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Chemical, biological and sensory evaluation of biscuit enriched with, iraqi dates, sweet lupine and defatted soybean flour.

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ABSTRACT

The objective of this study aimed to increase the nutritional and biological value of low cost biscuits to fight malnutrition among infants and young children. To produce biscuits, two of legumes flour (defatted soybean flour, whole meal flour of sweet lupine) and date (at tamr stage), were utilized in replacing part of wheat flour at three levels (5,10 and 15%). The products were analyzed for chemical, organoleptic and biological properties. The results indicated that legume flours were extremely rich in minerals iron, calcium, zinc and Phosphorous as compared with wheat flour, while skim milk powder has highest percent of Calcium (893.2 mg/100 g) , whereas, date has highest percent of phosphorous (676.09 mg/100 g). Protein content of Legume flours were about 2 to 5 times fold content of the cereals wheat flour. Legume flours were extremely rich in all essential amino acids than wheat flour. Results indicated that minerals contents of biscuits increased by increasing the level of replacement of wheat flour also this with legumes flour and date, The high content of minerals in these materials raise the nutritive value of biscuits. Biscuits made with legume flours had more leucine, total aromatic amino acids and tryptophan, while all biscuits samples had less lysine than FAO/WHO (1973) reference pattern. Therefore, these samples require more supplementation with complementary protein of they are to be used as food sources. The hedonic scores of all biscuits were generally high indicating a strong consumer appeal. The results show that the diets containing biscuits with flour substitutes caused a significant increase in hemoglobin and erythrocyte. The obtained results cleared that iron deficiency led to an extensive alteration that mostly resulted in clinically failure in liver and kidney functions in the affected animals.

keywords: Biscuit, Chemical, biological, sensory evaluation, Iraqi dates, sweet lupine and defatted soybean.

INTRODUCTION

Micronutrient malnutrition affects over 2 billions people in the developing world. Iron (Fe) deficiency alone affects >47% of all preschool aged children globally, often leading to impaired physical growth, mental development, and learning capacity. Zinc (Zn) deficiency, like iron, is thought to affect billions of people, hampering growth and development, and destroying immune systems. In many micronutrient-deficient regions, wheat is the dominant staple food making up >50% of the diet (Cakmak *et al .,*2010).

Iron deficiency is the single most common nutritional disorder world-wide and the main cause of anemia in infancy, childhood and pregnancy. It is prevalent in most of the developing world and it is probably the only nutritional deficiency of consideration in industrialised countries. In the developing world the prevalence of iron deficiency is high, and is due mainly to a low intake of bioavailable iron. However, in this setting, iron deficiency often co-exists with other conditions such as, malnutrition, vitamin A deficiency, folate deficiency, and infection. In tropical regions, parasitic infestation and haemoglobinopathies are also a common cause of anaemia. In the developed world iron deficiency is mainly a single nutritional problem. The conditions previously mentioned might contribute to the development of iron deficiency or they present difficulties in the laboratory diagnosis of iron deficiency (Olivares *et al.,* 1999).

In general, cereals and legumes take a large place of human food consumption. Animal proteins being more expensive, especially people in developing countries depend largely on plant to fulfill their protein requirements. Grain legumes alone contribute to about 33 % of the dietary protein nitrogen needs of humans. Moreover, it is also a good source of minerals (Kirmizi and Guleryuz, 2007). Besides being a good source of nutrition, there is a considerable interest in the relationship between plant-based diets and the prevention of certain human diseases, in which increased levels of radicals are implicated. Likewise legumes seem to be responsible for improving health and can prevent chronic diseases (Frias et al., 2005).

Lupine has the potential to be a source of vegetable protein due to its similar protein content to soy, the common source of vegetable protein used in the food industry. Investigation of its functional properties is essential to validate the potential application in the food industry (Jayasena *et al.,* 2010).

Lupinus seeds are mainly used to fortify foods and counteract nutrient deficiencies. Lupinus seed flour is increasingly used in cereal-based foods. The main interest in lupin for foods is related to its high content of protein content (Ruiz and Sotelo, 2001). Lupinus seed flours have been used for the production of protein isolates with good functional and nutritional properties In same way, protein digestibility of lupine proteins is good in vitro and compares favorably with soy protein (Lqari et al., 2002).

Soybean cultivars are classified as grain type, which are conventional soybeans for oil and animal feeding, and food type, which are those for human consumption in fermented foods and non fermented foods. Therefore, soybean cultivars for human consumption should present special chemical, physical and sensory characteristics. Protein, fatty acids (oleic, linoleic and linolenic), sugar contents, enzymes, grain size, and cooking time are some of the characteristics that can be modified through genetic breeding in order to obtain suitable soybean cultivars for human consumption. These characteristics must be confirmed by chemical, physical and sensory analysis (Meneguce *et al.,* 2005).

Soybean contains ferritin, a multimeric iron storage protein. It is now well proven that the iron from soybean ferritin is as much absorbed and bio-available as much it is from the animal products. Therefore, soybean is recommended to be incorporated in the diet of people suffering from anemia (Lonnerdal, 2009).

The date palm (Phoenix dactylifera L.) is one of mankind's oldest cultivated plants. It has been used as food for 6000 years (Amer, 1994). It could be used for generations to come due to its remarkable nutritional, health and economic value in addition to its aesthetic and environmental benefits. Every part of the date palm is useful. Dates offer useful prospects for fighting hunger and diseases. The importance of the date in human nutrition comes from its rich composition of carbohydrates, salts and minerals, dietary fibre, vitamins, fatty acids, amino acids and protein Al-Shahib and Marshall (2003). Biscuits and cakes are a group of snack food usually consumed by children and students because of their formers, high acceptability and content of major required nutrients. Biscuit is most popular bakery product worldwide. They are high in carbohydrates, fat and calorie but low in fiber, vitamin, and mineral which make it unhealthy for daily use. Because of its acceptability in all age group specially child, longer shelf life, better taste and its position as snacks it is consider as a good product of for protein fortification and other nutritional improvement (Mishra and Chandra, 2012).

Hence, the objective of this study was to produce biscuits by replacing wheat flour with different levels (5, 10 and 15%) of different legumes kinds (defatted soybean flour or whole meal flour of, sweet lupine) or date. The effect of replacement of wheat flour with these blends on the chemical, technological, sensory and nutritional characteristics was studied.

MATERIALS AND METHODS

MATERIALS

1. Raw materials:

a. Seeds of sweet lupine (*Lupinus termis*) and defatted soybean flour were obtained from Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

b. Dates were obtained from local market of Iraq.

c. Other materials include wheat flour (72% extraction), sugar, whole egg, vanilla, backing powder, butter, and salt were purchased from local market, Giza, Egypt.

d.Female rats were obtained from the Food Tech. Res. Institute of Agric. Res. Center, Giza, Egypt.

2. Kits: were obtained from Diamond Diagnostics Company, Germany.

METHODS

1. Preparation of raw materials

All seeds were cleaned, freed from foreign matter and ground separately using a Maxy Hermetic Mill Grinder, patent N: 53985B, Italy to pass through 60 mesh sieve. The powdered samples were kept in dark glass bottles until chemical analysis.

2. Preparation of biscuits:

Control Biscuits sample were prepared included loog of ingredients with sugar powder 40g, Salt 0.5g, fat (yellow butter) 35g, Ammonium bicarbonate 1.5g, Vanillin 0.25g, Water 15g, according to the method described by (Wade (1988). The supplemented biscuits were prepared using the same formula expect for wheat flour (72%) with sweet lupine, defatted soybean and dates flour at 5, 10 and 15 levels (Table 1). The biscuits were backed at 170 -180 ºC for 12 min. and allowed to cool at room temperature.

Table (1): The formula of biscuits by gram (g).

Ingredients / Samples	Wheat flour (ex.72%)	Skim milk powder	Sweet lupine flour	Defatted soybean flour	Date
Control	95	5	--	--	--
1	90	5	5	--	--
2	90	5	---	5	--
3	90	5	--	--	5
4	85	5	10	--	--
5	85	5	--	10	--
6	85	5	--	--	10
7	80	5	15	--	--
8	80	5	--	15	--
9	80	5	--	--	15

3- Gross Chemical composition

Moisture, protein, fat, ash and fiber of raw materials and biscuits were determined according to A. O. A. C (2007).

Zinc, iron and calcium content were determined using a Pye Unicom Sp 19000 atomic absorption spectroscopy techniques after dry ashing according to the methods described in the A.O.A.C. (1995). Phosphorus was determined according to the methods described by Herbet et al. (1971).

Carbohydrates were estimated by difference as follows:

Available carbohydrates = 100 – (% protein + % fat + % ash + % fiber) according to Chatfieled and Admas, (1940).

Amino acids content were determined according to the method described by Pelltt and Young (1980), using a LKB 4151 Alpha plus Amino Acid Analyzer, Regional Center For Food & Feed. Agric. Res. Center, Giza, Egypt.

Amino acid score (AAS) was calculated according to the FAO/WHO (1973) as follows:

$$\text{AAS (\%)} = \frac{\text{mg amino acid in 1gm test protein}}{\text{mg amino acid in 1gm reference protein}} \times 100$$

Protein Efficiency Ratio (PER) was estimated using the equation reported by Alsmeyer et al.(1974):PER =0.684 + 0.456 (Leucine)- 0.047 (Proline).

Biological value (BV) was estimated using the equation suggested by Mitchell and Block (1946)BV= 49.9+ 10.53 (P E R)

Energy value:

The energy value was calculated from the following equation as reported by Hawk, *et al*. (1949). Energy value= 4 (total carbohydrates + protein)+ 9 x fat

4. Physical measurements of the produced biscuits

Physical measurements were carried out according to Abdel-Magied (1991) as following thickness, Diameter (both average of 15 bicuits) (cm), weight of 15 biscuits (g) and stock height of 154 biscuits (cm). The diameter and thickness of biscuits measured by Plano meter to the nearest mm.

5.Organoleptic Evaluation:

Sensory characteristics evaluation of the produced biscuits were subjectively assessed by a panel of ten judges. Panelists were asked to use the control sample as the basis for determining acceptance by first assigning score it and then evaluating each test sample in comparison to control. The quality attributes, crust color, taste, odor, texture, crust appearance and over all acceptability of biscuits were organoliptically evaluated as cited from Kassim, (2002).

6. Biological evaluation of tested materials:

1. Animal experiments

Thirty (30) male albino rats weighting 75-90 gm each were used in this experiment. Animals were housed in cages under normal health laboratory conditions house (at 25 Cº, 12-h light and dark cycle) at Food Technology Research Institute, Giza, Egypt. The animals were feed on basal diet for 10 days (Table 2). To improve iron absorption, all rats were first made anemic through bleeding of rates 1 ml every two days and free iron diets were used in adaptation period for two week prior to start of experiments. Rats were fed on standard synthetic diet (as presented in Table (2) and supplied with tap water). Salt and vitamin mixtures that recommended by A.O.A.C (2000) were used as presented in Tables (3) and (4).

Table (2): Composition of the basal diet:

Ingredient	**Basal diet(g/100g)**
	%
Casein	15
Starch	68
Fat (Corn oil)	7
Mineral mixture	4
Vitamin mixture	1
Cellulose	5

Table (3): Composition of salt mixture.

Salt	**Weight (g)**
NaCl	139.300
K1	000.790
KH_2PO_4	389.000
$MgSO_4$	057.000
CaO_3	381.000
$FeSO_4$. $7H_2O$	027.000
$MuSO_4$. H_2O	004.010
$ZnSO_4$. $7H_2O$	000.548
$CuSO_4$. $5H_2O$	000.470
$COCl_2$. $6H_2O$	000.023

***Salt mixture.**

Table (4): Composition of vitamins mixture.

Vitamins	Weight(g)
Vitamin A palmitate (500.000 Iu / g)	00.80
Vitamin D3 acetate (1000 Iu / g)	01.00
Vitamin E acetate (500 Iu / g)	10.00
Menadione sodium Bisul-fate (62.5% menacione)	00.08
Thiamine HCl	00.60
Riboflavin	00.60
Pyridoxine HCl	00.07
Nicotinic acid	03.00
Calcium pantothenate	01.60
Folic acid	00.20
Biotin , 1%	02.00
Cyano Cobalamine 0.01%	01.00
Sucrose	978.42
Total	1000

***Vitamins mixture.**

2. Experimental design

After the adaptation period, the animals (30 rats) were divided into 6 groups, each group consisted of 5 rats. The rats were feed with the different tested diets for 30 days. The composition of the control and different tested diets were shown in Table (5).

Group 1(control +): The rats were fed on experimental diet (basal diet).

Group 2 (control -): The rats were fed on free iron experimental diet

Group 3: The rats were fed on biscuit produced by substituting 5% of wheat flour with skim milk powder.

Group 4: The rats were fed on biscuit produced by substituting 10% of wheat flour with 5% skim milk powder and 5% defatted soy flour.

Group 5: The rats were fed on biscuit 10% of wheat flour with 5% skim milk powder and 5% sweet lupine flour

Group 6: The rats were fed on diet contained dried Biscuit 15 % of wheat flour with 5% skim milk powder and 10% date.

Table (5): Composition of the experimental diets (g/100g).

Ingredient	1 Control +	2 control free iron	3	4	5	6
	%	%	%	%	%	%
Casein	15	15.0	7.10	5.99	6.25	7.05
Starch	68	68.0	-	-	-	-
Fat (Corn oil)	7	7.0	-	-	-	-
Mineral mixture	4	4.0(-Fe)	3.42(-Fe)	3.47(-Fe)	3.47(-Fe)	3.44(-Fe)
Vitamin mixture	1	1.0	0.86	0.87	0.87	0.86
Cellulose	5	5.0	3.0	2.9	2.6	2.67
Biscuit control	-	-	85.6	-	-	-
Biscuit with 5% defatted soy flour	-	-	-	88.0	-	-
Biscuit with 5% sweet lupine flour	-	-	-	-	88.1	-
Biscuit with 10% date	-	-	-	-	-	86.3

The actual weight of food intake was calculated. The rats were weighed weekly to determine body weight gain, (feed eefficiency = weight gain x 100/feed intake). At the end of experiment, the animals were killed by decapitation, and the organs (brain, heart, lungs, spleen, kidney, stomach, testes and eyes) were weighted.

3. Hematological parameters of experimental animals

Blood samples were collected from the killed animals at the end of each experiment. Blood samples were obtained and placed immediately on ice. Heparin was used as an anticoagulant. Plasma was obtained by centrifugation of samples at 860 g for 20 min, and was stored at -60°C until used for analyses. Non coagulated blood was tested, shortly after collection, for hemoglobin (Hb), total erythrocyte count (TEC) and total leukocyte counts (TLC). Blood Hb concentration was determined by the cyanomethemoglobin procedure (Wintrobe, 1965). Erythrocytes were counted on hemocytometer using a light microscope at 40 x 10 magnification. Blood samples were diluted to 200 times by physiological saline (0.9% sodium chloride solution) before counting. Leukocytes were counted on AO Bright line hemocytometer using a light microscope at 10 x 10 magnification after diluting blood samples to 20 times with a diluting. Fluid (1% acetic acid solution and little of Leishman's stain) before counting **(Hepler, 1966).** All the parameters were calculated as mean of five readings.

4. Blood biochemical measurements

Stored plasma samples were analyzed for total protein (TP) by the Biuret method according to Gornal *et al*. (1949). Albumin (A) concentration were determined by the method of Doumas (1971). Globulin (G) concentrations were determined by difference (Total protein-albumin) and A/G ratio was calculated. Concentration of urea was determined by the method of Fawcett and Scott (1960). Plasma total bilirubin was measured using the method of Walter and Gerade (1970). Plasma calcium was determined by the method of Gindler and King (1972).

The activities of plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel (1957). Alkaline phosphatase (AlP) activity was measured in plasma by the method of Belfield and Goldberg (1971).

5-Statistical analysis

Data were analyzed according to Steel Torrie (1981). Statistical significance of the difference in values of control and treated animals was calculated by (F) test with 5% significance level. Data of the present study were statistically analyzed by using Duncan's Multiple Range Test (SAS, 1986).

RESULTS AND DISCUSSION

Chemical composition of raw materials:

The proximate chemical composition of raw materials used in making biscuit were shown in table (6). The data indicated that the moisture content of different materials was in the range of 3,85-20,33, the dates shows the highest content of moisture (20,33%). Protein, fat, crude fiber and ash contents of defatted soybean flour and whole meal of sweet lupine and skim milk powder were higher than wheat flour. Defatted soybean flour has the highest percent of protein (45,9%) and crude fiber, while wheat flour has highest percent of total carbohydrates. Protein contents of Legumes flours were about 2 to 5 times fold content of the cereals wheat flour.

Table (6): Chemical composition of raw materials used in making biscuit (on dry weight basis).

Samples No.	Moisture %	Protein %	Fat %	Crude fiber %	Ash %	Total carbohydrates %
Wheat flour 72% ex. (WF).	11.13	10.04	0.84	0.76	0.52	87.44
Dates (at tamr stage) (T)	20.33	3.27	2.77	5.5	2.1	86.36
Sweet lupine flour (SL)	10.04	18.68	4.77	5.49	3.45	67.61
Defatted soybean flour (DS)	6.1	45.9	1.85	4.03	6.58	41.64
Skim-milk powder (SMP)	3.85	32.65	0.61	-	7.64	59.1

All above results are found to be closely near that obtained by, Zahran (2000) who found that the defatted soybean flour contained 8.62% moisture, 6.72% ash, 4.21% fiber, 52.1% protein, 3.38% fat and 33.59% carbohydrates (on dry weight basis).

Barakat , (2003) who found that the protein, fat, total dietary fiber, ash and available carbohydrates contents of Siwi dates at tamr stage(on dry weight basis) were 3.97, 3.25, 14.9, 4.08 and 73.8%, respectively. While Fe, Ca and Zn contents were 1.53, 977.7 and 10.5 mg/100g, respectively (on dry weight basis).

Minerals content:

Results in Table (7) show that wheat flour has lower amounts of minerals than other raw materials with some exceptions. For other raw materials, defatted soybean flour had the highest amounts of iron (26.87 mg/100g) followed by sweet lupine flour (7.84 mg/100g), then date (3.28 mg/100g).

Table (7): Minerals contents of raw materials used in making biscuit (on dry weight basis).

Minerals content mg / 100 g	Wheat flour (WF)	Dates (at tamr stage) (T)	Sweet lupine flour (SL)	Defatted soybean flour (DS)	Skim milk powder
IRON (Fe)	2.1	3.28	7.84	26.87	0.6
Calcium (Ca)	26.7	134.54	388.27	255.6	893.2
Zinc (Zn)	0.707	0.619	3.47	6.26	5.515
Phosphorous (P)	244.12	676.09	222.1	656.04	669.1

The results indicated that legumes flours were extremely rich in minerals as compared with wheat flour. While skim milk has highest percent of Calcium (893,2 mg/100g), whereas, date has highest percent of phosphorous(676.09 mg/100g).These data are in agreement with (Meneguce *et al.,* 2005). Who found that the Soybean contains about 5% minerals. It is relatively rich in K, P, Ca, Mg, and Fe. Soy ferritin can supplement reasonable quantities of iron

Amino acids content:

Amino acids composition of the ingredients used in making biscuit are show in Table (8). The amino acids profiles of legumes flours were similar or only slightly different. However, legumes flours and skim milk powder are rich in total essential amino acids lysine, threonine, and total aromatic amino acids as compared with wheat and dates flour profiles.

Legumes flours were extremely rich in all essential amino acids than wheat flour 72% ex., except metionine and cystine. The high content of legumes flours from indispensable amino acids especially lysine (2.4 –3.7 times fold of lysine content of wheat flour) makes it useful for supplementing cereal protein low in lysine.

Wijeratne (1994). found that defatted soybean flour contained the essential amino acids in the amounts of 5.1, 7.7, 6.9, 1.6, 5.0, 4.3, 1.3, 5.4 and 2.6 (g/16 gN) for Isoleucine, Leuceine, Lysine, Methionine, Phenyalanine, Threonine, Tryptophan, Valine and Histidine, respectively.

Table (8): Amino acids content of raw materials used in making biscuit (on dry weight basis).

Amino acids	g/100g of total protein				
	Wheat flour 72% ext	Dates (at tamr stage) (T)	Sweet lupine flour (SL)	Defatted soybean flour (DS)	Skim milk powder
Iso-leucine	3.71	4.65	4.00	3.8	6.46
Leucine	7.46	7.4	7.00	5.6	10.83
Lysine	1.86	2.6	4.60	5.9	8.76
Methionine +Cystine	3.52	3.22	1.91	3.5	3.75
Phenyle alanine +Tyrosine	7.48	6.3	9.44	7.5	10.37
Threonine	3.04	3.5	3.67	3.5	4.79
Tryptophane	1.2	1.0	0.80	1.3	-
Valine	4.5	3.7	3.94	5.1	7.01
Total E.A.A	32.77	32.37	35.36	36.2	51.97
Arginine	3.2	7.5	8.54	6.8	3.5
Aspartic acid	3.47	7.5	10.91	9.6	6.6
Serine	4.47	4.67	4.84	4.3	4.45
Glutamic acid	37.35	33.41	24.83	26.7	19.52
Proline	11.0	5.69	3.81	4.4	10.26
Glycine	3.57	1.92	4.24	3.7	1.69
Alanine	2.7	3.42	4.04	3.6	2.93
Histidine	2.34	3.76	3.39	4.7	3.16

Sensory evaluation and Physical properties of biscuit:

Sensory characteristics:

Average sensory panel scores of appearancy color, Crust, appe, Texture, Crispness, Taste, Odor and Overall acceptability for biscaits formylas contained wheat flour and its mixtures with dates, sweet lupine , defatted soybean flour and skim milk powder are tabulated inTable (9) Control sample (95% wheat flour +5% skim milk powder) showed the best overall acceptability (92%) followed by the sample No (6) which contain 90% wheat flour + 5% skim milk powder +10% datas (89%). Sample (2) contain 90% wheat flour +5% skim milk powder + defatted soybean (87.50%) and sample (1) which contain 90% wheat flour +5% skim milk powder + 5% sweet lupine (86%) while the overall acceptability for the rest samples ranged between 78 to 84% these variations related to levels of raw materials.

Table (9):.organoleptic evaluation of experimental biscuit samples made form wheat flour (72% extraction) and its mixtures.

Samples No.			Color 10	Crust appe. 20	Texture 20	Crispness 15	Taste 20	Odor 15	Overall acceptability 100
Con.		95% wheat flour + 5% SMP.	10.0^{a}	18.0^{a}	17.5^{bc}	13.5^{a}	19.0^{a}	14.0^{a}	92.00^{a}
5% wheat flour substitution	1	90% WF + 5% SMP + 5% SL	9.5^{ab}	16.0^{bc}	17.0^{cd}	13.0^{a}	18.0^{ab}	12.5^{abc}	86.00^{abcd}
	2	90% WF + 5% SMP + 5% DS	10.0^{a}	17.0^{ab}	16.0^{def}	12.5^{ab}	18.0^{ab}	14.0^{a}	87.50^{abcd}
	3	90% WF + 5% SMP + 5% date	9.0^{abc}	17.0^{ab}	17.0^{cd}	13.0^{a}	19.0^{a}	14.0^{a}	89.0^{abc}
10% wheat flour substitution	4	85% WF + 5% SMP + 10% SL	9.0^{abc}	16.0^{bc}	17.0^{cd}	12.5^{ab}	17.0^{bc}	11.5^{bc}	83.00^{abcd}
	5	85% WF + 5% SMP + 10% DS	9.0^{abc}	16.0^{bc}	16.0^{def}	13.0^{a}	18.0^{ab}	12.0^{abc}	84.00^{abcd}
	6	85% WF + 5% SMP + 10% date	8.0^{cde}	15.0^{cd}	18.0^{abc}	13.5^{a}	19.0^{a}	14.0^{a}	87.50^{abcd}
15%wheat flour substitution	7	80% WF + 5% SMP + 15% SL	8.0^{cde}	16.0^{bc}	16.0^{def}	11.0^{b}	16.0^{cd}	11.0^{c}	78.00^{cd}
	8	80% WF + 5% SMP + 15% DS	9.0^{abc}	16.0^{bc}	16.0^{def}	12.0^{ab}	15.0^{d}	11.5^{bc}	79.50^{bcd}
	9	80% WF + 5% SMP + 15% date	6.5^{fg}	13.0^{ef}	18.5^{a}	13.0^{a}	18.0^{ab}	13.0^{ab}	82.00^{abcd}
		LSD at 0.05	**1.27**	**1.60**	**1.41**	**1.58**	**1.603**	**2.11**	**11.38**

Physical properties:

Biscuits were subjected to physical measurements including weight, volume, specific volume, diameter, thickness and spread factor. Measurements of substituted flour biscuits at various levels are shown in table (10). Results indicated that specific volume (which relates with good crispness and texture) of wheat substituted flour biscuits were lower than that of control biscuit. A negative relationship could be noticed between flour substitution level and biscuits specific volume. Biscuits specific volume of control sample was 1.82 cm^3/gm and ranged from 1.45 to 1.77cm^3/gm for biscuit samples containing flour substitution at levels from 5 to 15% of dates or legumes flour. Such decrease in biscuits specific volume could be attributed to humidity and water holding of legumes flours. For other measurements, it could be noticed that biscuits spread ratio increased as wheat flour replacement level increased. Biscuits spread ratio increased from 6.0 for control sample and ranged from 5.58 to 7.69 for wheat flour substituted at levels from 5 to 15% of date or legumes flour. The changes in baking properties may be due to the changes in the quality and quantity of protein with the added ingredients and also attributed to gas retention of dough during baking process (Sai *et al.,* 1997 and Hussien *et al.,* 2010**).**

So, according to obtained results of above studies of sensoncal and physical properties of different biscuit mixtures samples, it was decided to select samples, No 1, 2 and 6 beside control due to their high acceptability and good physical properties to continue different analysis and evaluations.

Table (10): physical measurements of experimental biscuit samples made form wheat flour (72% extraction) and its mixtures.

Samples No.			Specific volume			Spread ratio		
			Weight (gm)	Volume (cm^3)	Specific volume (cm^3 / gm)	Diameter (Width) (cm)	Thickness (cm)	Spread ratio W/T
Con.		95% wheat flour + 5% SMP.	3.076	5.6	1.82	6.0	1.0	6.0
5% wheat flour substitution	1	90% WF + 5% SMP + 5% SL	2.82	5.0	1.77	6.0	0.8	7.5
	2	90% WF + 5% SMP + 5% DS	2.65	4.2	1.58	5.6	0.96	5.83
	3	90% WF + 5% SMP + 5% date	3.214	5.2	1.62	6.3	1.0	6.3
10% wheat flour substitution	4	85% WF + 5% SMP + 10% SL	2.754	4.88	1.77	6.0	1.0	6.0
	5	85% WF + 5% SMP + 10% DS	3.156	4.8	1.52	6.0	1.0	6.0
	6	85% WF + 5% SMP + 10% date	3.094	5.0	1.62	6.4	0.9	7.11
15% wheat flour substitution	7	80% WF + 5% SMP + 15% SL	2.418	3.8	1.57	5.8	0.9	6.44
	8	80% WF + 5% SMP + 15% DS	3.098	5.0	1.61	5.8	1.04	5.58
	9	80% WF + 5% SMP + 15% date	2.718	4.4	1.62	6.0	0.8	7.5

***Chemical samples components of biscuit samples on dry weigh basis:**

The results in Table (11) showed that protein content of biscuit was increased from (6.35mg/100gm) for control sample to 6.6 mg/100gm and 7.42 mg/100gm for biscuits with 5 % sweet lupine and 5% defatted soybean flour, respectively. While protein content percentage decreased compared to control 5.67 mg/100gm for biscuits with replacement level of 10% dates.

Table (11): Chemical composition of biscuit produced from wheat flour 72% ex. (WF), and its mixtures (on dry weigh basis).

Samples No.		Energy cal./100gm biscuit	Moisture %	Protein %	Fat %	Crude fiber %	Ash %	Total carbohydrates %
Recommended Daily Dietary Allowances for children 7-10 years (1989)*		2000	-----	28 gm	------	-----	-----	-----
Con.	95% wheat flour + 5% SMP.	454.7	6.3	6.35	18.34	0.4	1.21	73.7
1	90% WF + 5% SMP + 5% SL	454.8	6.2	6.6	18.44	0.54	1.3	73.12
2	90% WF + 5% SMP + 5% DS	454.4	6.2	7.42	18.39	0.5	1.39	72.3
3	85% WF + 5% SMP + 10% date	452.9	6.6	5.99	18.53	0.64	1.3	73.54

***Food Nutrition Board, National Academy of Sciences-National Research Council, (R. D.D. A) (1989).**

100 gm dry basis of biscuit samples with 5% replacement of wheat flour with legumes flour had from 23.57- 26.5% of protein, respectively and 22% of energy of Recommended Daily Dietary Allowances (R.D.D.A., 1989) for children 7-10 years, 28 Kg weight and 132 cm height.

The protein content of all biscuit samples was lower than that of the wheat flour and its raw materials Table (6). These losses of protein during baking may be due to the maillard type browning reaction i.e, the reaction of suger with amino acids (Hussein, 1987) .Also the data show that the fat content of all biscuit samples was high than wheat flour and its raw materials due to the butter yellow used in the biscuit formula. Data show that the fat content of all biscuit samples was high than that of wheat flour and its raw materials due to the yellow butter used in the biscuit formulas. These results that are In agreement with Ghonim (2002**).**

Minerals content:

Two levels 5% of legumes flour (defatted soybean flour and whole meal flour of sweet lupine) and 10% dates were used to substitute wheat flour in biscuits making. biscuit samples and control were analyzed for their minerals content, i.e., iron, calcium, zinc and Phosphorous. The obtained results are shown in Table (12).

The iron content was found to be 1.17 mg/100gm of control samples. It is clear that it increased in the range from 4.3 mg/100gm to 62.4 mg/100gm , in 5% biscuits with sweet lupine flour or defatted soybean flour and 10% dates (at tamr stage) compared to control.

Table (12): Minerals content of biscuits samples .(mg / 100 g (dry basis).

Samples No.		**Fe**	**Ca**	**Zn**	**P**
Recommended Daily Dietary Allowances for children 7-10 years (1989)		12	800	10	800
Con.	95% wheat flour + 5% SMP.	1.17	46.27	0.55	155.07
1	90% WF + 5% SMP + 5% SL	1.33	56.47	0.62	154.02
2	90% WF + 5% SMP + 5% DS	1.9	52.96	0.70	166.93
3	85% WF + 5% SMP + 10% date	1.22	52.69	0.538	180.69

***Food Nutrition Board, National Academy of Sciences-National Research Council, (R. D.D. A) (1989).**

The calcium content was found to be 46.27 mg/100gm in control, while, it was increased to 56.47 mg/100 gm for 5 % sweet lupine flour, defatted soybean flour or 10% dates (at tamr stage). 52.96 and 52.69 mg/100 g in biscuits samples. Flour replacement with 10% dates decreased biscuit content of Zinc to 0.538 mg/100 gm compared to control, while flour replacement with 5% legumes flour increased Zinc content of biscuits samples to 0.62 to 0.70 mg/100gm

The phosphorous content of biscuit was found to be 155.07mg/100gm in control sample. It was decreased from control to 154.02 mg/100gm for flour replacement with 5% sweet lupine, and increased to166.93 and 180.69 mg/100gm for flour replacement with 5% defatted soybean flour and 10% date, respectively.

Results indicated that minerals content of biscuits increased by replacement of wheat flour with legumes flour and dates, such increase in minerals mainly due to high content of minerals of replacing ingredients as compared with wheat flour in (Table 6). While skim milk has highest percent of Calcium (893.2 mg/100gm) whereas, dates has highest percent of phosphorous (676.09 mg/100 gm). The high content of minerals in these materials raise the nutritive value of biscuits.

100gm (dry basis) of biscuits sample 5% replacement of wheat flour with defatted soybean flour had 15.83, 6.62, 7.0 and 20.86% of Fe, Ca, Zn and P, respectively of Recommended Daily Dietary Allowances (R.D.D.A., 1989) for children 7-10 years, 28 Kg weight and 132 cm height.

Amino acids content:

Amino acids composition of biscuits produced by substitute wheat flour 72% ex., with 10% dates (at tamr stage) and 5% defatted soybean flour (DS) or whole meal flour of sweet lupine (SL) are show in Tables (13). The addition of legume flours to wheat flour appeared to increase the concentration of most of the essential amino acids (isolucine, lysine, cystine, methionine, threonine, tryptophan and valine), compared to the control. The amino acids profiles of control biscuit sample and all biscuit samples at 10% wheat flour replacement with dates were similar or only slightly different.

Table (13): Amino acids content of biscuits samples(g/100g protein).

Amino acids	95% WF + 5% SMP. Control		90% WF + 5% SMP + 5% SL		90% WF +5% SMP +5% DS		85% WF +5% SMP +10% date		Amino acids@ pattern of FAO/ WHO/UNU 1985 (E)
	C	AAS (%) C/E	1	AAS (%) 1/E	2	*AAS (%) 2/E*	3	*AAS (%) 3/E*	
Iso-leucine	3.83	95.75	3.85	96.25	3.83	95.75	3.87	96.75	4.0
Leucine	7.98	114	7.93	113.3	7.57	108.1	8.02	114.6	7.0
Lysine	2.95	53.6	3.11	56.5	3.53	64.18	3.04	55.27	5.5
Methionine +Cystine	3.55	101.4	3.42	97.7	3.55	101.4	3.55	101.4	3.5
Phenyle alanine +Tyrosine	7.93	132.2	7.37	122.8	7.87	131.2	7.93	132.2	6.0
Threonine	3.31	82.75	3.35	83.75	3.36	84.0	3.35	83.75	4.00
Tryptophane	1.01	101	0.99	99.0	1.06	106.0	0.99	99.0	1.0
Valine	4.91	98.2	4.85	97.0	4.96	99.2	4.91	98.2	5.0
Total E.A.A	35.47	98.5	34.87	96.9	35.73	99.3	35.66	99.1	36.0
Arginine	3.25		3.67		3.9		3.37		
Aspartic acid	3.96		4.54		5.02		4.01		
Serine	4.67		4.5		4.44		4.47		
Glutamic acid	34.52		33.62		32.98		34.21		
Proline	10.88		10.31		9.69		10.72		
Glycine	3.27		5.64		3.34		3.20		
Alanine	2.74		2.84		2.90		2.75		
Histidine	2.55		2.55		2.88		2.52		
PE R	**3.81**		**3.82**		**3.685**		**3.84**		
B.V	**90.02**		**90.12**		**88.70**		**90.31**		

@ Food and nutrition board: Recommended Dietary Allowances, 9th ed. National Academy of Sciences-National Research Council, Washington,D.C.,1980,p.43.

1-WF: wheat flour (72% extraction) 2-LS: Whole meal lupine seed
3-DS: defatted soy flour 4- CP: Whole mealchickpea
5-FG: Whole meal fenugreek flour

Comparison of the amino acids composition of legume flours and dates biscuits showed that legume flours products contained similar or higher total essential amino acids levels than the FAO/WHO [21] pattern, except for a few modest deficiencies like lysine. The deficiency of these amino acids was not the result of legumes flours substitution, but for the level of substitution. Biscuits made legume with flours had more leucine, total aromatic amino acids and tryptophan, while all biscuits samples had less lysine than FAO/WHO (1973) reference pattern. Therefore, these samples require more supplementation with complementary proteins food sources.

From the same table it could be noticed that all amino acid scores (AAS) increased compared with the control ones probably due to the improvement in lysine and other essential amino acids except sulfur containing amino acids (methionine and cystine), glutamic acid and proline. On the other hand both calculated protein efficiency ratio (PER) and biological value (BV) of biscuits ranged between increment and decrement compared with control sample.

Biological evaluation:

1. Effects of different diets fed to male rats on hematological parameters

Changes in blood hemoglobin (Hb mg/dl), total erythrocyte count (red blood cells count RBcs x10^6/mm^3),serum iron and serum total iron binding capacity **(**TIBC **(**μg/dl)) of rats fed on different diets for 35 days are shown in Table (14).

Table (14):Effects of different diets fed to rats on hematological parameters

Parameter / Rat group		Hb* (gm/dl)	RBcs** (million/ml^3)	Serum iron (μg/dl)	"TIBC" (μg/dl)
1 (Control +) Rats fed basal diet	Zero time	10.50^{c}	4.14^{ab}	211.02^{cd}	459.40^{bcd}
	End time	12.63^{ab}	4.20^{ab}	254.33^{ab}	474.33^{bc}
2 (Control -)Rats fed basal diet free iron	Zero time	8.76^{d}	3.36^{def}	178.41^{e}	377.10^{e}
	End time	7.46^{e}	3.42^{def}	181.67^{e}	424.34^{d}
3 Rats fed biscuits (control)	Zero time	8.51^{d}	3.48^{de}	193.11^{de}	443.77^{cd}
	End time	8.22^{de}	3.59^{cd}	205.35^{cde}	443.66^{cd}
4 Rats fed biscuits with 5% defatted soy flour	Zero time	8.16^{de}	3.19^{def}	230.66^{bc}	473.05^{bc}
	End time	13.36^{a}	4.26^{a}	277.12^{a}	557.01^{a}
5 Rats fed biscuits with 5% sweet lupine flour	Zero time	8.40^{d}	3.12^{ef}	213.34^{cd}	448.44^{cd}
	End time	13.11^{a}	4.07^{ab}	255.67^{ab}	491.66^{b}
6 Rats fed biscuits with 10% date	Zero time	8.06^{de}	3.02^{f}	214.33^{cd}	466.35^{bc}
	End time	12.40^{ab}	3.84^{bc}	224.33^{cd}	479.02^{bc}
L.S.D		0.80	0.29	20.99	20.70

*** Numbers in the same column followed by the same letter are not significant different at *P*< 0.05**

**** Hb = Serum hemoglobin.** ***** RBcs = Red blood cells count.**

******"TIBC" = Serum total iron binding capacity.**

Hb = (normal value = 11.5- 15.5g/dl).

RBCs = (normal = 4.5- 5.2 millions / ml^3). **TIBC =(250 -450 ug / dl)**

From Table (14) it was clear observed that hemoglobin levels decreased after deptetion period to less than 10 gm/100 ml due to deficiency of iron from their diets. It can be also noticed that, there were significant differences between groups at the end repletion period had a decline in hemoglobin (Hb) and total erythrocyte cells count (RBcs).

Blood hemoglobin (Hb) and erythrocyte count (RBcs) of rats fed positive basal diet, rats fed on diet containing biscuits with 5% defatted soy flour, rats fed on diet containing biscuits with 5% sweet lupine flour and rats feed biscuits with 10% date were 12.63, 13.36, 13.11 and 12.4 mg/dl and 4.2, 4.26, 4.07 and 3.84 x 10^6 mm^3, respectively after five weeks. This means that the diets containing biscuits with flour substitute caused a significant increase in hemoglobin and erythrocyte. In case of serum iron and TIBC (Serum total iron binding capacity)were 181.67µg/dl and 424.34µg/dl, respectively for rats fed on diet containing free iron basal diet (2) after five weeks, while in case of rats fed on diet contain biscuits made from wheat flour (3) were 205.35µg/dl and 443.66µg/dl, respectively.

Serum iron and TIBC (Serum total iron binding capacity) of rats fed positive basal diet, rats fed on diet containing biscuits with 5% defatted soy flour, rats fed on diet containing biscuits with 5% sweet lupine flour and rats fed biscuits with 10% date were 254.33, 277.12, 255.67 and 224.33µg /dl; and 474.33, 557.01, 491.66 and 479.02µg/dl, respectively after five weeks. This means that the diets containing biscuits with flour substitute caused a significant increase in serum iron and TIBC (Serum total iron binding capacity), which lead to the same trend of hemoglobin and erythrocyte.

Rucker *et al.,* (1994) who found that the effect of absorption aids on the bioavailability of iron require high iron content, because iron homeostasis is primarily regulated through its absorption and iron cannot be excreted via other ways in iron deficient anemic rats. Utilization of legumes flour and date to substitute wheat flour to produce biscuits increased iron absorption in rats fed a containing diets. In rats fed the positive basal diet, the increase in iron absorption was only slight but significant.

2.**Effects of different diets fed to rats on plasma parameters**

The mean values of liver and kidney functions of rat and serum total protein (TP), (albumin (A), globulin (G), and A/g ratio) as affected in rats fed on different diets for 35 days are shown in Table (15) and Table (16).Data illustrated that all groups of rats at the end of experimental time had an increase of total protein content (g/dl), albumin (g/dl) and globulin (g/dl) and that increase was significant. Group (1) which, fed on basal diet had the highest increment in total protein, albumin and globulin, followed by group (4) and group (5) then group (6), while group (3) had the lowest contents of total protein, albumin and globulin (g/dl) at the end of experimental time.

It can be observed also that there was positive relationship between uric acid and serum total protein, thus uric acid had the same trend of total protein of blood serum. Meanwhile, creatinen (mg/dl) was significant increase by the end of experimental time, but there was no relationship with total serum protein. Liver enzymes activity was increased in groups (1), (4), (5) and group (6) at the end of experimental time. It can be observed also that liver enzymes activity was decreased for rats on groups (2) and (3) at the end of experimental time. Rats fed on diets containing biscuits made of flour substitute with legume flour caused the increased blood uric acid concentration in rats, there by changing of the nitrogen balance.

Table (15): Effects of different diets fed to rats on plasma parameters

Rat group (Parameter)		Total serum protein: Total protein (g/dl)	Albumen (A) (g/dl)	Globulin (G) (g/dl)	A/G ratio
1 (Control +) Rats fed basal diet	Zero time	7.07^{c}	3.56^{cd}	3.51^{b}	1.01
	End time	8.36^{b}	4.69^{b}	3.67^{bc}	1.30
2 (Control -)Rats fed basal diet free iron	Zero time	5.47^{e}	3.23^{d}	2.24^{def}	1.44
	End time	6.16^{d}	4.17^{bcd}	1.99^{efg}	2.09
3 Rats fed biscuits (control)	Zero time	4.86^{f}	3.33^{cd}	1.53^{gh}	2.18
	End time	6.13^{d}	4.33^{bc}	1.80^{fgh}	2.41
4 Rats fed biscuits with 5% defatted soy flour	Zero time	4.80^{f}	3.46^{cd}	1.34^{h}	2.58
	End time	8.14^{b}	5.50^{a}	2.64^{cd}	2.08
5 Rats fed biscuits with 5% sweet lupine flour	Zero time	6.60^{d}	3.87^{bcd}	2.73^{cd}	1.42
	End time	7.43^{c}	4.66^{b}	2.77^{cd}	1.68
6 Rats fed biscuits with 10% date	Zero time	6.13^{d}	3.66^{bcd}	2.47^{de}	1.48
	End time	7.36^{c}	4.33^{bc}	3.03^{bc}	1.43
L.S.D		0.44	0.64	0.54	-

*** Numbers in the same column followed by the same letter are not significant different at *P*< 0.05 Total protein = (5.7- 8.2 g/dl) A= Albumen(3.2-4.8g/dl) A/G= ratio (1-1.8)**

Table (16): Effect of feeding rats on six groups of diets

Rat group (Parameter)		Kidney functions: Uric acid	Creatinen	Liver enzymes activity: GOT U/dl	GPT U/dl	Alkaline phosphatase U/dl
1 (Control +) Rats fed basal diet	Zero time	4.73^{d}	0.62^{cde}	87.35^{b}	17.68^{c}	174.68^{c}
	End time	6.42^{c}	0.70^{bc}	123.66^{a}	35.03^{a}	200.36^{bc}
2 (Control -)Rats fed basal diet free iron	Zero time	3.90^{d}	0.58^{de}	68^{d}	17.66^{c}	184.01^{c}
	End time	3.96^{d}	0.85^{a}	63.40^{d}	16.76^{c}	81.05^{d}
3 Rats fed biscuits (control)	Zero time	4.86^{d}	0.61^{cde}	88^{b}	16.67^{c}	173.33^{c}
	End time	5.10^{d}	0.86^{a}	67.32^{d}	21.67^{bc}	96.00^{d}
4 Rats fed biscuits with 5% defatted soy flour	Zero time	4.13^{d}	0.44^{f}	88.01^{b}	17.33^{c}	238.66^{a}
	End time	7.26^{b}	0.83^{a}	91.30^{b}	25.01^{b}	191.35^{c}
5 Rats fed biscuits with 5% sweet lupine flour	Zero time	4.44^{d}	0.69^{bcd}	81.05^{c}	16.66^{c}	173.34^{c}
	End time	7.27^{b}	0.76^{ab}	92.07^{b}	20.02^{c}	249.03^{a}
6 Rats fed biscuits with 10% date	Zero time	4.33^{d}	0.56^{e}	89^{b}	17.17^{c}	173.33^{c}
	End time	6.11^{c}	0.81^{a}	90.33^{b}	18.69^{c}	224.10^{ab}
L.S.D		0.84	0.08	4.82	3.91	26.33

*** Numbers in the same column followed by the same letter are not significant different at *P*< 0.05**

Uric acid = (2.3- 6.0) Creatinen=(0.5- 1.1) Alkaline phosphatase= (30-129u/dl)

So, from obtained results, it could be concluded that iron deficiency led to an extensive alteration that mostly resulted clinically into failure if liver and kidney functions in the affected animals. Also, it was concluded that the used legumes flour and date led to some but not complete antagonistic or protective effects against the iron deficiency, as a moderate changes were seen in the studied organs of these cases.

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التقييم الحسي والكيميائى والبيولوجي للبسكويت المدعم بدقيق الترمس الحلو – فول الصويا منزوع الدهن والتمر العراقي.

فاتن يوسف ابراهيم ، عبد الحميد ابراهيم عبد الجواد ، محمود بدوي دومه و حسن ياسين نمر
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الهدف من هذا البحث هو دراسة امكانية استخدام خليط من دقيق الحبة الكاملة لكل من الترمس الحلو ودقيق فول الصويا منزوع الدهن وكذلك العجوة (البلح في مرحلة التمر) بنسب استبدال ٥، ١٠، ١٥% لكل منها من دقيق القمح (استخلاص ٧٢%) في تصنيع البسكويت منخفض التكاليف عالي القيمة الغذائية. وتمت دراسة تأثير عملية الأستبدال بهده النسب علي الصفات الكيميائية والتكنولوجية والحسية بالأضافة للقيمة الغذائية للبسكويت الناتج. أظهرت النتائج ارتفاع محتوي دقيق البقوليات من البروتين تروا‌حت ما بين ٢-٥ مرات مثل دقيق القمح. كما اشارت النتائج إلي ان البقوليات كانت أغني من دقيق القمح في محتواها من العناصر المعدنية، بينما كان اللبن الفرز المجفف الأغني في محتواه من الكالسيوم (٨٩٣.٢ ملجم/ ١٠٠) في حين كان التمر الأغني في محتواه من الفسفور(٦٧٦.٠٩ملجم/١٠٠). تميز كل من دقيق البقوليات واللبن الفرز المجفف ارتفاع محتواه في الأحماض الأمينية الأساسية الكلية ومن الحامض الأميني الليسسن والثريونين وفي مجموع الأحماض الأمينية العطرية (الأروماتية) مقارنة بدقيق القمح والتمر واشارت النتائج إلي أرتفاع المحتوي من العناصر المعدنية لعينات البسكويت التي تم فيها عملية إستبدال بدقيق البقوليات او التمر وترجع هذه الزيادة لأرتفاع محتوي هذه المواد من العناصر المعدنية، مما يرفع من القيمة الغذائية للبسكويت الناتج والبسكويت المصنع بإستبدال دقيق البقوليات تميز بارتفاع محتواه في الأحماض الأمينية الليوسين وفي مجموع الأحماض الأمينية العطرية (الأروماتية) والتربتوفان، في حين نقص محتوي العينات في الليسين مقارنة بنموذج الـــ FAO/WHO، لذا وجب زيادة نسبة الإستبدال بمصادر غذائية مكملة وكذلك حازت غالبية عينات البسكويت المصنعة بإستبدال دقيق القمح بدقيق البقوليات علي درجة عالية من القابلية الحسية بالنسبة للمستهلكين كما أشارت النتائج إلي انه في حالة الفئران المغذاة علي وجبات بها بسكويت يحتوي علي إستبدال لدقيق القمح حدثت تغيرات معنوية تمثلت في زيادة مستوي الهيموجلوبين وعدد كرات الدم الحمراء وحدث زيادة معنوية في نسبة عنصر الحديد في السيرم وفي قدرة السيرم الكلية علي ربط الحديد.