Consumption of Bread Fortified with Vitamins and Minerals Improves Biochemical Nutrient Levels of Healthy Adults: A Pilot Randomised Clinical Trial

Vitamin ve Mineraller ile Zenginleştirilmiş Ekmek Tüketimi Sağlıklı Yetişkinlerin Biyokimyasal Besin Ögesi Düzeylerini İyileştirir: Pilot Randomize Klinik Çalışma

Gül Biringen Löker[1], Filiz Açkurt[1], Gülçin Satır[1,2], Birdem Amoutzopoulos[1]

[1] *TUBİTAK, Marmara Research Centre (MRC), Food Institute (FI), Gebze, Turkey*
[2] *Süleyman Demirel University, Engineering Faculty, Department of Food Engineering, Isparta, Turkey*

ABSTRACT

Aim: *The main objective of this pilot randomised clinical study was to determine the effects of vitamin and mineral fortified commercial Turkish bread on some blood parameters of healthy adults.* ***Subjects and Methods:*** *Twenty-nine healthy adults were participated into the study and divided into two groups: trial (n=16) and control (n=13). Trial group consumed fortified bread (176 g/day) as part of their usual diet for 73 days while control group did not change their diet. Biochemical parameters were analysed in blood samples at the beginning and end of the intervention period.* ***Results:*** *In the trial group, significant increase was determined in blood levels of vitamins B_1, B_2, B_6, B_{12} and C and minerals iron, calcium, zinc at the end of the intervention period ($p<0.05$). Change in serum folic acid levels was not significant. Daily consumption of vitamin and mineral fortified commercial Turkish bread led to remarkable increase in the blood vitamin and mineral concentrations, apart from folic acid.* ***Conclusion:*** *Although this pilot study has several limitations in terms of study design, the study results indicate a need for the long term and large scale randomised studies for a clear definition of the effects of fortified bread in daily nutrition.*

Keywords: *Food fortification, micronutrients, clinical research, bread*

ÖZET

Amaç: *Bu randomize klinik pilot çalışmanın ana amacı vitamin ve mineraller ile zenginleştirilmiş ticari bir Türk ekmeğinin sağlıklı yetişkin bireylerin bazı kan parametreleri üzerindeki etkilerinin belirlenmesidir.* ***Bireyler ve Yöntem:*** *Yirmi dokuz sağlıklı yetişkin birey çalışmaya katılmış ve iki gruba ayrılmıştır: Deney (n=16) ve kontrol (n=13). Yetmiş üç gün boyunca, deneme grubu günlük diyetlerine ek olarak zenginleştirilmiş ekmek (176 g/gün) tüketmiş, kontrol grubu diyetinde değişiklik yapmamıştır. Biyokimyasal vitamin ve mineral parametreleri müdahale sürecinin başlangıcında ve sonunda, toplanan kan örneklerinde analiz edilmiştir.* ***Bulgular:*** *Uygulama sonunda, deneme grubunun bazı kan vitamin (B_1, B_2, B_6, B_{12} ve C vitaminleri) ve mineral (demir, kalsiyum, çinko) düzeylerinde önemli artışlar saptanmıştır ($p<0.05$). Serum folik asit düzeylerindeki değişim önemli bulunmamıştır. Vitamin ve mineral ile zenginleştirilmiş ticari Türk ekmeğinin günlük tüketimi, folik asit hariç, kan vitamin ve mineral konsantrasyonlarında belirgin artışlara neden olmuştur.* ***Sonuç:*** *Bu pilot çalışmanın, çalışma dizaynı açısından pek çok kısıtlamaları olsa da, araştırma sonuçları günlük diyette zenginleştirilmiş ekmeğin etkisinin daha net bir şekilde tanımlanabilmesi için uzun süreli ve büyük ölçekli randomize çalışmaların gerekliliğini işaret etmektedir.*

Anahtar kelimeler: *Gıda zenginleştirme, mikro besin ögeleri, klinik araştırma, ekmek*

INTRODUCTION

It has been well known that vitamin and mineral deficiencies affect about 1/3 of the world population for a long time. Inadequate intake of vitamin and mineral may cause decrease in learning ability and increase the risk of infections, mental retardation, low working capacity, anaemia and birth defects (1). Particularly micronutrient deficiency is one of the most significant public nutrition based health problem for various risk groups in Turkey (2,3). In some of the industrialized countries, food fortification programmes solved the nutrition deficiency problem by fortifying staple foods with specific nutrients (4).

İletişim/Correspondence:
Gül Biringen Löker
TUBİTAK Marmara Research Centre (MRC) Food Institute (FI), PK 21, 41470, Gebze, Kocaeli, Turkey
E-posta: *Gul.Biringen@tubitak.gov.tr*
Geliş tarihi/received: *20.01.2014*
Kabul tarihi/accepted: *24.04.2014*

The main objective of food fortification studies is to fortify widely consumed foods by deficient nutrients for certain population groups or for the whole population. Food fortification must also be low-cost, and the sensory characteristics of foods should not be altered by fortification (5). Fortified bread used for food fortification programmes in several countries, as bread is a commonly consumed food within all ages in the population. And the bread fortification has a practical application compared to other food products in the view of standardisation (6,7).

In 1974, bread was one of the staple foods of Turkey and its average daily consumption was about 350-400 g (8). According to the recent records of "Survey on Bread Waste of Turkey" bread is still the staple food of Turkey however daily consumption of the product is decreasing gradually. The daily consumption of bread was estimated about 331 g in 2008 and 319 g in 2012 (9). In Turkey there has been several debate considering bread fortification programmes. A committee on food fortification was founded in 2003 by the collaboration of Ministry of Development and other organisations. This committee took further action plans on food fortification practices in Turkey (10). The food industry has fortified a range of foods voluntarily. However, the lack of consumer and government information on the prevalence of nutrient deficiencies adversely effects to take strategic decisions on food fortification (5). Nowadays, bread is fortified with nutrients in commercial basis and not for the public in Turkey (11). Recently published Report of Turkey Nutrition and Health Survey indicate deficient intake of some nutrients in specific population groups of Turkey (12). These recent results can raise the awareness about micronutrient deficiencies and interventions to address and take preventive actions, specifically by food fortification. In 2005, a pilot study for commercial bread fortification was carried out on commercial basis (13). The publication of the results of this previous study could be used as a reference for governmental bodies or further intervention studies on food formulation in Turkey. This presented study aimed to determine the effects of commercial Turkish bread (CTB) fortified with specific vitamins and minerals on specific nutrients status in blood and in other biochemical parameters of healthy adults.

SUBJECTS and METHODS

The sample size of study groups was calculated according to a previous study about the effects of folic acid fortified wheat cereals on serum folate status of women (14). According to the two-sided paired sample t-test, it was estimated that a sample size of 15 would achieve 90% power to detect a significance difference in folate levels (α=0.05). Thirty-four healthy adults were enrolled in the study based on the following criteria: 1) general good health 2) aged between 25 and 45 years; 3) having Body Mass Index (BMI) between 20–25 kg/m^2; 4) consuming ≤2 alcoholic beverages/ week; 5) not diagnosed with any chronic diseases; 6) not taking medications; 7) not following a special diet (eg. vegetarian) and planning to change dietary habits; 8) not allergic to any foods and 9) not taking multivitamins or other dietary supplements before and during the study (13,15). After explanation of the nature of the study, written informed consent was read and signed by the participants. The subjects were randomly divided into 2 groups: a trial group (TG, n=17) and a control group (CG, n=17). Of the 34 subjects included, 5 subjects withdrew from the study due to illness, lost to follow up or not being able to give blood.The physical characteristics of the 29 participants included in the study are as follows; TG, male: 8 female: 8; age: 33.31±5.0 years, BMI: male 25.00±0.55, female: 22.16±2.37 and CG, n=13; male: 7, female: 6; age, 32.77±7.33 years, BMI: male 21.45±2.57, female: 21.87±0.84. The study was conducted in 2005 by a research team of TUBITAK MRC, in accordance with The Helsinki Declaration II and approved by the report of The Ethical Committee of Marmara University, School of Medicine (No: MAR-YÇ-2004-0142). During seventy-three days of intervention period, TG consumed 8 to 10 slices (176 g/day) of fortified commercial Turkish bread (FCTB) as part of their usual diet, whereas CG was assigned to follow their usual diet (16). An industrial bread manufacturer provided the FCTBs. FCTB was fortified with seven vitamins: vitamin B_1, B_2, B_6, B_{12} C, niacin, folic acid and three minerals: iron, calcium, zinc. The nutrient composition of FCTB used in the

study are detaied in Table 1. The 24 hours food record and food frequency questionnaire were obtained at baseline of the intervention by the dietitian. All subjects were asked to continue their regular diet during the study. Subjects were instructed to avoid intake of fortified products and any type of nutrient supplements during the study. Systolic and diastolic blood pressure, height (subjects standing, feet and heels together, buttocks and the top of the back against to immobile tape) and body weight (subjects wearing the minimum amount of clothing) of the participants were measured both at the beginning and at the end of the intervention period.

Collection of blood samples, biochemical analysis and statistical analysis

A venous blood sample (33 mL) was collected after a 12-h overnight fast at the beginning and end of the intervention period. As indicator of supplemented vitamins and minerals; vitamin B_1, B_2, B_6, B_{12}, C, and folic acid, niacin, iron, zinc and calcium analyses were conducted in the collected blood samples. For the assessment of any indirect effect of fortified bread; haemogram, blood lipid profile (total cholesterol, triglyceride, HDL and LDL cholesterol), blood glucose, urea and alkaline phosphatase analyses were also performed. Whole blood samples (2 mL) were transferred to EDTA containing tubes for haemogram tests. Blood samples (4 mL) were taken into gel separation tubes for assay of blood glucose, lipids, urea and alkaline phosphatase. Blood samples (10 mL) were taken into aluminium wrapped tubes containing EDTA for the determination of vitamins B_1, B_2, B_6 and centrifuged 10 minutes at 4500 g. The upper phases were transferred into Eppendorf tubes wrapped with aluminium foil (1 mL) and stored at -40°C. Blood samples (5 mL) for the determination of vitamin C were taken into lithium-heparinized tubes and centrifuged 10 minutes at 10.000 g, the upper phases were put into Eppendorf tubes wrapped with aluminium foil and stored at -40°C. Blood samples (10 mL) for the determination of minerals were taken into polyethylene tubes, centrifuged 10 minutes at 4500 g. The upper phases transferred into 10 mL tubes and stored at +4°C. Blood samples (4 mL) for the determination of vitamin B_{12}, folic acid and niacin were taken into gel separation tubes, centrifuged 15 minutes at 4000 g and stored at +4°C. After bringing to the room temperature, the blood samples were analysed.

The System XT-2000i blood counter measured haemogram with flouroscence flow cytometry method (17). Blood lipids (total cholesterol, HDL, LDL), triglyceride, urea, alkaline phosphatase and fasting blood glucose were measured by using a DADE Behring Dimension RxL Chemistry variability analyzer, with reagents and calibrators supplied by Dade Behring Diagnostics (Sydney, Australia) (18-22). High performance liquid chromatography (HPLC) was used to determine plasma vitamin B^1, B^2, B^6 and niacin (Recipe Chemicals&Instruments GmbH, Munich, Germany) and vitamin C (HPLC-Analytic, Immundiagnostic AG, Bensheim, Germany) (23-27). Serum levels of iron, zinc and calcium were measured by Atomic Absorption Spectrophotometry A Analyst 700 equipped with a FIAS 100 flame injection system and a hollow cathode lamp power supply (Perkin Elmer, Norwalk, CT) (28,29). Serum vitamin B12 (30) and folic acid levels were measured with a chemiluminescence immunoassay (Roche diagnostics, Germany) on an Elecsys 2010 automated analyzer (Roche Diagnostics, Germany) (30). Statistical analyses were undertaken with SPSS 13.0 soft-ware (SPSS Inc., Chicago, IL, USA). A paired t test was used to compare the differences within group. The comparison of the differences between and within groups was tested by the repeated major ANOVA technique.

RESULTS

According to the study results, no significant changes of BMI were determined in both groups at the start and end of the study. The assessment of the food records completed at the beginning of the study period are given in Table 2. Based on the assessment of food frequency questionnaires, 42% and 53% of the participants were consuming vegetable and fruits each day respectively, while 75% and 53% were consuming milk and dairy products. Participants were consuming meat, meat products and legumes more than one time in a week. The daily consumption of tea (83%) and coffee

(88%) was high among the participants. Most of the participants were consuming white bread (67%) in their daily diet. According to the fortification levels of FCTB, some blood vitamin (vitamins B_1, B_2, B_6, B_{12}) and mineral (iron, zinc, calcium) levels of the subjects in TG ($P<0.05$) increased significantly while folic acid levels did not change after the intervention period. Although it was not-significant, the niacin levels of TG increased. At the end of the study, the mean blood vitamin B_1, B_2, and folic acid, iron and calcium levels differed significantly between the subjects of CG

Table 1. Energy and nutrient composition of FCTB and its vitamin-mineral contribution as percentage of Recommended Dietary Allowances (RDA)*

Energy and nutrients	FCTB (100g)	FCTB (RDA %)
Energy (kcal)	245	-
Water (g)	37.5	-
Ash (g)	1.26	-
Protein (g)	7.83	-
Fat (g)	1.58	-
Dietary fibre (g)	1.9	-
Carbohydrate (g)	49.93	-
Vitamin B_1 (mg)	0.39	30
Vitamin B_2 (mg)	0.47	31
Vitamin B_6 (mg)	0.51	28
Vitamin B_{12} (mg)	0.63	32
Niacin (mg/100g)	5.1	30
Folic acid (mcg)	58.67	31
Vitamin C (mg)	17.2	29
Iron (mg)	4.2	32
Calcium (mg)	286	36
Zinc (mg)	4.05	29

**Average values for adults*

Table 2. Average daily nutrient intake of the CG (n=13) and TG (n=16) at the beginning of intervention*

Energy and nutrients	CG		TG	
	M	F	M	F
Energy (kcal)*	2888	1727	1749	1851
Protein (g)	122.29	69.95	64.65	79.91
Fat (g)	107.59	71.61	69.96	78.03
Carbohydrate (g)	348.59	200.68	215.11	207.25
Dietary fibre (g)	28.06	24.49	22.76	19.25
Monounsaturated fatty acids (g)	38.08	27.58	24.57	27.81
Polyunsaturated fatty acids (g)	25.17	14.28	11.26	11.70
Cholesterol (mg)	305.89	199.49	162.72	218.8
Carotene(mg)	3.04	3.87	4.57	3.47
Vitamin A (µg)	1235.9	1155.2	1446.9	1125.0
Vitamin E (mg)	10.19	9.32	7.72	7.65
Vitamin B_1 (mg)	1.11	0.93	1.18	1.29
Vitamin B_2 (mg)	1.81	1.22	1.71	1.95
Niacin (mg)	24.1	14.57	17.50	19.85
Vitamin B_6 (mg)	1.58	1.47	1.77	1.95
Vitamin B_{12} (µg)	1.74	1.76	2.96	2.46
Pantothenic acid (mg)	5.74	4.45	4.10	4.42
Biotin (µg)	45.61	30.33	29.15	29.48
Folic acid (µg)	364.59	295.95	289.77	287.25
Vitamin C (mg)	68.28	71.43	82.05	87.36
Iron (mg)	10.09	10.99	15.26	16.11
Calcium (mg)	796.16	817.69	1000.64	1227.53
Zinc (mg)	16.56	9.06	14.11	16.74
Sodium (mg)	4954.3	3523.2	3726.27	4098.14
Potassium (mg)	3076.6	2869.3	2160.32	2341.62
Magnesium (mg)	427.44	305.14	273.85	249.28
Phosphorous (mg)	810.2	856.8	1026.26	1175.10
Copper (mg)	2.77	1.82	1.68	1.75
Manganase (mg)	3.92	4.31	4.94	4.87
Iodine (µg)	198.34	157.5	128.29	152.38

**M=Male, F=Female*

Table 3. The biochemical findings of the CG (n=13) and TG (n=16)*

	CG (n=13)				TG (n=16)			
	1	SD	2	SD	1	SD	2	SD
Glucose (mg/dL)	82.62	18	83.92	6.96	82.43	7.43	81.36	6.08
Total cholesterol (mg/dL)	183.36†	21.53	182.09†	23.11	208.36	30.89	207.21	23.48
LDL cholesterol (mg/dL)	111.09†	23.27	116.27	26.62	133.14	28.78	133.07	17.56
HDL cholesterol (mg/dL)	50.54	11.21	54.08	13.48	49.5	11.2	50.86	10.91
Total cholesterol /HDL cholesterol	3.86	1.22	3.75	0.95	4.39	1.03	4.35	1.11
Triglyceride (mg/dL)	138.92	108.98	103.46	35.1	128.64	51.14	122.43	39.95
Urea (mg/dL)	25.69	3.71	26.62	3.31	27	7.21	25.43	5.17
Alkalen phosphatase (ALP) (U/L)	87.46	14.3	86.85	16.07	85	18.91	88.64	15.14
Erythrocyte (mil/µL)	4.85	0.43	5.01‡	0.48	4.71	0.48	4.95‡	0.45
Haemoglobin (g/dL)	14.08	1.32	14.99‡	1.54	13.79	1.42	14.79‡	1.33
Haematocrit (%)	41.18	3.88	42.04	3.63	40.49	3.69	42.5‡	3.34
Leukocyte (1000/µL)	6.6	1.33	6.38	1.72	6.96	2.45	6.11	1.66
Thrombocyte (1000/µL)	248.77	45.02	217.46‡	44.95	251.78	76.75	219.85‡	62.04
Vitamin B_1 (µg/L)	49.34	13.14	50.54†	13.19	48.92	13.75	61.27‡	13.1
Vitamin B_2 (µg/L)	116.24	12.57	116.59†	14.04	113.61	10.04	138.88‡	13.48
Vitamin B_6 (µg/L)	20.32	4.76	20.02	3.89	18.45	4.69	26.44‡	10.98
Niacin (µg/L)	17.1	6.21	17.43	5.91	19.3	6.47	21.43	8.6
Folic acid (ng/mL)	8	2.77	6.15†‡	1.68	8.94	4.39	9	4.03
Vitamin B_{12} (pg/mL)	424.69	210.61	425.85	195.43	391.88	225.99	491.75‡	260.72
Vitamin C (mg/L)	6.1	2.67	6.28	1.93	4.37	2.71	5.8‡	2.4
Iron (mg/L)	1.44	0.24	1.47†	0.21	1.4	0.28	1.78‡	0.24
Calcium (mg/L)	116.7	7.64	111.66†	8.26	113.8	14.53	134.58‡	13.28
Zinc (mg/L)	1.57†	0.21	1.61†	0.17	1.2	0.12	1.87‡	0.23

**SD: Standard deviation, 1- The start of study, 2-The final of study, † Significant differences between group means (P < 0,05), ‡ Significant differences within groups (p < 0,05)*

and TG ($p<0.05$). Some blood vitamin (vitamins B_1, B_2, B_6, B_{12}) and mineral (iron, zinc, calcium) levels did not changed in CG, although folic acid levels decreased significantly ($p<0.05$) (Table 3).

In this study, other biochemical blood parameters such as glucose, total cholesterol, HDL cholesterol, LDL cholesterol, triglyceride, urea, alkaline phosphatase, erythrocyte, leucocyte, and thrombocyte levels were not changed significantly. Thus, other parameters related with bread consumption except vitamins and minerals used for fortification would not be responsible for the effects of FCTB on blood nutrients (Table 3).

DISCUSSION

At the end of the intervention trial statistically important changes were observed for most of the blood nutrients (Table 3).

The folic acid content of FCTB was 58.67µg/100g, which was about 30% of RDA of the normal adults (31,32) (Table 3). In a dietary trial, individuals were fed with bread fortified with 138 µg of folic acid, and the fortified bread was improved serum folate concentrations by 45% (33). During this study, due to the inadequate level of folic acid fortification or bioavailability, no significant change was observed in the serum folic acid concentrations of TG. In various countries with inadequate folic acid intake, folic acid fortification of staple foods like bread and grain products is supported by food laws (34-37). In this study, FCTB was formulated in the view of RDA levels for adults (31), nutrient composition of bread ingredients, production process, potential nutrient-nutrient interaction and prevalence of nutrient deficiencies in Turkish population.

In 1996, FAO reported folic acid in combination with vitamin B_6 and vitamin B_{12} to decrease plasma homocysteine levels which is inversely associated with several health risks such as developing ischemic heart diseases (4). In a public survey conducted in 1998, flour and cereal grain products were fortified with vitamin B complex, folic acid and iron, ultimately, various health impacts such as decline in neural-tube defects were observed in the same population according to the follow-up studies (38). In this study CTB was fortified with vitamin B complex (B_1, B_2, B_6,

B_{12}) and folic acid and significant changes were observed in TG as expected ($p<0.05$) (Table 3).

Iron deficiency anaemia is particularly common in the countries where diets are cereal-based (39). The strategy of iron fortification of food is used in many developed countries to prevent iron deficiency (40,41). Therefore, CTB was also fortified with iron. In TG, significant increases were determined on serum iron levels at the end of the intervention study ($p<0.05$).

The iron bioavailability of the foods fortified with iron is not only associated with the product, it is also in relation with the total dietary composition. Ascorbic acid is one of the most important factors increasing the iron absorption. In this study, blood haemoglobin and haematocrit levels of TG increased which might be supported by the positive effects of vitamin C in the content of FCTB on the iron bioavailability (41). Vitamin C was added into the formulation to increase the iron bioavailability of bread. In addition, vitamin C content of bread was providing approximately 30% of RDA levels for adults. Fortification of CTB with vitamin C can be a factor supporting the increase in blood haemoglobin and haematocrit levels of TG ($p<0.05$) (Table 3).

Calcium intake may have a negative effect on both nonheme- and heme-iron absorption, as shown by several studies. This effect of calcium was observed particularly on the diets deficient in iron. It is highlighted that this negative effect of calcium should be taken into consideration whereas iron deficiency is one of the common problems especially for the specific risk groups in developing countries (42,43). In this study, it was observed that iron absorption was not affected negatively although the calcium level of the fortified bread formulations was higher than 200 mg/100g CTB (Table 3).

According to an analysis on national food supplies, it was seen that the diets of ~20% of the global population are zinc-deficient. In many developing countries, zinc deficiency symptoms appear because of low consumption of animal-based foods that are the rich source of zinc and high intake of cereals and legumes that contain substantial amount of phytates that may inhibit zinc absorption (44). At the end of this study, serum calcium and zinc levels of TG were found over the anticipated levels. The increase on the blood concentrations of these minerals is unexpected which would be caused by the inadequate number of subjects in TG.

One of the limitations of this study was the lack of following dietary nutrient intakes. As this study was in a pilot scale dietary intake was evaluated only at the beginning of study. In further clinical trials it is necessary to observe the dietary intake of participants in order to determine the effect of food supplementation more clearly.

The present study indicates the effects of daily consumption of a fortified bread on some nutritional and biochemical markers in healthy adults. The daily consumption of fortified bread elevated plasma concentrations of nutritional parameters in subjects consuming FCTB except some nutrients such as folic acid and niacin. It would thus indicate the importance of clinical trials for testing the efficiency of formulation of fortified products. This pilot study would be used as a source for food formulation studies and developing National Public Policies to prevent nutritional deficiencies (45). Fortification of commercial bread with vitamin and minerals may improve the blood levels of nutrients and this can be considered as an effective public health approach for the control and prevention of widespread nutrient deficiencies in Turkey. Although this study has several limitations in terms of study design, the results warrant the long term and large-scale randomised studies to confirm the effects of fortified bread in daily diet.

Acknowledgement/Teşekkür: *We thank TUBITAK MRC research team; study nurse, Mrs. Münevver Yılmaz and research technicians, Şenol Erdoğan, Tamer Işık and Muhlis Tekel for their efforts during the study and Ege University, Faculty of Medicine, Biostatistics and Medical Informatics Department for the statistical evaluation of the study. The authors also thank to Assoc. Prof. Dr. Cesarettin Alaşalvar from TUBITAK MRC for reviewing the manuscript.*

Conflict of interest/Çıkar çatışması: *There is no conflict of interest.*

Funding sources/Maddi destek: *This study was supported by TÜBİTAK MRC Food Institute Project no: 5044106, 2005.*

Author contributions/Yazarlık katkısı: *GBL, project director has the supervising responsibility for all aspects of this project, performed the data collection, evaluation and the writing of the manuscript, FA, project consultant, advised on study design and reviewed the manuscript, GŞ performed mineral analysis and data compilation, BA contributed on the writing and reviewing of the manuscript.*

REFERENCES

1. WHO. Fortification of flour to control iron deficiencies in the Middle East and North Africa. Report of a Joint WHO/UNICEF/MI/ILSI Workshop Beirut, 13-16 June 1998. Alexandri, WHO Regional Office for the Eastern Mediterranean; 1999.
2. Açkurt F, Wetherilt H, Löker M, Hacıbekiroğlu M. Biochemical assessment of nutritional status in pre-and post-natal Turkish women and outcome of pregnancy. Eur J Clin Nutr 1995;49:613-622.
3. Pekcan G. Food and nutrition policies: what's being done in Turkey. Public Health Nutr 2006;9(1A):158–162.
4. FAO. Food Fortification: Technology and Quality Control. Rome, FAO Food and Nutrition Paper; 1996.
5. Liberato SC, Pinheriro-Santana HM. Fortification of industrialized foods with vitamins, Rev Nutr, 2006; 19 (2), 215-231.
6. Hertrampf E, Cortes FF, Erickson JD, Cayazzo M, Freire W, Bailey LB, Howson C, Kauwell GA, Pfeiffer C. Consumption of folic acid–fortified bread improves folate status in women of reproductive age in Chile, J Nutr 2003;133: 3166–3169.
7. Czeizel AE. Bread is fortified with folic acid in Hungary, BMJ 2002;325(7360):391.
8. Köksal O. Nutrition in Turkey-national nutrition-health and food consumption survey of Turkey 1974. Ankara, UNICEF; 1977.
9. TMO. Türkiye'de ekmek israfı araştırması. Ankara, Toprak Mahsulleri Ofisi Genel Müdürlüğü, Ocak, 2013.
10. DPT. Ulusal gıda ve beslenme stratejisi çalışma grubu raporu. Ankara, DPT; 2003.
11. UNICEF. UNICEF and the Flour Fortification Initiative Support Improving Iron Status through Flour Fortification. Press Centre; 6/2012. Available online at http://www.unicef.org.tr/basinmerkezidetay.aspx?id=2244&dil=en&d=1
12. TBSA. Turkey Nutrition and Health Survey 2010. Ankara, Republic of Turkey Ministry of Health, General Directorate of Health Surveys, Hacettepe University Faculty of Health Sciences Department of Nutrition and Dietetics;2014. Available online at http://www.sagem.gov.tr/TBSA_Beslenme_Yayini.pdf
13. Löker GB, Açkurt F, Toklu G. Project report-5034149. Gebze, TÜBİTAK Marmara Research Center; 2003.
14. Johansson M, Witthoft CM, Bruce A, Jagerstad M., Study of wheat breakfast rolls fortified with folic acid-The effect on folate status in women during a 3-month intervention, Eur J Nutr 2002; 41(6): 279-286.
15. Donald S, Young MB. Implementation of SI units for clinical laboratory data. Ann Intern Med 1987;106:114-129.
16. Hansen M, Samman S, Madsen LT, Jensen M, Soresen SS, Sandström B. Folic acid enrichment of bread does not appear to affect zinc absorption in young women. Am J Clin Nutr 2001;74:125-129.
17. Oshiro I, Takenaka T, Maeda J. New method for haemoglobin determination by using sodium lauryl sulfate (SLS). Clin Biochem 1982;15:83-88.
18. Flegg HM., An investigation of the determination of serum cholesterol by an enzymatic method, Ann Clin Biochem 1973; 10:70-84.
19. Roschlou P. Enzymatic bestimung des gesamtcholesterins in serums. J Clin Chem Biochem 1974;12:403-407.
20. Kunst A. UV methods with hexokinase and glucose-6-phosphate dehydrogenase, Meth Enzym Anal 1983;6:163-172.
21. Talke H, Schubert GE. Enzymaticsche. Blood and serum in optischen test nach Warburg, Klin Wschr 1965;41:174.
22. Bowers GN, McComb RB. A continuous spectrophotometric method for measuring the activity of serum alkaline phosphatase, Clin Chem 1966;12:70.
23. Clin Rep. Instruction manuel for the determination of Vitamin B1 in whole blood by HPLC, Version, 1.3.05/04. Munich, Recipe chemicals+instruments GmbH; 2004.
24. Clin Rep. Instruction manuel for the determination of Vitamin B_2 in whole blood by HPLC, Version, 1.0, 08/02. Munich, Recipe chemicals+instruments GmbH; 2002.
25. Clin Rep, Instruction manuel for the determination of Vitamin B6 in plasma/whole blood by HPLC, Version, 1.3. 02/03. Munich. Recipe chemicals+instruments GmbH; 2003.
26. Hofmann P, Brodhag C, Schüep W. Determination of B vitamins in tablets-version 1.2, Roche Vitamins Ltd, pp. 1/5-5/5; 1988.
27. Immundiagnostik AG, Kit-Vitamin C for colorimetric determination of Vitamin C in Li-heparine plasma, serum and urine, Bensheim; Immundiagnostik AG, 2005.
28. AOAC. Official Methods of Analysis of the Association of Official Chemists, 15th ed. Gaithersburg, AOAC; 1995.
29. AOAC. Official Methods of Analysis of AOAC International, 7th ed. Gaithersburg, AOAC; 2000.
30. Roche. Elecsys 2010 System Menu. Germany, Roche Diagnostics; 2005.
31. National Research Council. Subcommittee on the 10th ed. of the RDAs. Washington, National Academy Press; 1989.
32. Baysal A. Beslenme. Ankara, Hatipoğlu Yayınevi; 1996.
33. Winkels R. Dietary folate intake: is there a need for folic acid fortification in the Netherlands? Ph.D. Thesis. Wageningen, Wageningen University; 2008.
34. Rader JI, Weaver CM, Angyal G. Total folate in enriched cereal-grain products in the United States following fortification. Food Chem 2000;70:275-289.
35. Finglas PM, Witthöft CM, Vahteristo L, Wright AJA, Southon S, Mellon FA. et al. Use of an oral/intravenous dual-label stable-isotope protocol to determine folic acid bioavailability from fortified cereal grain foods in women. J Nutr 2002;132:936-939.
36. Cho S, Johnson G, Song WO. Folate content of foods: comparison between databases compiled before and after new FDA fortification requirements. J Food Compost Anal 2002;15(3):293-307.
37. Mills JL, Von Kohorn I, Conley MR, Zeller JA, Cox C, Williamson RE et al. Low vitamin B12 concentrations

in patients without anemia: the effect of folic acid fortification of grain. Am J Clin Nutr 2003;77:1474-1477.
38. Tulchinsky TH, Varavikova EA. The new public health. 2th ed. London, Elsevier Academic Press; 2009.
39. Zimmermann MB, Chaouki N, Hurrell RF. Iron deficiency due to consumption of a habitual diet low in bioavailable iron: a longitudinal cohort study in Moroccan children. Am J Clin Nutr 2005;81:115-121.
40. Ballot DE, Macphail AP, Bothwell TH, Gillooly M, Mayet FG. Fortification of curry powder with NaFe (111) EDTA in an iron-deficient population: report of a controlled iron-fortification trial. Am J Clin Nutr 1989;49:162-169.
41. Nayak B, Nair KM. In vitro bioavailability of iron from wheat flour fortified with ascorbic acid, EDTA and sodium hexametaphosphate, with or without iron. Food Chem 2002;80:545-550.
42. Hallberg L. Does calcium interfere with iron absorption? Am J Clin Nutr 1998;68:3–4.
43. Mendoza C, Pearson JM, Brown KH, Lönnerdal B. Effect of a micronutrient fortificant mixture and 2 amounts of calcium on iron and zinc absorption from a processed food supplement. Am J Clin Nutr 2004;79:244-250.
44. De Ramana DL, Lönnerdal B, Brown KH. Absorption of zinc from wheat products fortified with iron and either zinc sulphate or zinc oxide. Am J Clin Nutr 2003;78:279-283.
45. Alp A, Ergün C. Evaluation of nutritional policies of some developed countries. Journal of Nutrition and Dietetics 2013;41(1):83-90.