IN VITRO AND IN VIVO BINDING CAPACITY OF WHEAT BRAN, CORN AND SOY HULLS FOR CA, FE AND ZN

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ABSTRACT

Wheat bran (WB), corn hull (CH) and soy hull (SH) were analyzed for its proximate constituents and mineral content and evaluated for its binding capacity for calcium (Ca), iron (Fe) and zinc (Zn). Protein, soluble and insoluble dietary fiber, and mineral contents varied significantly among the three dietary fiber sources. Wheat bran (WB) contained significantly more Ca and Zn than CH and SH sources. Overall, WB bound significantly more Ca, Fe and Zn (alone or in combination with other minerals) than other dietary fiber sources. Re –acid washing stripped most of the bound minerals from the dietary fiber sources as in vitro experiment. In vivo experiment was designed to study the absorption of minerals by wister rats fed on pan bread substituted with 10,15 and 20% WB, CH and SH for 4 weeks. Serum, liver, tibia and feces level of Fe and serum Zn were significantly decreased for rats fed pan bread +20% dietary fiber sources. On the other hand, serum, tissues and feces levels of calcium were still unchanged by any type of dietary fiber used in the present study. In conclusion WB, CH and SH did not have negative effect on mineral absorption at 15%dietary fiber sources in vivo.

Key words: Mineral binding, Wheat bran, Corn hull, Soy hull, Dietary fiber, Tissue minerals

INTRODUCTION

Interest in dietary fiber and its physiological effects is growing rapidly. The positive effects attributed to increase dietary fiber intakes include reduction of diverticulosis symptoms, increase in fecal bile acid excretion, and control of diabetic symptoms. The negative effect is the reduction of trace mineral absorption

to the body from the gastrointestinal tract. (Thompson & Weber 1979 and Torre et al 1992).

Dietary fiber includes cellulose, hemicellulose, pectines, gums, and mucilages. These are biosynthesized from hexose, pentose, and uronic acid and liginin which built from phenylpropane units such as cinamyl and couramyl alcohol. Proportions of these constituents

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vary generally among of dietary fiber sources and can markedly influence the absorption of minerals. Antinutrients associated with dietary fiber such as phytic acid, oxalic acid, and proteins, which could affect to certain extent mineral bioavailability. Minerals can also interact and influence their absorption. Dietary fiber limit mineral bioavailability by binding, diluting and trapping minerals within dietary fiber particles or shorting the transit time of nutrients through the intestine (Idouraine et al 1996; Debon & Tester 2001 and Luccia & Kunkel 2002)

Iron, calcium, and Zinc their relative scarcity in tissues, perform important function in health and resistance to disease. Although the dietary intake of these minerals is adequate, deficiencies are noticed in populations of low socioeconomic groups who consume cereals and millets as their staple foods. This may be due to lowered bioavailability of these minerals from their diets. Fiber and phytate are known to decrease bioavailability of minerals from cereal and millet diets (Lakshmi & Sumahi 1997; Levarate-Verny et al 1999 and Siener et al 2001)

The present work was, carried out to study, in vitro the binding capacity of wheat bran, corn and soy hulls for Ca, Fe and Zn alone and in different combination at pH 7. Also the biological evaluation of pan bread substituted with 10, 15 and 20% of wheat bran, corn hull and soy hull on calcium, iron and zinc absorption on serum and tissues.

MATERIAL AND METHODS

Materials

Wheat flour 72% extraction, wheat bran and corn hull were obtained from

South Cairo milles Company, soy hull was obtained from Agriculture Research Center Giza Egypt the hulls and bran were ground and all samples passed through a 60 mesh screen.

Preparation of pan bread

Pan bread was prepared according to the method described by **Kent-Jones** and **Amos** (1967).

Chemical composition

Protein, crude fiber, crude fat and ash were analyzed according to the methods out lined in AOAC (1998). Carbohydrate content were determined by difference.

Determination of phytic acid

Phytate were extracted and determined according to the method described by Camire and Clydesdale (1982) and modified by Mohamed et. al. (1986).

Determination of cellulose

Cellulose was determined according to the method described by Updegraff (1969).

Determination of lignin

Lignin was determined using the method described by Tanaka et al (1985).

Determination of hemicellulose

Hemicellulose was determined by subtracting the sum of the weigh of cellulose, lignin, protein, fat and ash from that of different component (Ali, 2002).

Determination of dietary fiber

Soluble (SDF), insoluble (ISDF), and total dietary fiber (TDF) were determined in fat-free dried samples as described in AACC (2000) method.

Mineral binding

Mineral binding was determined as described by Idouraine et al (1996). Defatted samples were acid washed with HCL solution (pH1, ratio 7:1w/v) by shaking the slurry overnight and filtered. The residue was washed several times with distilled deionized water until the filtrate being neutral (pH 7). Samples were dried overnight in vacuum oven at 50°C A known weight (6g) was shaken separately or in mixture for 3h with calcium, copper, iron, and zinc solutions alone (8ml of 1000ppm mineral solutions / g of sample). The slurry was centrifuged at 2500xg for 15 min. Supernatants were discarded while the residues were washed three times with deionized water (pH 7), transferred to weighing dishes.

Biological assay

The biological evaluation was conducted using 78 male albino rats (60-70gm) in the Environmental Toxicology Research Unit Faculty of Agric. Ain Shams Univ. After feeding on basal diet for two weeks as adaptation period, the rats were divided into 13 groups (n = 6). Animals fed different formulas as shown in Table (1) for 4weeks. Feces were collected every day, dried, ground and kept till analysis.

At the end of experiment period, rats were fasted and anesthetized. Hearts, livers, kidney and tibia were removed carefully and weighed.

Table 1. Groups of eight wisteria male rats.

Group	Treatment
Group	(pan bread made from)
1	Normal control
2	Normal control-Ca
3	Normal control -Fe
4	Normal control -Zn
5	Wheat flour 72% + 10% WB
6	Wheat flour 72% + 15% WB
7	Wheat flour 72% +20%WB
8	Wheat flour 72% + 10% CH
9	Wheat flour 72% + 15% CH
10	Wheat flour 72% + 20% CH
11	Wheat flour 72% +10% SH
12	Wheat flour 72% + 15% SH
13	Wheat flour 72% + 20% SH

WB.= Wheat bran

C.H.= Corn hull

S.H. = Soy hull.

Mineral analysis

A known weight of defatted, acid-washed, mineral bound, and re-acid washed materials were wet ashed as described by (Idouraine et al 1995). Meanwhile organs, tibia and feces were dry ashed according to the method described by Shah et al (1991). Ca, Zn and Fe were determined in the above mentioned material according to the methods out lined in A.O.A.C. (1998) using Atomic absorption Spectrophotometer(Perkin – Elmer Model 4100 ZL).

Statistical analysis

Statistical analysis was done according to the method describe by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Chemical composition of raw materials and pan bread substitute with wheat bran, corn and soy hulls.

Table (2) Showed that hemicellulose is the major component of wheat bran and corn hull being 57.09 and 52.34 %, respectively. Meanwhile soy hull recorded the lowest percentage of hemicellulose

(20.59). On the other hand soy hull had higher amount of cellulose 40.5%. These results are agreements with Doner and Hicks (1997) they reported that corn fiber contain 53.1, 15, and 13% hemicellulose, cellulose and lignin respectively, and nearly to Blaney et al (1996) they reported that wheat bran contained 8.8% and 3.4% cellulose and lignin respectively. The highest phytic acid contents was found in wheat bran (4.13%) while the lowest ones was observed in corn hull (2.64%). Sorour (1997) found that the high phytic acid and phytate phosphate found in whole grain might be due to kernel wheat 85% of it being associated with aleurone layer.

Table 2. Fiber constituents and phytic acid content of wheat bran, corn and soy hulls.

Sample	Cellulose %	Lignin %	Hemicelluose %	Phytic acid %
Wheat bran	10.90	9.62	57.09	4.13
Corn hull	22.33	12.32	52.34	2.64
Soy hull	40.50	20.18	20.59	3.93
L.S.D.	0.675	0.913	0.813	0.147

Table (3) summarized the chemical Compositions (protein, ash, crude fiber, fat and total carbohydrate), of raw materials and pan bread made from substituted wheat flour 72% with 10, 15 and 20%wheat bran, corn hull and soy hull. From these results it could be noticed that protein contents of soy hull and wheat bran were significantly higher than that of corn hull. Fat of raw materials ranged between 0.44 to 4.39%, while ash content ranged from 0.50 to 4.59%. Fiber con-

tents of soy hull had significantly higher than wheat bran and corn hull.

The chemical compositions of pan bread substituted with 10, 15, and 20% wheat bran, corn and soy hulls are presented in Table (3). The crude fiber, ash, fat, content were increased with increasing substituted level. While the total carbohydrates were decreased by increasing substituted levels of different dietary fiber sources. These results are in agreement with those of Latif (1997); Khalon &

Table 3. Chemical compositions of raw materials and pan bread substituted with different levels and sources of dietary fiber sources.

				F	Raw mater	rials		
Samples	Replace- ment level %	Mois- ture	Pro- tein*	Fat*	Ash*	Crude*	Total carbohy drate*	Calories Kcal/100g
WF		12.57	11.25	1.44	0.50	0.49	86.32	403.24
WB		11.36	13.99	3.94	4.46	9.63	67.98	363.34
C.H.		10.94	7.39	4.39	1.23	8.73	78.26	382.11
S.H.		10.38	10.27	3.75	4.59	32.55	48.84	270.19
L.S.D.		0.158	0.271	0.032	0.026	0.978	0.485	
		Pan bread						
Control	0.0	35.81	12.20	1.49	1.13	0.75	84.43	399.93
WB	10	37.21	12.42	1.78	1.69	1.68	82.43	395.42
WB	15	38.10	12.50	1.92	1.89	2.10	81.69	394.04
WB	20	39.54	12.59	2.06	2.09	2.58	80.68	391.62
C.H.	10	36.91	11.76	1.89	1.24	1.53	83.58	398.37
C.H.	15	37.47	11.51	2.04	1.36	1.97	83.32	397.68
С.Н.	20	38.64	11.10	2.29	1.45	2.39	83.17	397.69
S.H.	10	36.33	12.00	1.69	1.62	3.77	80.92	386.89
S.H.	15	37.15	11.89	1.84	1.83	5.24	79.20	380.92
S.H	20	38.21	1179	1.96	2.01	6.81	77.43	374.52
L.S.D.		0.578	0.192	0.034	0.019	0.028	0.542	

WB = Wheat bran

C.H. = Corn hull.

S. H.= Soy hull

= gm/100g On dry basis.

WF = Whea: flour

Chow (2000) and Madruga & Camara (2000).

Soluble, insoluble and total dietary fiber

Results of soluble (SDF), insoluble (ISDF) and total dietary fiber (TDF) of,

wheat bran, corn and soy hulls were presented in Table (4). Corn hull showed the highest ISDF (67.11%) among the fiber sources. Soy hull had higher SDF and ISDF (53.88 and 10.73%, respectively). The substituted wheat flour with different dietary fiber sources caused an increase

Table 4. Dietary fiber content (% dry weight basis) of different raw materials and pan bread.

To one di onto	Replacement		Fiber fraction	ons
Ingredients	level %	TDF %	SDF %	ISDF %
Wheat flour		3.21	0.36	2.85
Wheat bran		48.20	8.08	40.12
Soy hull		64.61	10.73	53.88
Corn hull		73.30	6.19	67.11
L.S.D.		2.175	0.257	0.897
		Pa	n bread	
Control		4.87	0.56	4.31
WB	10	8.23	1.12	7.11
WB	15	10.73	1.53	9.20
WB	20	12.94	1.98	10.96
C.H.	10	10.31	1.10	9.21
C.H.	15	13.89	1.41	12.48
C.H.	20	17.36	1.69	15.67
S.H.	10	9.46	1.48	7.98
S.H.	15	12.58	1.95	10.63
S.H.	20	15.61	2.31	13.30
L.S.D.		0.094	0.026	0.346

WB = Wheat bran.

C.H.= Corn hull.

S. H .= Soy hull..

SDF = Soluble dietary fiber

ISDF = Insoluble dietary fiber.

TDF = Total dietary fiber

in TDF, SDF, and ISDF of produced pan bread. The increasing of TDF, SDF, ISDF, were respect to substitution levels and type of fiber sources. These results confirmed with those of Mongeau and Brassard (1990). They reported that ISDF, SDF and TDF for wheat bran were 40.78, 7.39 and 48.17 %, respectively. Meanwhile it much lower than those of corn hull (Khalon and Chow, 2000).

- I- The binding capacity of wheat bran, corn hull and soy hull for Ca, Fe and Zn separately or in combination in vitro
- A- Mineral contents of raw and acidwashed dietary fiber

Calcium, iron and zinc of defatted and acid – washed wheat, corn and soy fiber are listed in Table (5). Defatted wheat bran had higher content in Ca (2472.16

(μ g/g) while soy fiber had higher Fe (357.18, μ g /g). Zn content ranged from 115.68 to 25.3 μ g/g of wheat bran and corn hull respectively. These results are paralleled with **Thompson and Weber** (1981) they reported that Fe for wheat bran, corn hull and soy hull was 160.8, 73.8 and 395.6 μ g/g, respectively.

Acid washed of the hulls were studied which showed that Ca appeared more efficiently removed with the acid solution (90-93 % reduction) than Fe and Zn (60-78% reduction). This might suggest that Ca have lower energy bonds These results are in agreement with that found by Idouraine et al (1996).

B- Binding capacity of different dietary fiber sources for Ca, Fe and Zn.

The binding capacity of wheat bran, corn and soy hulls for Ca, Fe and Zn separately or in combination are presented in Table (6). Fiber sources bound more calcium when it was alone than in combination with iron. Wheat bran bound more calcium (5186 ppm), suggesting that wheat bran and soy hull might have more specific sites for Ca than other fiber sources or it have the same number of sites but they have greater affinity for Ca than the binding sites on other fibers. These results are in agreement with Blaney et al (1996) and Claye et al (1998). They reported that rice bran, wheat bran, oat fiber, apple fiber and tomato fiber were bound Ca at ranging from 800 µg /g for rice bran to 10097μg/g for tomato fibers. They also suggested that the sequential of calcium bound differently to the various fiber sources as follows hemicellulose > lignocellulose > cellulose > lignin. These results differ from Luccia and Kunkel

(2002). They reported that oat bran, wheat bran, and lignin in the presence of protein significantly reduced ionic calcium, while pectin and cellulose had no effect on ionic calcium under the same condition.

It is worth to note that the binding capacity of fiber sources for Ca was affected by the presence of other mineral. Wheat bran and soy hull bound less Ca when it was combined with iron and Zn. This might be due to the higher protein content of wheat and soy hull compared to other fiber sources. Phytic acid, oxalic acid, and other components have been implicated in mineral binding. Siener et al (2001), suggested that dietary fiber makes no important contribution to calcium-binding capacities, except for soy and oat barns.

Data in Table (6) revealed that binding capacity of different fiber sources for Fe resulted in wheat bran bound significantly of Fe when it was alone or combined with other minerals than soy and corn hulls, suggest that wheat bran might have more specific binding sites for Fe than soy or corn hulls. Torre et al (1995) and Debon and Tester (2001), reported that the presence of ionised carboxyl (uronic / pyruvic acids) and sulphate groups, in polyanionic polysaccharides significantly binding with Fe and Zn in acidic condition presumably due to complexation (chelation), wheat bran and soy fiber bound less Fe when it was added separately compared with combination with Ca.

Also, binding capacity of fiber sources for Zn showed that binding of Zn alone or in combination with other minerals was high in all studied fiber sources. Although the binding capacity values for the three fiber sources were similar,

wheat bran and soy hull had significantly higher binding values than those of corn hull. The finding of **Idouraine** et al (1996); Rendleman & Grobe (1998) and Debon & Tester (2001), confirmed the present data.

Table 5. Minerals content of defatted and acid washed wheat bran, soy and corn hulls.

			cid washe	ed .		
Minerals	Wheat	Com hull	Cau bull	Wheat	Corn	Carr built
(μg/g)	bran	Corn hull	Soy hull	bran	hull	Soy hull
Ca	2472.16	60.4	2164.43	151.0	57.0	149.8
Zn	115.68	25.3	35.83	34.70	5.06	8.91
Fe	155.29	59.25	357.18	357.18	14.9	78.56

Table 6. Binding capacity of wheat bran, corn and soy hulls for Ca, Fe and Zn separately or in combination (in vitro).

_	Ca (μg/g)						
Combined	Whea	Wheat bran		· Corn hull		y hull.	
Minerals	Bound	Re-acid	Bound	Re-acid	Bound	Re-acid	
Ca alone	5186	508	2567	205	3936	314	
Ca + Fe	2150	107	1256	62	2050	102	
Ca +Zn	2397	167	1304	78	2102	126	
_	Fe (μg/g)						
Fe alone	3136	690	1960	431	2960	651	
Fe + Ca	3307	727	2256	496	3011	662	
Fe + Zn	1399	307	1055	189	1211	242	
	-		Zn	(μg/g)			
Zn alone	4156	731	2416	531	3017	663	
Zn + Ca	3969	665	2121	381	2155	387	
Zn + Fe	2201	396	1227	220	1744	331	

II. The biological evaluation of pan bread substituted with different levels of dietary fiber from different sources

Table (7) showed a significant decrease in the amount of serum iron in the case of wheat flour 72% substituted with 20% wheat, corn, and soy hull. The same trend was observed regarding tissues and feces iron. These data was nearly to Morris & Ellis (1989) and Levarate-Verny et al (1999), they found that no

adverse effects of wheat bran on iron metabolism. These results were similar to those cited by Hayashi et al (2001); Abdel-Kader et al (2002) and Hurrell et al (2002). They found that Fe absorption was not suppressed by phytate, however feeding of the corn husk and water melon skin promoted Fe absorption in rats fed phytate-free diets, and they suggested that plasma iron showed a significant decrease in rats fed whole meal and wheat flour 82% extraction + 20% wheat bran.

Table 7. Fe concentration in serum and tissues of the rats.

Groups	Serum	Liver*	Heart*	Kidney*	Tibia*	Feces
Groups	μg/dl					(µg/day)
G +	99.25	251.06	135.14	110.14	56.5	171.8
G –	52.01	137.3	71.2	68.16	10.33	
W.B. 10	96.70	248.90	133.9	108.41	56.01	173.3
W.B. 15	95.35	241.22	130.36	106.20	53.60	176.3
W.B. 20	86.88	219.80	111.80	85.44	43.30	203.0
C. H. 10	96.20	247.12	132.32	107.4	53.20	175.5
C.H. 15	94.77	240.01	131.84	101.20	49.60	177.3
C.H. 20	84.01	212.20	101.74	77.31	37.65	199.4
S.H. 10	98.01	250.1	136.6	111.1	54.30	174.9
S.H. 15	97.20	248.7	132.2	109.01	50.60	176.4
S.H.20	89.23	227.7	119.24	89.95	42.01	198.3
L.S.D.	4.621	8.123	5.746	5.147	3.99	5.95

WB = Wheat bran

S. H.= Soy hull

C.H.= Corn hull.

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^{* =} $(\mu g/g \text{ Wet. Wt.})$

Table (8) showed calcium concentration in serum, tissues, tibia and feces of the rats. Serum, tissues and feces calcium concentration was unchanged by any type of the dietary fiber used. These results are in agreement with Levarate-Verny et al (1999) and Abdel-Kader et al (2002).

Serum, liver, kidney, heart, tibia and feces Zn were observed in Table (9). The data show that 20% of wheat bran, soy hull and corn bran were significantly reduced serum Zn concentration, tibia Zn and feces but had no effect on Zn tissues. These results are in agreement with Jiang (1986) and Levarate-Verny et al (1999). They reported that 12% cellulose in the diet resulted in significant lower

plasma Zn concentration. They noticed that the effects of digestive enzymes (hemicellulase, pepsin,and trypsin) released over half of the bound minerals, suggesting that released minerals should be available for resorption in vivo

Fiber association with phytic acid bound a number of mineral in vitro, generally phytic acid is consumed with various complex carbohydrates and it is likely that their fermentation, lowering the colon pH, favors phytase activity. Moreover, microbial fermentation of carbohydrate can increase the solubility of divalent cations and enhance their absorption in coecume (Younes et al 1996).

Table 8. Calcium concentration in serum and tissues of the rats.

Treatment	Serum mg/dl	Liver*	Heart*	Kidney*	Tibia mg/g	Feces mg/day
G +	9.99	32.10	40.20	32 6 .15	140.1	12.62
G	4.70	15.01	17.30	68.4	9.80	
W.B. 10	9.93	31.92	39.90	326.18	139.0	12.87
W.B. 15	9.91	31.80	38.45	325.15	134.8	12.67
W.B. 20	9.93	30.24	38.32	311.40	130.3	12.91
C.H. 10	9.97	31.86	38.95	324.9	134.9	12.89
C.H.15	9.90	30.75	37.86	322.71	131.3	13.03
C.H. 20	9.88	30.01	37.71	304.85	128.4	13.11
S.H. 10	9.93	31.90	39.20	325.92	136.6	12.39
S.H. 15	9.91	31.27	38.44	323.90	132.5	12.51
S.H.20	9.89	30.12	37.49	309.82	129.3	12.38
L.S.D.	0.432	1.781	1.994	8.713	6.596	0.991

WB= Wheat bran. S. H.= Soy hull C.H.= Corn hull.

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^{* =} $(\mu g/g \text{ wet. wt.})$

Table 9. Zn concentration in serum and tissues of the rats.

Groups	Serum (µg/dl)	Liver*	Heart*	Kidney*	Tibia*	Feces (µg/day)
G+	130.23	81.33	18.5	90.3	202.66	171.5
G –	70.19	28.3	8.01	32.6	74.7	
W.B. 10	128.93	80.1	18.01	88.96	199.01	171.2
W.B. 15	124.81	79.26	17.27	87.01	195.8	175.8
W.B. 20	109.32	78.00	15.9	86.40	169.1	189.4
C. H. 10	125.22	79.55	17.65	88.5	198.40	169.1
C.H. 15	115.73	78.8	16.44	87.2	193.8	172.6
C.H. 20	104.20	76.8	14.20	66.01	171.20	192.3
S.H. 10	127.9	79.8	17.95	88.61	198.65	170.9
S.H. 15	119.24	78.2	16.8	86.2	194.20	174.8
S.H.20	106.1	77.15	15.4	84.01	166.8	198.2
L.S.D.	6.881	3.236	1.981	4.631	6.974	5.342

WB= Wheat bran.

C.H.= Corn hull.

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S. H.= Soy hull..

 $^{* = (\}mu g/g \text{ wet. wt.})$

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دراسات على قدره رده القمح و قشره الذرة و الصويا على الارتباط بالحديد و الزنك و الكالسيوم داخل وخارج الجسم

[\$ 7]

تم فى هذه الراسة تقدير المكونات الكيميائية و والمعادن لكل من رده القمح، قشره كل من الذرة، الصويا ودراسة تأثير إرتباطها بالكالسيوم، الحديد، الزنك.

وجد أن هناك اختلاف معنوي بين مصادر الثلاثة لألياف الغذائية المستخدمة في كلا من المحتوى البروتيني ، الألياف الغذائية غير الذائبة المحتوى من المعادن. كما وجد أن رده القمح تحتوى على أكبر نسبة من الزنك و الكالسيوم بالمقارنة بقشره كل من الذرة، الصوبا.

وعموما وجد أن رده القمح أكثر قدره على ربط كل من لحديد و الزنك والكالسيوم (منفردة أو مرتبطة) وذلك بالمقارنة بقشره الذرة، الصويا. بينما أوضحت النتائج أن أعاده الغسيل بالحامض تؤدى ألى إزالة معظم المعادن المرتبطة بلألياف الغذائية المستخدمة وذلك خارج الجسم.

كما درس تأثير هذه الألياف على المتصاص الحديد والزنك والكالسيوم في الفئران المغذاة على خبز القوالب حيث تم الستبدال دقيق القمح بمصادر الألياف الغذائية المستخدمة بنسب ١٠، ١٥، ١٠ ١٠ معنوي في نسبة الحديد في السيرم، الكبد معنوي في نسبة الحديد في السيرم، الكبد السيرم عند الفئران المغذاة على خبز القوالب + ٢٠ الألياف الغذائية المستخدمة ومن ناحية أخرى وجد أن الألياف الغذائية المستخدمة السيرم على نسبة الكالسيوم في السيرم، الأنسجة ، ، البراز.

وكنتيجة نهائية وجد أن رده القمح وقشره كل من الذرة و الصويا ليس لها تأثير سلبي على امتصاص الحديد و الزنك والكالسيوم حتى ١٥% من مصادر الألياف الغذائية المستخدمة في الدراسة داخل الجسم.

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