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CHEMICAL AND BIOLOGICAL EVALUATION OF SUPPLEMENTED MAIZE BREAD

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Abstract

Cooked maize (Giza-2 nixtamal) was grinded to prepare maize dough (masa) and baked. The produced maize bread was chemically and nutritionally evaluated.

Supplementation was carried out with 1-lysine 0.5%, methionine 0.3% and a mixture of the same two materials at same concentrations, together with a control. The mixture gave the best gain in body weight, PER and FER.

INTRODUCTION

Ranhotra and Loewe (1974) reported that, all essential amino acids except tryptophan showed some loss during baking process. Also, they indicated that, the loss of lysine occurred during the baking process was estimated by 10-15 percent of lysine content in both enriched and unriched bread.

El-Husseiney *et al.*, (1976) studied the fortification of whole wheat flour with three levels of lysine-HCl (0.25%), (0.50%) and (0.75%) on the growth rate and body composition of chickens. The results showed that, gain/feed ratio increased gradually when the added lysine level 0.50%, then drops sharply for diet fortified with 0.75%. Protein efficiency ratio (PER) increased noticeably by raising the lysine level in the diet to 0.50% then decreased after that for diet supplemented with 0.75% to a value lower than that for the unfortified sample.

Sugiyama *et al.*,(1997) indicated that, there was no significant difference in the growth of rats between the three diet groups (casein, soybean protein or soybean protein plus methionine) for 14 days. Relative liver weight was significantly greater in rats fed the casein diet than in rats fed the other two diets containing soybean protein. The plasma total cholestrol concentration was significantly lower in rats fed on the soybean protein diet than in rats fed on the casein diet. Methionine supplementation significantly increased the plasma total cholestrol concentration, but the value was still lower than that of casein group. The plasma triglyceride concentration was significantly higher in rats fed the casein diet than in rats fed on the other two diets.

Aim of study:

The aim of this study is to fortify maize bread with essential amino acids especially lysine and, methionine, and detecting the most efficient ratio of fortification through the biological assay.

MATERIALS AND METHODS

White maize kernels (Giza-2 variety) were obtained from Field Crops Research Institute; Agricultural Research Centre; Giza, Egypt.

The samples were treated by autoclaving as follows: Electrically Heated Sterilizer (Model ssr-3A-MC) was used at different treatments to obtain cooked maize by placing maize kernels samples of 600g/1800 alkaline water (1.0% Ca $(OH)_2$) in stainless steel Jar (previously adjusted at 1.0 kg/cm^2 (120°C) and 1.5 kg/cm^2 (125°C) for 30, 60, 90 and 120 min., respectively. The second cooking treatment of maize kernels was pre-soaked for 24hr. before autoclaving, then the pre-soaked samples were autoclaved with steam at 100°C without pressure. Then the cooked maize were washed with tap water to produce nixtamal. Then the nixtamal was grinded by stone mill to obtain the maize dough (masa) and then fortified with O.5% 1-lysine - HCl, 0.3%, L-Methionine and mixed both of them at the same level and the last one was considered as control.

Determination of Amino Acids:

Amino acids determination other than tryptophan was performed according to the method of Winder and Eggum (1966). The apparatus used for analysis was High Performance Amino Acid Analyzer, Beckman 7300.

Determination of tryptophan:

Tryptophan was determined colorimetrically in the alkali hydrolysate according to the method of Blauth *et al.*, (1963).

Experimental animals:

Rats were obtained from Helwan Animal Experimental Station, Ministry of Health, a total of (30) white weanling male albino rats, with body weight average (40-50 g), were kept under healthy laboratory conditions and fed casein diet for 7 days as adaptation period.

Diet preparation:

The composition of the tested diets used in this experiment were as recommended in A.O.A.C. (1990).

The first group (6 rats) were kept as control (negative control), while the second group rats were randomly divided into four subgroups (each of 6 rats), one of them was left as positive control and the other subgroups were fed on test diets. Five diets were formed to be equal in the test samples as recommended in A.O.A.C. (1990).

Determination of calcium:

Calcium content was performed according to the method described in A.O.A.C. (1990). The gain in body weight, PER, FER, S.GOT, S.GPT, uric acid and serum total protein were determined.

Preparation of blood samples for analysis:

The blood samples were subjected to plasma separation for determination biochemical analysis and enzyme assays.

The protein content of plasma was determined according to the Method of Cornall *et al.*, (1949).

The serum glutamate pyruvate and oxaloacetate transaminase (GPT-ALT) and (GOT-AST) were measured colorimetrically according to the method of Reitman and Frankel (1957).

The kidney function serum uric acid was determined according to the method of Caraway (1955).

RESULTS AND DISCUSSION

Table 1 shows the amino acids composition of bread produced after treating maize with alkaline and pressure compared to the original raw material. The mentioned data revealed that the essential amino acids in raw maize, arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine threonine, valine and tryptophan were found in amounts of 3.74%, 3.13%, 3.13%, 10.44%, 2.52%, 2.43%, 3.82%, 2.61%, 3.83% and 0.63% respectively. While these amino acids were found in the maize bread (produced from maize treated by 1.0kg/cm2 at 90min.) in amounts of 2.97%, 2.38%, 2.72%, 8.5%, 1.95%, 1.87%, 3.23%, 2.21%, 3.14% and 0.54%, respectively. Generally all the essential amino acids decreased after the alkaline treatment. These results agree with Sanderson *et al.*, (1978) who studied the effect of alkaline processing of corn on its amino acids, and mentioned that, a new profile of amino acids could be generated as concerning the effect of alkaline processing. He illustrated that lanthionine and ornithine amino acids derived from alkaline breakdown of cysteine and arginine. The most important limiting amino acids in maize are lysine, tryptophan and methionine. Therefore maize bread was supplemented by lysine and methionine to compensate the natural deficiency of these amino acids. The disulfide bonds of cystine link the matrex protein, it was found that the digestion of alkaline treated corn proteins by enzymes proceeds more rapidly than with normal corn meal which may be due to better accessibility to the corn proteins caused by starch gelatinization and changes in the protein matrix.

Tryptophan decreased after alkaline cooking. This may be due to the transformation of some of the tryptophan to niacin. (FAO. 1997).

Finally it could be mentioned that all amino acids content of nixtamal corn flour decreased when it was baked to maize bread with one exception of cystine which is increased from 1.39 to 1.7%.

Calcium content (mg/100gm) of maize bread as affected by autoclaving:

From table 2 it could be noticed that calcium content had been increased from five to eight folds as compared with the untreated original corn sample. The increase in calcium content was accelerated as the time of heat treatment is increased. When maize was cooked at 1.0 kg/cm^2 for 30 min., calcium content was found in amount of 100 mg/100g. While when cooking was increased up to 120 min. calcium content became 150mg/100g. The untreated original corn sample showed calcium content of 20.1 mg/100g. The other two treatments behaved in the same trend as the first treatment but the levels of increased calcium content were higher. From table 2 it is clear that, when pressure is increased from 1kg/cm^2 to 1.5kg/cm^2, calcium content increased from 100mg/100g to 140 mg/100g, respectively, when time of heat exposure was 30 min. The increase in calcium content due to increasing of heat exposure, may be related to the increased penetration of $Ca(O\text{-}H)_2$ present in the media during cooking process of maize kernels. The same increase in calcium content occurred when cooking pressure was increased.

These results agree with Serna-Saldivar *et al.*, (1988), who mentioned that, calcium content increased from 0.02% in whole grain of maize to 0.15% in nixtamal of maize bread.

When tortilla bread was compared with the local maize bread produced in Egyptian country-side, known as "Merahrah", which ground fenugreek grains were added in. It was found that calcium content was 92.11 mg/100g in the latter, while in the tortilla it ranged from 100 to 175 mg/100g.

The local maize bread was produced by adding 1% soltani yeast, then it was fermented for $^1/_2$ hr. before baking.

Protein efficiency ratio (PER):

Data presented in table 3 revealed that, the best improvement trial for PER value was observed in the group of rats fed on fortified corn with 1-lysine and 1-methionine (2.78) which has 94.56% of relative value comparing with PER value of casein group (2.94). Other fortified groups produced PER value lower than the above group while the lowest PER value was recorded for maize group (1.97). The improvement in fortified maize with 1-lysine and 1-methionine may be attributed to that addition of the previous amino acids, which enhance body weight and increase the protein efficient ratio of maize. These results agree with those obtained by Guircius (1978), who illustrated that,the fortification with lysine will be improve mainly protein quality.

Food efficiency ratio (FER):

Table 3 indicates food efficiency ratio as the second nutritional parameter of maize fortified with lysine, methionine and mixture of lysine and methionine. From table 3 it could be observed that the best relative value for FER was recorded in the group of rats received fortified maize with mixture of lysine and methionine (94.88%) compared with FER value of casein group, while the other relative FER values were 80% and 75% for fortified maize with lysine and methionine, respectively. Generally the results of FER took the same trend of the results of PER for all tested groups under investigation. The improvement in FER may be due to the addition of lysine and methionine to the maize, which increased growth of tested rat groups. These findings agree with Bressani and Elias (1969).

Liver is an organ, which is actively concerned with food ingredient metabolism. Animal organs like brain, liver and kidney have generally constant value of enzyme activity, these activities can be changed under the influence of the diet fractions. Table 4 shows the ratio of liver wt./body wt., of different groups of rats fed on maize fortified with lysine, methionine and mixture of lysine and methionine. It could be observed that all groups of rats have liver wt./body wt. ratio compared with the same results of control group. These results mean that there were no harmful effect could be induced in the liver of animals fed on maize fortified with different amino acids under investigation. These data agree with those obtained by Shaker *et al.*, (1989).

For biochemical evaluation serum protein, albumin, globulin, serum transaminase enzymes GOT, GPT (AST, ALT) and serum uric acid were evaluated.

Plasma protein and albumin are synthesized in the liver by extracting extrahepatic tissues. The synthesis of protein and albumin were affected by food consumption. The major effect of low serum albumin concentration (hypo-albuminemia) which occurs frequently in the liver and kidney disease, is soft tissue edema due to the administered inter vascular colloid Osmotic pressure (David and Martin 1985).

Data presented in table 5 indicate the different values of serum protein albumin and globulin of different groups of rats fed on maize bread fortified with lysine, methionine and mixture of them. The table cleared that maize bread with fortification produced~the lowest serum protein value (5.6 mg/100ml) while maize bread fortified with mixture of lysine and methionine, produced the nearest serum protein value (6.98 mg/100ml) to the same value of control group (7.04 mg/100ml). The table also indicates that the increase in fortified maize groups are highly significant. On the other hand, table 5 recorded that maize fortified with lysine showed the highest albumin value (4.41 mg/100ml), while the lowest value of serum albumin was for maize bread without fortification (3.56 mg/100g). These results regarding to albumin increasing may be attributed to the addition of lysine to maize bread. Data in Table 5 concerning serum globulin indicate that the highest serum globulin value was seen in the group of rats received maize bread fortified with the mixture of lysine and methionine (2.95 mg/100ml), followed by the value of control group (2.90mg/100ml), while the lowest globulin value was noticed in the group received unfortified maize bread (2.03mg/100ml). The increase in serum globulin values may be attributed to the addition of methionine and lysine. These findings agree with those obtained by Gabr (1993).

Transamination is an important reaction of protein metabolism, and has a wide distribution and is normally presented in blood serum. Aspartate amino transferase (AST) is aboundant in the liver, the heart and skeleton muscles. Alanine amino transferase (ALT) is more restricted in its distribution, being found almost exclusively in the soluble fraction of the cell and in high concentration only in the hepatyte. Table 6 shows the results of AST & ALT values of different groups of rats received maize fortified with lysine, methionine and mixture of both amino acids. It could be observed from the same table that there was no significant difference in serum AST value between test group and control group; i.e. adding amino acids (lys. & meth.) in maize fortification processing has no harmful effect.

Also, Concerning serum ALT values, in table 6, it is clear that serum ALT values of all tested groups occupied the normal range and there was no significant difference could be observed between serum ALT values of tested groups and control group. These results agree with those obtained by Gabr (1993).

Determination of serum uric acid concentration is useful in the diagnosis of gout in the assessment of renal function. The increase of serum uric acid levels is observed in all forms of nephritis with nitrogen retention. Uric acid is formed from purine basis and the later is produced from amino acids synthesized in the liver. Table 7 indicates the different values of serum uric acid of all groups of rats received maize bread fortified with lysine and methionine and mixture of them. It could be noticed that there were no significant differences between uric acid values of tested groups and the control group, in spite of that all uric acid values in table 7 were in the normal range.

Table 1. Amino acids composition of raw white maize (Giza-2) and maize bread produced from lime triated maize.

Amino acids Composition	Raw white maize		maize bread	
	%	% of protein (8.7)	%	% of protein (8.5)
Essential amino acids:				
Arginine	0.43	3.74	0.35	2.97
Histidine	0.36	3.13	0.28	2.38
Isoleucine	0.36	3.13	0.32	2.72
Leucine	1.2	10.44	1.00	8.5
Lysine	0.29	2.52	0.23	1.95
Methionine	0.28	2.43	0.22	1.87
Phenylalaunine	0.44	3.82	0.38	2.23
Threionine	0.3	2.61	0.26	2.21
Valine	0.44	3.83	0.37	3.14
Tryptophan	0.072	0.63	0.064	0.54
Non-essential Amino acids:				
Alanine	0.67	5.83	0.58	4.93
Aspartic acid	0.62	5.39	0.59	5.01
Glutamic acid	1.98	17.22	1.7	14.45
Glycine	0.35	3.04	0.32	2.72
Proline	-	-	-	-
Serine	0.37	3.22	0.26	2.21
Tyrosine	0.34	2.96	0.28	2.38
Cystine	0.16	1.39	0.2	1.7

Table 2. Calcium content (mg/100g) maize bread produced from white maize (Giza-2) by different cooking treatments.

Treatments	Time of treatments (mins)	Calcium content (mg %)
Control	-	20.1
A *	30	100
	60	120
	90	136
	120	150
B * *	30	140
	60	150
	90	160
	120	172
C * * *	30	120
	60	140
	90	160
	120	175

* A: means 1.0 kg/cm^2.
** B: " 1.5 kg/cm^2.
*** C: presoaking for 24 hrs. before autoclaving without pressure.

Table 3. The average value[a] of growth, food and protein effeciency ratio (FER & PER) of rats fed on different diets.

Group No.	Diets	Gain in body wt. G	Food intake (gm)	FER Value	PER Value
1.	Casein diet (negative control)	64.66 $\pm 1.03^{b}$	258.8 $\pm 6.17^{b}$	0.2498 $\pm 9.09^{b}$	2.94 $\pm 0.07^{b}$
	Relative value	100%	100%	100%	100%
2.	Maize bread diet	46.50 $\pm 6.37^{b}$	276.6 $\pm 12.29^{b}$	0.168 $\pm 0.020^{b}$	1.97 $\pm 0.171^{b}$
	Relative value of casein	71.92%	106.88%	67%	67%
3.	Maize bread + lysine	52.66 $\pm 0.186^{b}$	263.3 $\pm 5.16^{b}$	0.2 $\pm 5.24^{b}$	2.35 $\pm 0.044^{b}$
	Relative value of casein	81.44%	101.74	80%	79.93%
4.	normal diet maize bread + methionine	50.5 $\pm 2.16^{b}$	266.6 $\pm 7.52^{b}$	0.189 $\pm 6.51^{b}$	2.22 $\pm 0.055^{b}$
	Relative value of casein	87.10%	103.01%	75.86%	75.5%
5.	maize bread + lysine + methionine	62 ± 2.52^{b}	262 ± 7.16^{b}	0.237 ± 6.17^{b}	2.78 ± 0.052^{b}
	Relative value of casein	95.89%	101.28%	94.88%	94.56%

a: Each value is the average of six animal per group.
b: Mean ± standard diviation (S.D.).

Table 4. The ratio of liver weight/body weight of rats fed on maize bread fortified with lysine and methionine.

Group number	Diets	Liver weight (gm)	Body weight	Liver weight/ Body weight ratio
1.	Basa diet negative control	3.80	108	3.51±0.32*
2.	Normal diet positive control	3.70	104	3.25±0.525*
3.	Normal diet maize bread + lysine	3.80	107	3.55±0.518*
4.	Normal diet maize bread + methionine	3.77	105	3.59±0.265*
5.	Normal diet maize bread + lysine + methionine	3.70	104	3.55±0.566*

* S.D. (Standard Diviation).

Table 5. Serum protein albumin, globulin and albumin/globulin ratio of rats fed on different diets.

Sample description	Protein mg/100ml	Albumin mg/100ml	Globulin mg/100ml	Alb/Glb ratio
Basal diet (casein) (Negative control)	7.04±0.29*	4.13±0.25*	2.9±0.27*	1.42
Maize bread (Positive control)	5.60±0.27*	3.56±0.28*	2.03±0.24*	1.75
Maize bread + Ly.*	6.79±0.36*	4.41±0.43*	2.37±0.35*	1.86
Maize bread+Met.**	6.65±0.21*	3.96±0.29*	2.68±0.22*	1.47
Maize bread + Ly. + Met.*	6.98±0.15*	4.02±0.17*	2.95±0.18*	1.36

* S.D. (Standard Diviation)
* Lysine
** Methionine

Table 6. Serum transminases enzymes (S.AST & S.ALT) of rats fed on different diets

Sample description	AST**(GOT)	ALT**(GPT)	AST/ALT**
1. Basal diet (casein)	34.66±2.16*	44.66±0.82*	0.776
Negative control	31.50±1.64*	43.30±2.80*	0.727
2. Maize bread Positive control	34.16±1.72*	43.80±1.10*	0.779
3. Maize bread + Ly.	34.83±2.85*	43.80±1.10*	0.779
4. Maize bread+Met	34.83±2.85*	43.80±1.60*	0.795
5. Maize bread + Ly + MET	33.60±2.73*	44.10±0.75*	0.761

* Mean ± standard diviation (S.D.).
** No significant changes were observed.

Table 7. Serum Uric of rats fed on different diets (mg %)

Serum* Uric acid	Sample description				
	Basal diet Negative Control	Maize bread positive control	Maize bread+ Ly**	Maize bread+ Met	Maize bread +Ly+Met**
Mean	5.50	5.43	5.52	5.52	5.48
S.D	±0.82	±0.40	±0.28	±0.67	±0.79

* No significant changes were observed between values.
** Ly = Lysine, Met = Methionine.

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التقييم الكيماوى والبيولوجى لتدعيم خبز الذرة

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تم تدعيم العجينة الناتجة من طبخ وفرم حبوب الذرة باستخدام الحامض الأمينى اللايسين والحامض الأمينى المثيونين بتركيز ٠,٥ ٪ ، ٠,٣ ٪ لكل منهما على التوالى وكذلك خليط منهما بنفس التركيز حيث تم انتاج الخبز مقارنة بالكنترول غير المدعم والكنترول باستخدام الكازين حيث تمت تغذية فئران التجارب لمدة ٢٨ يوم وكذلك تم تقدير الكالسيوم والأحماض الأمينية والزيادة فى الوزن وكفاءة الغذاء ونشاط انزيمات الكبد ووزن الكبد إلى وزن الجسم وحامض البوليك فى الدم والبروتينات الكلية.

ووجد أن نسبة الكالسيوم تزيد بأكثر من ٥–٨ مرات فى الخبز الناتج باضافة هيدوكسيد الكالسيوم مقارنة بخبز الذرة العادى (المرحرح)، ووجد أيضاً أن التغذية على الخبز المدعم بحامض اللايسين والمثيونين معاً بتركيز ٠,٥٪ و٠,٣٪ على التوالى قد أعطى أحسن النتائج مقارنة بأى منهما منفرداً من حيث كفاءة البروتين أو الزيادة فى الوزن والبروتينات الكلية. لكن لم توجد فروق معنوية من حيث انزيمات الكبد أو حمض البوليك.