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EFFECT OF FORTIFICATION OF TORTILLA WITH PLANT PROTEIN AND SOME MINERALS ON GROWTH AND BIOLOGICAL EVALUATION THROUGHOUT TWO SUCCESSIVE GENERATIONS OF RATS

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By
N. M. Abd El-Motaleb

Food Technology Research Institute, Agricultural Research Center , Giza , Egypt

ABSTRACT

Tortillas fortified with protein (defatted soybean and peanut flour) and mineral salts (zinc ascorbate and ferrous fumarate) were chemically analyzed for there initial ingredients and also the final product of tortillas. The essential amino acids were in high content in all the fortified tortillas, corn tortilla fortified with plant protein (CTFP) or corn tortilla fortified with plant protein and some minerals (Zn, Fe) (CTFPM) compared with the unfortified ones.

The biological evaluation resulted in development of Protein efficiency ratio (PER), Net protein utilization (NPU), Digestibility coefficient (DC) and Biological value (BV) regarding plant protein either in the presence or absence of minerals in the two successive generation's periods. Number of pups per litter, weight, growth of lactating pups and survival rate of second generation were elevated for rats fed on fortified tortilla. The effect of fortification on blood hemoglobin of rats during all stages (in two generations) were determined. The present data clearly demonstrate that the fortification of tortilla with protein, zinc and iron is important to improve the nutritional value for celiac patients.

***Key words**: biological evaluation, fortification, peanut, rats, soybean, tortilla, two generations,*

1. INTRODUCTION

Maize (corn) is a staple food for millions of people around the world. It is especially important in America, Mexico and some countries of Africa and Asia where it is the main source of energy and protein for the population (Serna-Saldivar *et al.*, 1990). Tortilla and bakery products containing gluten free flours are especially produced for celiac disease population in Egypt (El Tawil *et al.*, 2001). In Mexico, tortilla per capita consumption in some groups is higher than 120 kg per year. In rural areas, maize provides ≈ 70% of the calories and 50% of the daily protein intake (Serna- Saldivar *et al.*, 1988).

Unfortunately, maize tortillas are not perfect food because they lack in both protein quality and the levels of iron, zinc and some vitamins (A and some of B). From the practical viewpoint, the consumption of tortilla alone might cause Kwashiorkor in children (especially celiac patients). This is due to the lack of two essential amino acids, lysine and tryptophan. Supplementation of maize tortilla with beans or other legumes or animal products would be the best alternative to alleviate protein malnutrition (Chryssanthi *et al.*, 2002).

In the developing countries, fortification of common staple foods would upgrade the nutritional status of the population. The nutritional quality of maize tortillas can be improved by protein fortification and utilizing good quality protein (Sproule *et al.*, 1988 and Serna -Saldivar *et al.*, 1990). Moreover, Juan *et al.* (2003) reported that the diet of tortilla fortified with 4% defatted and cooked soybean flour had a positive effect on nutritional properties through improving the growth and development of experimental animals.

Peanut press cake can be used for human food if it is processed from food-grade peanut under hygienic environments (Lusas 1979). Peanut flour has relatively high protein content, blend flavor and light tan color which allow it to be incorporated into a wide range of food (Prinyawiwatkul *et al.*, 1995).

The objective of this investigation was to study the effect of fortification of tortilla with defatted soybean, peanut and minerals (zinc and iron) on its nutritional value. The biological evaluation of the fortified tortilla on the two successive generations rats was also studied.

2. MATERIALS AND METHODS

2.1.Materials

Corn (maize) grains were obtained from Field Crops Res. Inst.,

Agric. Res. Center, Giza, Egypt. The grains were cleaned, then milled in a laboratory Mill Junior and sieved through 60 mesh sieve to give a fine powder corn flour. Wheat flour 72% extraction was obtained from International Co., For Processing Grain Products (Flour land Co. 6 th Oct., Giza, Egypt).

Commercial peanut seeds were purchased from local market in Giza. The seeds were crushed and pressed by hydraulic laboratory press to extract the oil. The residual materials as defatted peanut cake was dried in an air oven at 130 °C for 5 min. according to Cheewapramong *et al.,(*2002). The dried peanut cake was milled and sieved through 60 mesh sieve to give a fine powder peanut flour and was stored at 3°C until used. Defatted soybean was obtained from the Soy Processing Unit, Food Tech. Res. Inst., Agric Res. Center, Giza, Egypt.

2.2. Methods

2.2.1. Preparation of tortilla

Four types of tortilla were prepared according to the method described by Khan *et al.,* (1992) as modified by El-Tawil and Atia (1999)., and baked at 250° C for 5-7 min. in an electric oven as follows: The first and second types of tortilla were composed of 100% wheat flour (wheat tortilla "WT") and 100% corn flour (corn tortilla "CT"). The third tortilla was prepared from corn flour (94%) plus defatted soybean flour (3%) and defatted peanut flour (3%) (corn tortilla fortified with plant protein "CTFP"). The fourth tortilla was similar to the third tortilla plus ferrous fumarate salt (3 mg absolutely iron /100g) and zinc ascorbate (3 mg absolutely zinc/100g) (corn tortilla fortified with plant protein and minerals "CTFPM").

2.2.2. Chemical analysis

Protein, ash, crude fiber and ether extract contents of the raw materials and the four types of tortilla were determined using the methods outlined in the A.O.A.C (1990). Total carbohydrates were calculated by difference. Amino acids of tortillas were determined according to the procedure described by Olison *et al.* (1978) using amino acid analyzer model Beckman 7300. Tryptophan was determined colorimetrically according to Blauth *et al.* (1963).

Minerals (zinc and iron) were determined in the four types of tortilla according to the method outlined in A.O.A.C. (1998). The Perkin Elmer Model 4100 zl Atomic Absorption spectrophotometer was used for the determinations of the minerals.

2.2.3. Nutritional experiments

Albino weanling (76) rats ranging in weight between 50-55 g. (36 females and 40 males) were obtained from faculty of Veterinary Sci., Cairo University, Giza, Egypt.

2.2.3.1.The first generation of rats

2.2.3.1.1. Protein efficiency ratio (PER)

The basal diet consisting of corn starch (75%), casein (10 %), corn oil (5%), salt mixture (4%), vitamin mixture (1%), and cellulose (5%) was prepared according to A.O.A.C. (1990). Experimental rats were fed on the basal diet for one week and divided into two experiments, as shown in the following scheme (Fig1).

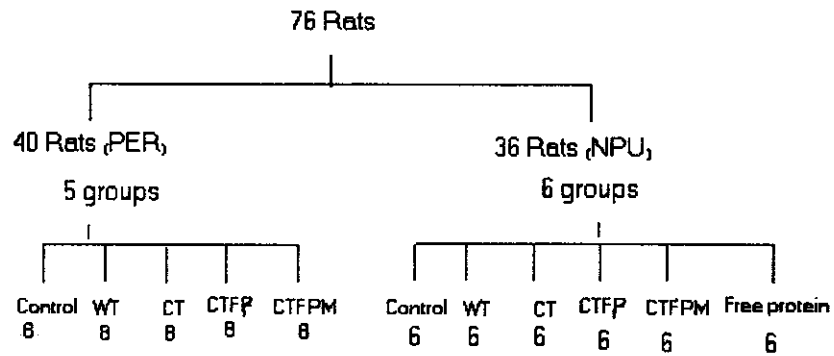

Fig (1): Experimental design of the PER and NPU

The first experiment contained five groups each consisting of 6 females and 2 males. The first group was fed on basal diet as control rats. The other groups were fed on WT , CT , CTFP and CTFBM tortilla respectively. After 28 days, body weight of each rat and feed consumption were recorded for determining protein efficiency ratio (PER) according to Campell (1961). Meanwhile blood samples were collected from each group using tubes containing heparin for determining hemoglobin (the first experiment stage).

2.2.3.1.2. Net protein utilization (NPR)

Another experiment of the first generation of rats was designed to evaluate Net protein utilization (NPU), digestibility coefficient (DC) as described by Miller and Bender (1955) and biological value (BV) was

calculated according to the equation of Metchell and Block (1946). Six groups of rats (each contained 6 rats) were prepared. Five groups had the same diets as described above and the sixth group fed on free protein diet. The experimental period was 10 days, after that the rats were sacrificed with chloroform and the carcasses were oven dried at 105° C, then ground and used for estimation of total nitrogen using the macro Kjedahl method. From the total feed consumption, the protein and nitrogen intake by each rat were calculated, and feces nitrogen was determined.

2.2.3.2. The second generation of rats

After the end of the first experiment stage (28 days), the remaining rats (6 females and 2 males) of each group were used for the second stage. These rats were fed individually on the tested diets for two months. Each 3 female rats were allowed to meet with one male for 5-7 days. After pregnancy, females were housed separately until the new weanlings (pups) were allowed to lactation. At the end of lactation, the weanling rats were fed on the tested diets (basal diet, WT, CT, CTFP and CTFPM) till each rat weighed 50-55 g. after that, five rats from each group were used to determined NPU, DC and BV such as in the first generation. The remaining rats from the five groups were fed another 28 days on the same tested diets to determine PER. Finally the rats were anesthetized and blood was collected to determine hemoglobin.

2.3. Determination of hemoglobin

Blood hemoglobin was determined by colorimetric method using. Kits supplied by Diamond Diagnostics, Egypt, according to Wintrobe (1965).

2.4. Statistical analysis

The data were statistically analyzed using analysis of variance and Duncan's multiple range tests according to the SAS program (1987).

3. RESULTS AND DISCUSSION

3.1. Chemical composition of raw materials and tortillas

The chemical analysis of the wheat flour 72%, corn flour, defatted soybean and peanut flour are shown in Table (1). The results show that the protein content varied between the different materials

used. The highest percentage of protein was recorded for defatted soybean flour (48.62%) followed by peanut flour (44.13%), meanwhile, the corn flour recorded the lowest percentage (9.35%). In addition, total carbohydrates were higher in wheat flour, corn flour and wheat tortilla than all other tortillas. It could be noticed that the chemical changes in tortilla were affected by source of flour or additives. Corn tortillas fortified with plant protein (CTFP) and (CTFPM) had the highest protein content (12.98% and 12.95% respectively). In this concern data show that, wheat tortilla (WT) had the lowest values of crude fiber, fat and ash. This may be due to fortification of corn tortilla with defatted soybean and peanut flours which increased protein, fat, ash and fiber.

Table (1): Chemical composition of raw materials and tortillas (% on dry wt. basis).

Samples	Protein	Crude fiber	Ether extract	Ash	Carbohydr--ates
Wheat flour 72%	11.82±0.12	0.79±0.03	1.02±0.03	0.56±0.06	85.81±0.24
Corn flour	9.35±0.33	2.24±0.11	3.88±0.20	1.51±0.08	83.02±0.72
Defatted soybean flour	48.62±0.45	2.82±0.30	1.47±0.21	4.60±0.31	42.49±1.27
~ peanut ~	44.13±0.06	2.50±0.07	1.32±0.16	4.08±0.03	47.97±0.32
Wheat tortilla(WT)	11.95±0.30	0.73±0.02	1.00±0.1	0.50±0.03	85.82±0.45
Corn tortilla (CT)	9.86±0.03	1.97±0.10	3.32±0.23	1.71±0.02	83.14±0.38
CTFP*	12.98±0.22	2.32±0.02	3.83±0.12	2.50±0.10	73.37±0.46
CTFPM**	12.95±0.13	2.34±0.08	3.92±0.78	2.45±0.15	78.34±1.14

* CTFP: Corn tortilla fortified with plant protein (3% soy bean + 3% peanut).
** CTFPM: Corn tortilla fortified with plant protein and some minerals.

3.2. Essential amino acids and some minerals of the different types of tortilla.

Table (2) shows the essential amino acids content of WT, CT, CTFP and CTFPM tortillas. The data revealed that the percentage of essential amino acids for CTFP recorded the highest values as compared with other tested tortillas. So it could be noticed that fortification of tortilla with plant protein could elevate the essential amino acids. These results are in agreement with those obtained by Cheewapramong *et al.*, (2002) who found high amounts of almost all essential amino acids especially lysine, in defatted peanut flour. From the same Table, it could be noticed that the minerals, iron and zinc contents were higher, in CTFPM than those in the tortilla produced from corn flour alone. On the other hand, CTFP had Fe, Zn higher contents than both wheat tortilla and corn tortilla.

tortilla and corn tortilla.

Table (2): Essential amino acids and some minerals of different tortillas.

Amino acid g/100g protein	Wheat tortilla	Corn tortilla	CTFP*	CTFPM **	FAO/WHO 1985
Arginine	4.25	3.15	5.12	5.13	
Histidine	2.01	2.46	2.65	2.59	
Isoleucine	3.37	2.78	3.88	3.83	4.00
Leucine	6.72	9.51	10.89	10.90	7.04
Lysine	2.53	2.37	3.46	3.41	5.44
Methionine + cystine	1.42	1.47	1.69	1.72	3.50
Phenylalanine + tyros.	4.65	3.75	4.48	4.39	6.10
Threonine	2.95	2.86	3.42	3.48	4.00
Tryptophan	1.40	0.71	0.85	0.89	0.96
Valine	4.28	3.67	3.97	3.90	4.96
Minerals(mg/100g)					
Iron	4.15	1.17	2.03	7.60	
Zinc	1.82	3.01	4.13	8.17	

* CTFP: Corn tortilla fortified with plant protein (3% soy been + 3% peanut).
** CTFPM: Corn tortilla fortified with plant protein and some minerals.

3.3. Biological evaluation of fortified tortilla proteins

Protein efficiency ratio (PER), net protein utilization (NPU), digestibility coefficient (DC) and biological value (BV) of first generation rats are summarized in Table (3). It could be noticed that the weight gain in case of first generation rats fed on CTFP and CTFPM diets were significantly higher (53.08 and 54.67 g) than rats fed on corn or wheat tortillas. Meanwhile, the weight gain of all treatments resulted in significant decrease compared with content.

It could be also noticed that all parameters including PER, NPU, DC and BV showed increases in the following order: rats fed on diets containing tortilla made from corn flour (CT) wheat flour (WT) CTFP < CTFPM<control fed on basal diet. This might be ascribed to the high available lysine content of peanuts compared to its level in the cereals (Johri *et al.*, 1988). The protein quality of corn meal is substantially improved by fortification with defatted soybean and peanut flour (Bookwalter *et al.*, 1998).

Table (3): Effect of fortified tortilla on weight gain and biological evaluation of protein.

Diets	Weight gain (g)	PER[(c)]	NPU[(d)] %	DC[(e)] %	BV[(f)] %
Control (basal diet)	60.35	2.35	78.13	91.91	85.00
Wheat tortilla*	32.14	1.69	65.52	87.07	75.16
Corn tortilla*	28.25	1.43	61.27	85.75	71.45
CTFP (a) *	53.08	2.15	71.08	88.45	80.36
CTFPM (b)*	54.67	2.28	71.64	88.53	80.92
L.S.D. (0.05)	5.321	0.202	2.236	1.253	3.024

(a): CTFP: Corn tortilla fortified with plant protein (3% soybean + 3% peanut).
(b): CTFPM: Corn tortilla fortified with plant protein and some minerals.
(c): Protein efficiency ratio. (d): Net protein utilization.
(e): Digestibility coefficient. (f): Biological value.
* Diets contained salt mixture which consists of an amount 3/4 of iron and zinc salts

3.4. Effect of feeding with fortified tortilla on rat growth

Number of pups, birth weight of pups, weaning weight, survival rate %, males % and females % are presented in Table (4). The results show that rats fed on diets containing CTFP or CTFPM gave high number of pups per litter (7 and 8 pups / litter) that is almost the same as with the rats fed with basal diet. On the opposite side rats fed with wheat or corn tortilla had the lowest number of pups. In this context the weight of pups as well as the weight of weaning pups fed with CTFP or CTFPM had high values (22.16 and 23.87) which were comparable with pups fed on WT or CT tortillas. This effect probably occurred due to protein and energy malnutrition and deficiency of vitamins (B12) and minerals (zn, fe) of mothers significantly retarded growth in second generation individuals (Kubena *et al.*, 1998). Rats fed on diets with CTFP, CTFPM and control diet had high values of survival (50.2%, 54.6% and 70.3%) while rats fed on diets including WT and CT tortilla diets had 42.0 and 38.0% survival ,respectively. The lower survival was associated with lower body weight of weaning born with mothers fed diets deficient in protein, vitamins and minerals. The lack of good quality protein, iron and folic acid have been associated with reproduction failure (Chryssanthi *et al.*, 2002). The high percentage of males were weaned by females fed CTFP and CTFPM (57.5 and 68.1%). These results are quite in agreement with those obtained by Chryssanthi *et al.*, (2002) who found that the high percentage of males

for mothers fed on tortilla based diet from protein fortified and enriched dry masa flour.

Table(4):Effect of fortified tortilla on number of pups/litter, weight of pups, weight of weaning pups and the survival rate.

Diets	Number of pups per litter	Birth weight of pups (g)	Weight of weaning pups (g)	Survival rat %	Males %	Females %
Control(Basal diet)	8	5.5	28.32	70.3	56.0	44.0
Wheat tortilla	6	4.9	17.13	42.0	53.4	46.1
Corn tortilla	4	4.5	14.95	38.0	50.0	50.0
CTFP*	7	5.2	22.16	50.2	57.5	42.5
CTFPM**	8	5.4	23.87	54.6	68.1	31.9

* CTFP: Corn tortilla fortified with plant protein (3% soybean + 3% peanut).
** CTFPM: Corn tortilla fortified with plant protein and some minerals.

3.5. Biological evaluation of protein in second generation rats

Effect of fortified tortilla on weight gain, protein efficiency ratio (PER), net protein utilization (NPU), Digestibility coefficient (DC) and Biological value (BV) of second generation post weaned rats, are illustrated in Table (5). It could be noticed that weight gain and PER of rats fed on corn tortilla were lower by about 50% than control rats fed on basal diet. This might be due to that corn tortilla had low protein quality. Meanwhile, feeding on CTFP and CTFPM tortillas showed lower weight gain of rats by about 40% compared to control. The values of PER for CTFP and CTFPM showed no significant difference compared with control (basal diet). This may be due to improving their nutritional quality by protein, iron and zinc fortification. The DC values showed a slight variation between the different diets, with the highest value noticed for the basal diet (89.34) and the lowest value for CT (80.72). The BV values showed a similar trend as that of NPU. These results agree with previous findings obtained by Serna Saldivar *et al.*, (1988) that the addition of soybean flour and sesame to corn tortilla gave better PER, NPU, BV and amino acid profile than regular tortilla and improved the protein quality and food utilization.

Table (5): Effect of fortified tortilla on weight gain and biological evaluation of protein in the second generation.

Diets	Weight gain (g)	PER	NPU %	DC %	BV %
Control(Basal diet)	58.16	2.00	74.18	89.34	83.03
Wheat tortilla	29.47	1.32	59.24	81.12	73.02
Corn tortilla	25.36	0.96	55.17	80.72	68.34
CTFP*	35.24	1.76	67.36	86.14	78.19
CTFPM **	37.12	1.70	66.84	86.76	77.04
L.S.D. (0.05)	4.021	0.305	3.131	3.514	4.125

*: CTFP: Corn tortilla fortified with plant protein (3% soybean + 3% peanut).
**: CTFPM: Corn tortilla fortified with plant protein and some minerals.

3.6. Effect of feeding rats on fortified tortillas on their blood hemoglobin

Table (6) shows the effect of feeding wheat or corn tortilla and tortilla fortified with plant protein (CTFP) or plant protein and minerals (CTFPM) on blood hemoglobin of rat during first and second generations adult rats and lactating females. For growing rats of the first generation, the blood hemoglobin was higher in case of rats fed diets containing high protein with minerals and rats fed on diets containing tortilla fortified with high protein than rats fed on tortilla made from wheat flour or corn flour without the addition and rats fed on basal diet (control).

Table (6): Effect of fortified tortilla on blood hemoglobin.

Diets	Level of hemoglobin			
	Growing rats during first generation	Adult rats	Lactating females	Growing rats during second generation
Control(Basal diet)	9.24±0.213	10.65±0.024	9.68±0.092	10.55±0.215
Wheat tortilla	8.92±0.272	9.77±1.600	8.72±0.172	9.65±0.234
Corn tortilla	8.04±0.118	9.07±0.124	8.20±0.120	9.01±0.152
CTFP*	10.48±0.271	12.35±0.018	10.15±0.075	12.16±0.166
CTFPM**	11.18±0.345	13.44±0.114	11.89±0.135	13.85±0.250

* CTFP: Corn tortilla fortified with plant protein (3% soybean + 3% peanut).
** CTFPM: Corn tortilla fortified with plant protein and some minerals.

In the same manner, adult rats, lactating females and growing rats during the second generation fed on diets containing tortilla fortified with high protein and minerals and fed on tortilla made using plant protein had higher values of blood hemoglobin than those fed on tortilla made from wheat flour or corn flour and basal diet (control).

The results obviously showed that the fortification of tortilla with protein and some minerals improved the growth of rats in two successive generations and biological evaluation of protein. The cost of fortification is economically recovered by the massive nutritional and health benefits that occur especially for children fed on corn tortilla for long time (children suffering from celiac disease).

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تأثير الطرطية المدعمة بالبروتين النباتي وبعض المعادن على النمو والتقييم البيولوجي لجيلين متعاقبين من الفئران

نادية محمود عبد المطلب

معهد بحوث تكنولوجيا الأغذية – مركز البحوث الزراعية – جيزة – مصر

ملخص

أجري تحليلا كيميائيا للطرطية المدعمة بمصادر نباتية عالية البروتين (دقيق فول الصويا ودقيق الفول السوداني المنزوع منهما الدهن) وكذلك بعض العناصر المعدنية مثل الزنك والحديد (إسكوربات الزنك – فيومارات الحديد). كما تم إجراء التحليل الكيميائي للمواد الداخلة في عمل الطرطية. وجد أن الأحماض الأمينية الأساسية مرتفعة القيمة في كل من الطرطية المدعمة بالبروتين(CTFP) وكذلك المدعمة بالبروتين مع العناصر المعدنية (CTFPM) بالمقارنة بطرطية الذرة(CT). أظهر التقييم البيولوجي وجود تحسن في NPU,PER ,DC ,BV فيما يخص البروتين النباتي وذلك في وجود أو عدم وجود العناصر المعدنية ، وذلك لجيلين متعاقبين من الفئران.

كما أظهرت الفئران التى غذيت على الطرطية المدعمة زيادة في كل من عدد المواليد لكل حامل – الوزن – النمو في الفئران الرضع – نسبة الفئران على قيد الحياة من الجيل الثاني مقارنة بالفئران التي غذيت على الطرطية غير المدعمة. بتقدير الهيموجلوبين في الدم زادت نسبته في كل مراحل النمو للفئران (في الجيلين) المتناولة للطرطية المدعمة(CTFPM) مقارنة بالطرطية غير المدعمة(CT). وهذا يبين مدى أهمية تدعيم طرطية الذرة في حالة التغذية عليها لفترات زمنية طويلة كما في حالة مرضى الحساسية لجلوتين القمح (السيلياك).

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (٥٧) العدد الثالث (يوليه ٢٠٠٦):٤٦٣-٤٧٦.