protein content of white wheat bread was similar to that reported by Pellet and Shadarevian (1970), and Takruri (2002). The fat content in bran was similar to that reported by Holland et al. (1990), Williams (1997), and Takruri (2002), while it was higher than values obtained by Pomeranz (1988), and Kent and Evers (1994). The fat content of white Arabic bread was similar to that reported by Pellett and Shadarevian (1970), and Takruri (2002). For crude fiber, untreated bran had similar content of that reported by Kent and Evers (1994), and Takruri (2002). However, processed brans (soaked, fermented and

"soaked and fermented") had lower percentages of crude fiber than that corresponding to untreated bran.

The variations in protein and fat content of different sources, were expected and could be attributed to the differences in wheat variety from which bran and bread flours were extracted or to the origin of wheat regarding geographical and climate conditions. As indicated by Takruri (2002), the variations also may be due to the presence of varying starch endosperm fractions in the bran which, accordingly, can also contribute to such slight differences in the composition.

Differences in crude fiber content among different prepared bran foods may be due to the effect of soaking, fermentation or "soaking and fermentation" processes on the chemical properties of the bran fibers. This is in agreement with Amaral-Collaco (1998) who found a reduction of both hemicellulosic and cellulosic fractions in tomato pomace when it was fermented with a mixed culture of *Trichoderma reesei* and *Chrysoporium pruinosum.*

Wang et al. (1993) found that insoluble dietary fiber was lower in wheat bran than in the raw sample suggesting that the decreased insoluble dietary fiber and increased soluble dietary fiber in extruded products could be the result of disruption of covalent and covalent bonds in the carbohydrate and protein moieties, leading to smaller and more soluble molecular fragments. This was consistent with the data obtained by Lena et al. (1997) who found increased soluble DF content of wheat bran by enzymatic treatment.

The proximate composition of experimental diets used in the feeding of experimental rats were made similar in order to exclude any confounding factors other than intended ones. The results show that all experimental diets are isocaloric, and provide practically equal amounts of protein, fat and other constituents, which is needed for ruling out of any metabolic changes resulting from energy content.

Body weight gain can be used as an indicator of nutrient utilization and bioavailability (Moeljopawiro *et al*, 1988). The results of the present study demonstrate no significant changes in body weight gain of rats fed the different treated and untreated bran diets compared to the casein and white wheat bread diets. These results are consistent with those reported by Nishina et al. (1991), Ahmad and Takruri (1991) and Anderson et al. (1994), who found that the weight gains, in rats, were similar or not significantly different for rats fed the basal diet or wheat bran diet. A strong relationship between dietary fiber and, weight gain has been reported in one of the recent study, where Sáyago-Ayerdi et al. (2014) found that weight gain in the *Agave tequilana* dietary fibre and *jamaica calyces* (ADF-JC) group, after consuming the test diets for 5 weeks, was significantly lower than in the other groups.

These results are also in agreement with the results obtained by Takruri (2002) who used similar bran treatments obtained from the same source; there was a lack of significancy among different test diets regarding weight gain data. However, there was a trend in present results for the increment in body weight gain for the processed or treated bran diets. This trend could be explained by the notice that untreated bran diet resulted in an obvious bulky feces in comparison with other test diets. It could be suggested that more processed wheat fiber decreases fecal output as compared with raw and unprocessed wheat bran fiber. This suggestion is consistent with that of Vuksan et al. (1999). These authors stated that processed wheat fiber produces a reduction in the amount of phytate, in addition to affecting fecal bulkiness as well as the transit time. Moreover, a study performed by Takruri and Hamad in 2005 to investigate the effect of different processing treatments in terms of soaking and fermentation of wheat bran on serum lipids and lipoproteins in Sprague-Dawley rats. The authors found that preparation of wheat bran foods by soaking or/and fermentation improved some physiological characteristics of insoluble fibers, including the cholesterol-lowering ability (particularly total cholesterol TC and low-density lipoprotein cholesterol LDL-C).

Accumulative food intake is an indicator for the palatability of the diet and the acceptability of animals for the diets. There was no significant difference in FER among all groups, although, there was a difference between groups in the accumulative food intake. These findings, regarding FER are in close agreement with the findings of Takruri (2002) who used similar bran treatments. Accumulative food intake values varied among the experimental diets although there was no significant difference in FER. This could be attributed to the adaptation and to a compensatory increase in food intake, which in turn works to maintain body weight gain to be constant, since, the FER depends on weight gain. Maybe if the experiment prolongs to more than 6 weeks, the results will give better picture about this effect. As shown in Table 5, soaked bran diet resulted in the highest and significant food intake among all test diets, including casein. This could be explained by the increased palatability of soaked bran as a result of soaking process in comparison with other treatments. Little information is known about the effect of soaking, fermentation and "soaking and fermentation" methods because of the scarcity of studies dealing with the effects of such methods on weight gain, accumulative food intake and food efficiency ratio in

Sprague-Dawley rats. In other studies, fermentation had resulted in a reduction of both hemicellulosic and cellulosic fraction, which may behave as a soluble fraction and as a result this could be effective in altering weight gain, accumulative food intake and food efficiency ratio (Amaral-Collaco, 1998). Other processes, like extrusion cooking have been reported to increase the soluble fiber content of wheat bran and other food by-products rich in insoluble dietary fiber.

5. Conclusion

The soaking, fermentation, and "soaking and fermentation" processes of wheat bran results in a favorable effect on accumulative food intake in Sprague-Dawley rats through affecting the physicochemical properties of wheat bran rendering insoluble fraction of fiber behave like a soluble one. Although there was no significant effect of soaking, fermentation and "soaking and fermentation" methods on weight gain and food efficiency ratio at this study, it seems possible that these treatments slightly affect rats performance. Therefore, further investigation is required to support our findings.

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