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EFFECT OF USING SEED COATS ON HYPOGLYCEMIC IN RATS

Sandak, R.N.; A.S. I. El- Shazely and E. R. Sheashea
Food Techno. Inst., Agricultural Research Center, Giza- Egypt.

ABSTRACT

Celluloses fiber substances were prepared from wheat and hull less barley bran. Sodium hydroxide solution was used and the precipitate was centrifuged and washed by water several time to remove the color in first stage followed by peroxidation in the second stage. The products were washed to pH 6.0 + 0.5 and dried using warm air in an electric oven.

The chemical and physical properties of the raw materials and celluloses fiber from all bran are characterized. The results showed that the highest cellulose and insoluble dietary fiber contents found in the celluloses fiber compared with native brans. Also, the water and oil holding capacity were found in the same trend in celluloses fiber. Moreover, the anti-nutrition factors were the lowest in both wheat and barley celluloses fiber.

Celluloses fiber from wheat and hull-less barley used as hypoglycemic agent in rats fed on balady bread contained on 5, 10, 15, 20% celluloses seed coats. The results showed that incorporated different levels of celluloses wheat and hull-less barley bran used in balady bread which significant decreased the glucose level in rats from 145.0 to 106.0 mg/100ml in cellulose wheat bran and 151.0 to 110.0 mg/100ml in cellulose barley bran, respectively. Moreover, lipid fractions, i.e. cholesterol, triglyceride and total lipids were significant decreased when the levels of celluloses wheat and hull-less barley bran increased.

From the above results, it could be recommended that the balady bread made from 15 and 20% celluloses wheat and barley bran are hypoglycemic agent and reduced lipid fractions in diabetic rats.

INTRODUCTION

Cereals bran are important ingredients providing dietary fiber. Wheat bran is one of the most common raw materials for increasing the level of insoluble dietary fiber which influences the digestibility and bioavailability of nutrients and phytochemicals. The outer layers of grain contain cellulose and lignin which influence both the taste and mouth feel of the bran (Ross *et al.,* 2004).

Moreover, the diet contain large amounts of dietary fiber tend to be bulky and have low energy density. Therefore, in individuals with a limited appetite, such as very young or very old persons, such diets will potentially satisfy appetite to readily and therefore make it difficult to achieve adequate in takes of energy and nutrients. However, in many health adults the consumption of fiber- rich foods is self limiting due to their bulking character. This characteristics applies to a lesser extent to foods enriched with fiber and much less to fiber supplements (Gry,2006).

Consumption of foods rich in whole grains and cereal fibers has in epidemiological studies shown to reduce the risk of chronic diseases such as diabetes, cardiovascular disease and certain cancers, as reviewed by Larsson *et al.* (2005).

Celluloses fibers are prepared from a variety of low coast agricultural such as hull and bran seed costs. It absorbs large amounts of water to give a gelatin, like product without giving calories and increasing fiber content. By using celluloses fiber, food are designed to give desirable taste and mouth feel properties similar to the usual full fat food. The gelatin like material is suitable for hamburger, meats, cheeses and some baked food. Large calorie reductions in the diet are possible by using celluloses fiber (Inglett and Carriere , 2001 and Carriere *et al.*, 2003) or Nu- Trim X (Hallfrisch *et al.*, 2003).

During recent years, epidemiological observation has led to belief that fibers may play an essential role in maintaining man's health. The ingestion of dietary fibers have been shown beneficial effects on coronary heart disease, obesity, diabetes, breast cancers and other bowel disorders (FDA, 2001).

The objective of this study was to determine the chemical and physical properties of insoluble celluloses product made from wheat, and hull-less barley bran. Moreover, the insoluble celluloses were applications as celluloses fiber on hyperglycemic rats.

MATERIALS AND METHODS

Materials :

Hull- less barley (Hordeum vulgare L.,)variety Giza 30 and wheat (Triticum astivum L.,)variety Sakha 69 were obtained from Field Crops Res. Inst.,Agric. Res.Center,Giza, Egypt.

Hull- less barley and wheat grains were milled in a laboratary Qndramate to a fine powder. The different whole meal were passed on different meshs to give bran .

Methods:

Preparation of celluloses fiber:

The cellulose fiber was prepared from wheat and hull less barley bran according to Inglett (1997). Firstly, the brans were soaked in sodium hydroxide solution at pH 9 for one hour at 100^{o} C. This treatment was carried out to fractionate the fibers into soluble and insoluble compounds which are highly colored. The mixture was centrifuged and washed to remove the colored materials.

The solids obtained from the final washed step of the first treatment were resuspended in water at about 15% solid contents and it were subjected to the second treatment at pH 8.In the second treatment , the solids were bleached using hydrogen peroxide (10% V/V) at room temperatures ranging from 50 to 70^{o}C for 100 min. The wet solids were centrifuged and the solids were washed to pH 6.0+ 0.5. the wet bleached celluloses fiber were dried using warm air in an electric oven to give dried powder insoluble celluloses fiber .

Chemical analysis:

The chemical analysis were determined in wheat and hull- less barley bran and their celluloses fiber. Protein, oil, ash and total hydrolyzable

cabohydrates were determined according to A.O.A.C.(2000). Total phenolic compounds and tannin as tannic acid were determined colorimetrically as descirbed by Valverde *et al.* (2002) and Burn (1971).

The fiber content, cellulose and hemicellulose were determined according to the methods by Chahal *et al.* (1979). Whereas, total dietary fibers, soluble and insoluble dietary fibers were determined according to Prosky *et al.* (1988).

Physical Properties :

The physical properties of native wheat and barley bran celluloses fiber were determined as water holiding capacity (WHC) and oil holiding capacity (OHC) according to Childs and Abajian (1979) and Racper (1979).

Preparation of balady bread using celluloses fiber:

Balady bread was prepared from celluloses wheat and barley bran at different levels 5,10,15,20%,respectively, and wheat flour 72% extraction. The loaves baked at 450-500 C for 2.0 min. and bread loaves wear allowed to cool at room temperature according to Faridi and Rbenthaler (1984). The loaves were packed in polypropylene bags and stored in refrigerator at 5.0 C to used in nutrition experiments .

Nutrition experiments :

Male albino rat (60 rats) weight ranging between 140-150 g. were obtained from Helwan Experimental Animal Station, Egypt. The basal diet, consisting of corn starch 70%, casein 10%, corn oil 10%, salt mixture 4%, vitamin mixture 1% and cellulose 5% according to A.O.A.C.(2000). Experimental rats were fed the basal diet for eight days, then randomly divided into two main group (6 rats) was fed the basal diet and was considered as the control group (control A). The other two main groups (54 rats) were injected with alloxan solution (150 mg/kg body weight) to induce hyperglycemia(Buko *et al.*, 1996). After forty eight hour, the main groups were subdivided into nine subgroups (6 rats each). The first diabetic subgroup (control B) was fed the balady bread free addition. The rats of second, third fourth and fifth subgroups were fed on balady bread containing 5,10,15 and 20 % celluloses wheat bran. Also, the rats of six, seventh, eighth and ninth subgroups were fed on balady bread mad from celluloses hull-less barley bran at levels 5, 10, 15 and 20%, respectively. Finely, the hyperglycemic rats were fed on basal diet contain of 20% from balady bread made from celluloses wheat and hull-less barley bran .The body weight and food consumption were recorded weekly for five weeks. At the end of experiment, the blood samples were withdrawn from the orbital plexus and centrifuged at 1500 r.p.m. to obtain the sera and kept in a deep freezer at -20 C until to their analyses.

Estimation of some serum constituents:

Serum constituents (glucose levels, triglycerides, total lipids and total cholesterol) were determined according to Titez (1986), Royer (1969), Knight *et al.* (1972) and Hewitt and Pardue (1973), respectively.

Statistical analyses :

All results from nutrition experiments were statistically analyzed using the method reported by Steel and Torri (1980).

RESULTS AND DISCUSSION

Chemical composition :

A number of by products especially that contained high fiber contents, was utilized for hypoglycemic in rats. Such replacement aims to reduce caloric intake by using cellulosic fiber prepared from wheat and hull-less barley brans. The chemical composition of wheat and hull-less barley bran and its powder celluloses fiber are shown in Table (1). The results represented in Table (1) show that the total protien and oil content gave higher amounts for wheat and barley bran (7.50 and 6.10%) than their celluloses fiber (1.54 and 1.12%). Also, ash and total fiber were the parallel trend. Moreover, the total phenol and tannins in celluloses fiber were reduced amounts from 35 to 40% .

The results reported in the same Table (1) show that the total and insoluble dietary fiber and cellulose found higher in wheat bran and its cellulosic fiber than barley bran and its celluloses. Whereas, insoluble dietary fiber and cellulose had contained the highest amount of celluloses wheat and barley bran (69.98-60.86%) and (54.27-47.17 %).

The major polysaccharide was cellulose which is a linear beta linked glucan of high molecular weight, ranging 500.000and 1 million daltons (Candido and Campos , 1996). On the other hand, the large insoluble fibers are usually risistance to fermentation in the colon and so increases fecal dry matter (Johnson and Southgate, 1994).

Table (1): Chemical analysis of native and celluloses fiber from wheat and barely bran on dry weight bases.

Chemical analysis	Wheat bran %	Barley bran %	Fiber %	
			Wheat	Barley
Protein	7.50	6.10	1.54	1.12
Oil	2.72	1.41	0.85	0.42
Ash	11.10	10.15	17.32	15.61
Total dietary fiber	55.15	47.34	73.15	70.27
T. H. C.	80.38	83.32	7.14	12.58
Tannin mg / 100 g	110.22	150.19	66.31	97.95
Total phenolic mg/100 g	143.14	170.19	90.74	105.72
I.D.F	46.08	32.27	69.98	60.86
S.D.F	6.07	15.07	3.17	6.41
Cellulose	35.72	23.12	54.27	47.17
Hemicelluloses	9.54	7.31	11.39	10.72

T.H. C. Total hydrolysable carbohydrates
I.D.F : Insoluble dietary fiber S.D.F : Soluble dietary fiber

Physical properties :

The physical properties , water and oil holding capacity for native wheat and barley brans and their cellulosic fiber were determined and the results are represented in Table(2).The results showed that the physical properties were increased in celluloses wheat and barley fiber. The water holding capacity found increased from 3.00 ml/g at 10^{o}C for 30 min to 4.90 mg/g at 66^{o} C for 150 min in barley fiber and also in wheat fiber had highly increased from 3.00 ml/g at 10^{o}C for 30 min to 5.10 ml/g at 66^{o}C for 150 min. The water and oil holding capacity had almost the same trend. These results

are in agreement with that published by Nyman and Svanberg (2002) showed that physiological effects of dietary fiber are greatly dependant on physico-chemical properties of the ingested material, e.g. the water- binding capacity, the molecular distribution and the viscosity.

Also, in the same table, it could be noticed that the celluloses wheat bran had the highest value of oil holding capacity 2.50 g/g at 66 C for 150 min. followed by celluloses hull-less barley bran contained 2.30 g/g at the same condition. The increases in these results may be due to the content of celluloses and insoluble dietary fibers in all samples.

Table (2): Physical properties of native brans and their cellulosic fiber

Materials	WHC. ml/g.			OHC. g/g		
	30 min10°c	90 min38°c	150 min66°c	30 min10°c	90 min38°c	150 min66°c
Barley bran	2.50	3.00	3.70	1.40	1.70	1.90
Barley fiber	3.00	3.50	4.90	1.80	2.00	2.30
Wheat bran	2.60	3.20	3.90	1.60	1.90	2.20
Wheat fiber	3.00	3.20	5.10	1.80	2.10	2.50

WHC Water holding capacity OHC Oil holding capacity

Biological evaluation :

The results concerning the body weight gain, food intake and food efficiency ratio at the end of experiment (35 day) are recorded in Table (3). The results revealed that the body weight gain of diabetic rats in control B and the feed different proportions of celluloses wheat and barley bran in balaldy bread were significantly less than control A. Food intake in diabetic rats (control B) and other treatments were parallel trend. Decrement in feed intake may be due to the presence of high amount contents of fiber in wheat and barley bran, which led to fill the rats stomach and reduce the food intake) (Morite *et al.,*1997) . This led decrease the feed efficiency ratio in diabetic rats which was significantly lower than the normal rats (control A).

The effect of administrating balady bread samples on blood glucose of hyperglycemic rats are illustrated in Table (4). The blood glucose level of healthy rats (control A) was 103.0 mg/ 100.ml after 35 day of being fed the basal died .Whereas, the blood glucose level was increased in diabetic rats (control B) 193.0 mg/100ml fed on balady bread made from wheat flour 72% extraction. Incorporation of 5.10.15 and 20% of celluloses wheat and barley bran used in balady bread which showed that significantly decrease the glucose level after 35 day from 145.0 to 106.0 mg/ l00ml in celluloses wheat bran and 151.0 mg/ l00ml in celluloses barley barn, respectively. This hypoglycemic effect due to the high insoluble fiber and cellulose in these diets which have been useful in regulating blood glucose and insulin response factors that factors that are responsible for the preventation and treatment of coronary heart diseases and diabetes (Drzikova *et al.*, 2005).

Table (3): Mean of body gain weight, food intake and feed efficiency ratio of diabetic rats fed on different diets for five weeks.

Diets	Initial weight (g)	Final weight (g)	Body gain weight (%)	Total food intake (g)	Feed efficiency ratio
Control A	149.15 ±5.38	181.1 ±4.67	42.09	455.5 ±9.88	9.24 ±0.64
Control B	142.08 ±5.67	162.86 ±5.35	20.78	420.05 ±7.07	4.95 ±0.15
Wheat bam celluloses 5	147.28 ±4.43	164.81 ±2.81	17.53	402.25 ±9.75	4.35 ±0.29
10	140.7 ±2.45	152.91 ±5.59	12.21	367.50 ±8.65	3.35 ±0.45
15	150.08 ±3.59	157.62 ±2.21	7.54	332.50 ±6.51	2.27 ±0.21
20	143.55 ±2.33	146.17 ±3.12	2.62	301.0 ±7.31	0.87 ±0.31
Barley bran celluloses 5	143.55 ±3.82	159.39 ±2.89	15.84	395.5 ±8.32	4.01 ±0.52
10	145.43 ±3.89	156.13 ±3.68	10.70	357.0 ±8.21	3.03 ±0.36
15	145.43 ±4.26	150.65 ±443	5.32	325.5 ±8.65	1.63 ±0.72
20	149.13 ±5.24	150.33 ±2.96	1.20	294.0 ±6.45	0.41 ±0.64

The effect of administrating of balady bread samples on serum cholesterol, triglycerides, total lipids contents of alloxan diabetic rats were determined and the results are given in Table (4). In the diabetic rats hypercholesterolemia was secondary to diabetes. Since bread samples made from wheat flour and its mixed with celluloses wheat and hull-less barley bran contain a great portion of insoluble dietary fiber and cellulose influencing hypoglycemia, the decrease of cholesterol level may be affected by improving diabetes, which have been provoked hypercholesterolemia in these rat. This is clear from the very high serum cholesterol level in control B, 193.0mg/dl. Versus occurred reductions in cholesterol level of diabetic rats to 133.59, 128.97, 112.80 and 105.57 mg/dl in rats fed on cellulose wheat bran and 140.46, 138.22, 121.27 and 109.25 mg/dl in rats fed on cellulose hull-less barley bran at 5, 10, 15 and 20%, respectively at the end of experimental.

Not only cholesterol, but also triglycerides and total lipids were significant reduced in the serum of rat groups fed of high fibers content. Lipid fractions i.e., cholesterol, triglycerides and total lipids were decreased significantly as the level of cellulose wheat and hull-less barley bran increased. These results are in agreement with Hallfrisch *et al.* (2003) .

From the above results, it could be recommended that the celluloses wheat and hull-less barley bran can be used at level 15 and 20% with wheat flour 72% extraction to hypoglycemic and reduced lipid pattern in diabetic rats.

Table (4): Means of blood glucose levels and total lipids pattern in hyperglycemic rats after five weeks.

Diets	Blood glucose levels (mg/100ml)	Total serum lipids (mg/dl)	Total serum Cholesterol (mg/dl)	Serum triglycerides (mg/dl)
Control A	103.0 ±5.44	297.96 ±15.84	81.30 ±5.52	81.57 ±3.10
Control B	193.0 ±2.73	581.55 ±12.57	193.29 ±12.71	242.33 ±7.86
Wheat barn celluloses 5	145.0 ±6.39	455.12 ±43.82	133.59 ±5.77	134.72 ±8.32
10	134.0 ±6.28	415.04 ±39.08	128.97 ±3.98	125.20 ±12.49
15	120.0 ±7.51	342.0 ±42.83	112.80 ±6.49	116.23 ±10.58
20	106.0 ±6.31	316.79 ±35.17	105.57 ±5.31	95.83 ±11.73
Barley bran celluloses 5	151.0 ±7.32	469.97 ±41.84	140.46 ±6.82	145.17 ±5.54
10	142.0 ±5.71	423.85 ±38.13	138.22 ±4.01	133.32 ±7.02
15	127.0 ±4.87	385.55 ±40.89	121.27 ±9.00	127.06 ±8.08
20	110.0 ±6.59	332.12 ±43.82	109.25 ±7.18	102.61 ±9.56

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تأثير أستخدام أغلفة الحبوب على خفض سكرالدم فى الفئران

رأفت نجيب سندق، عبد المنعم صالح إبراهيم الشاذلي و عماد الدين راغب شعيشع

معهد بحوث تكنولوجيا الأغذية – مركز البحوث الزراعية – جيزة

تم تحضير سليلوز الالياف من أغلفة القمح والشعير العاري وذلك باستخدام محلول الصودا الكاوية لمدة ساعة علي درجة حرارة ١٠٠° م ثم اجري للمترسب طرد مركزي والغسيل بالماء لعدة مرات وذلك لإزالة اللون في المرحلة الأولي تم استخدام الهيدروجين بيروكسيد وفي المرحلة الثانية ثم الغسيل بالماء حتي تصل درجة pH ٦ والتجفيف باستخدام هواء جاف في فرن كهربائي.

تم تقدير التركيب الكيمائي والفيزيقي في أغلفة القمح والشعير العاري وسيليلوز الألياف الناتج منهم أوضحت النتائج ارتفاع نسبة السليلوز والألياف الغير ذائية في سليلوز جيل الألياف مقارناً بالأغلفة وأيضاً وجد أن سعة امتصاص الماء والزيت ترتفع فيى الالياف عن الأغلفة .

سليلوز الالياف الناتج من أغلفة كل من القمح والشعير العاري استخدم كعامل خافض لسكر الدم فى الفئران المغذة على الخبز البلدى المصنع من،٥و ١٠و ١٥و ٢٠% من سليلوز ألياف أغلفة الحبوب .

النتائج أوضحت ان سليلوز ألياف القمح والشعير المستخدمة فى الخبز البلدي عملت على خفض جلوكوز سكر الدم فى الفئران من ١٤٥ الى ١٠٦ ملجرام /١٠٠ مل لسليلوز ألياف القمح ومن ١٥١ الى ١١٠ ملجرام / ١٠٠مل لسيليوز الياف الشعير العاري ونذيد على ذلك ان كلا من الكوليستيرول والترای جلسريد والليبدات الكلية انخفضت فى الفئران بزيادة نسبة سليلوز اغلفة الحبوب .

من النتائج السابقة يمكن ان نوصى باستخدام الخبز البلدى المصنع من ١٥ ،٢٠ % سليلوز الياف القمح والشعير كعامل خافض للسكر وليبدات الدم فى الفئران المصابة بارتفاع سكر الدم