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FORTIFICATION OF CERTAIN DAIRY PRODUCTS TO INCREASE BIOAVAILABILITY OF MINERALS AND ANTIOXIDANTS

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ABSTRACT

The present study aimed to determine in vitro iron (Fe), zinc (Zn) and calcium (Ca) bioavailability of some dairy products. This study used date syrup, pomegranate syrup and sesame paste (tahina) natural sources to fortify the yoghurt and fermented milk (Rayb). Total phenols and antioxidant activity contents of samples were analyzed. Bioavailability ofFe, Zn and Ca in the samples were determined by an in vitro method as gastrointestinal digestion. All data were analyzed statistically. The results showed that sesame paste (tahina) had the highest level of total phenols. The fermented milk had the highest level of total antioxidant activity (96.387%) by DPPH. However, yoghurt had the highest level (89.910%) by ABTS. Addition of fruit syrup or tahina to yoghurt resulted in increase in total phenols and antioxidant activity. Moreover, the results showed that, the date syrup had the highest percent of Fe bioavailability (40.863%). The maximum bioavailability of Zn was found in low fat milk and fermented milk (59.992% and 59.780%, respectively). Meanwhile, the fermented milk had the highest bioavailability of Ca (59.072%), then yoghurt (54.622%). Generally, fortification by fruit syrup or tahina resulted in an increase in Fe, Zn and Ca bioavailability. The yoghurt which fortified by 5% date syrup had the highest of Fe bioavailability of Zn (38.454% and 44.444%, respectively). While fortification by tahina (both white and red) had the highest percent of Ca bioavailability (71.913% and 73.855%, respectively).

INTRODUCTION

In general, the addition of one or more essential nutrients to food to levels higher than normal is aimed to preventing and correcting deficiencies in one or more nutrients in the society or specific population groups (Bonner et al., 1999).It could be mentioned that fortification of food products by fruits or cereal, is one of the ways used to improve the overall nutrient intake of food, and minimize the side effects (Nestle, 2013).

Fermentation has been used to provide longer shelf life for perishable foods and to improve the flavor and odor of fermented food products (Buckenhüskes, 1993).. As the result of fermentation, more digestible, stable and flavored foods with enhanced nutritional value are formeded. During the growth of the microorganisms responsible for fermentation ,i.e. molds, yeast or bacteria, the desired flavor and aroma are resulted in (Yousef andCarlstrom, 2003). Yogurt is more nutritious and considered as an excellent source of protein, calcium, phosphorus, riboflavin, thiamin, vitaminB12, folate, niacin, magnesium and zinc (Gahruie et al., 2015). Moreover, consumption of fermented milk products slightly decrease the pH of stomach with consequent reduction of the risk of pathogen transit (O'connell and Fox, 2001). Since fermented milk products are among highly-consumed food in the world, they have been used to deliver nutritional components into human diet.

In vitro bioaccessibility/bioavailability methods are useful to supply knowledge on the interactions between nutrients and food components. The effects of pH and enzymes on either micronutrient absorbability or bioaccessibility) (Etcheverry et al., 2012).

Plant foods (fruits and vegetables) were found to possess several benefits, closely related to their high contents of vitamins and other compounds such as vitamin C, carotenoids, phenolic compounds, vitamin E, with antioxidant properties (Carbonell-Capella et al., 2013).

Vitamin D was found to increase calcium bioavailability and the absorptionof iron and zinc. Calcium and vitamin D, on the other side, when fortified in combination, the iron and zinc bioavailability was increased Kaushik et al.(2014). There was

MATERIALS AND METHODS

Buffalo's milk was obtained from the Faculty of Agriculture, Cairo University. Fresh date, pomegranate fruits and sesame paste (tahina) were purchased from local market, Giza at Governorate.

Pure culture for yoghurt of Str. Salivarius sub sp. thermophilsand Lactobacillus delbrueckii subsp. bulgaricus1:1 and pure culture for fermented milk of LactobacillusAcidophallus, Befidobifidumand Str. thermophiles 1:1:1 were obtained from Chr. Hansen Laboratories, Denmark.

Pepsin, pancreatin, α-amylase, lipase and cetylpyridinium bromide were purchased from Sigma–Aldrich Chemical Co. (St. Louis, USA) and bile extracts from Win Lab Laboratory chemicals reagents (Mumbai, India). All other chemicals used were of analytical reagent grade. Folin-Ciocalteu phenol reagent (2N), quercetindihydrate (2-, 3-,4- dihydroxyphenyl), Gallic acid as compounds standard,sodium carbonate (99.8%), 2,29-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

Preparation of fruits syrup "Dibis" and pomegranate syrup were prepared according to Arafa et al., (2006) and Arafa (2013) TSS = 78.1 % and kept in jars till use.

Standardized Buffalo's milk (0.9 % fat) was heated at 90°C for 10 min., cooled to 40°C. The active starter (2% w/w) and the fruit syrups and tahina were added as following;

a) For making the yoghurt, the date syrup (5%) was added to milk according to Mottwly, et al. (2010), pomegranate syrup (5%) was added to milk according to Abd El-Rahman (2011) and 1% of white (from dehulled seeds) or red (from whole seeds) sesame paste (tahina) were added to milk according to Hashish et al. (2014), mixed well and incubated at 40°C for 2-3 hours until coagulation. Yoghurt was refrigerated at 5°C till analysis.

b) For making yoghurt drink, white and red tahina (1%) was added to milk according to Hashish et al. (2014), mixed well and incubated at 40°C for 2-3 hours until coagulation. Yoghurt after storage at 5°C for 24hrs added 6% sugar and 0.15% vanilla and blended for 2 min. Then, storage in refrigerator at 5°C till analysis.

c) For making fermented milk (rayb, Date syrup (15%) was added to milk after starter according to Salem et al. (2008). Strawberry and Cinnamon bark were added separately as flavor agents and mixed well and incubated at 40°C for 2-3 hours until fermentation. Fermented milkwas refrigerated at 5°C till analysis.

Total phenols in all samples were determined by Folin-ciocalteau's reagent according to Arnous et al., (2001).Antioxidant activity was determined by (DPPH) method as described by Brand-Williams et al. (1995). The ABTS (2, 29-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)); radical scavenging capacity; this assay is based on the reductive decolonization of the radical form (ABTS), which is water soluble as described by (Gobba et al., 2014).

The bioavailability of calcium, iron and zinc were determined by in vitro digestion method as described by Garrett et al. (1999)>

Data were analyzed statistically using SPSS 19 program software (SPSS, 2007). Data were expressed as means ± SEM and the Statistical analysis was performed using one-way analysis of variance followed by Duncan's tests.

RESULTS AND DISCUSSION

The results in Table (1) showed that, total phenols and total antioxidant activity in ingredients which were use in this study. The greatest content of total phenols were found in sesame paste (tahina). While, the yoghurt had the lowest of total phenols. The present study determined total antioxidant activity by two methods: ABTS; this assay is based on the reductive decolonization of the radical form (ABTS), which is water soluble and DPPH; this assay is based on the reductive decolonization of the radical form (ABTS), which is alcohol soluble.The results showed that, total antioxidant activity by ABTS for pomegranate syrup, tahina and yoghurt were higher than total antioxidant activity by DPPH. This result may be due antioxidant compounds which were water soluble higher than alcohol soluble. Milk low fat and fermented milk had a high level of total antioxidant activity by DPPH. These results are a line with Benguo et al. (2011) who reported that the most predominant scavenging capacity of sesame seeds were phenol in seed hulls. Therefore, removal of the hulls decreased the reducing percent in white tahina as found in present study. These results are a line with Olagunju and Ifesan (2013) who found increasing of reducing percent for whole sesame seeds. And, also mentioned that, Hashish et al., (2014) addition of tahina resulted in an increase in the reducing percent of free radical scavenging capacity and total phenols contents

Table (1): Total phenols and antioxidant activity for ingredients:

Items	Total phenols (mg Gallic acid / 100g)	Total antioxidant activity (%)	
		DPPH	ABTS
Milk low fat	314.977e	93.377b	89.240a
Fermented milk	165.463f	96.387a	63.337d
Yoghurt	110.093g	29.513g	89.910a
Date syrup	415.473d	91.243c	74.090c
Pomegranate syrup	643.327c	48.453e	60.567e
White tahina	1806.283b	37.120f	45.967f
Red tahina	2006.250a	67.123d	76.100b

- **Each value in a column followed by the same letter are not significantly different at ($p \leq 0.05$).**

The results in table (2) explanted that, the fortification fermented milk by date syrup (15%) increased in total phenols and total antioxidant activity compared fermented milk alone. Also, the additional of cinnamon bark powder (0.3%) as flavored caused increase in total phenols and total antioxidant activity compared to concentrate strawberry juice as flavored (10%). Fortification by red tahina in both yoghurt and yoghurt drink had higher than white tahina for total phenols and total antioxidant activity. These results are line with Hashish et al., (2014) who found that, the fortification with tahina had increase in total phenols and total antioxidant activity.

Table (2): Total phenols and antioxidant activity for some dairy products:

Items	Total phenols (mg Gallic acid / 100g)	Total antioxidant activity (%)	
		DPPH	ABTS
Yoghurt with 1% white tahini	253.647h	95.210a	93.807a
Yoghurt with 1% red tahini	314.290f	90.430d	87.033d
Yoghurt with 5% pomegranate syrup	312.747f	94.523ab	90.280c
Yoghurt with 5% date syrup	281.810g	92.447c	92.833ab
Yoghurt drink with 1% white tahini	351.907e	83.877e	85.557ef
Yoghurt drink with 1% red tahini	369.823d	93.237bc	86.483de
Fermented milk with 15% date syrup	725.353c	94.460ab	84.910f
Fermented milk with 15% date syrup and flavored by Strawberry	873.070b	89.133d	89.660c
Fermented milk with 15% date syrup and flavored byCinnamon bark	1342.970a	95.320a	92.260b

*** Each value in a column followed by the same letter are not significantly different at (p≤0.05).**

The data in table (3) showed the Fe, Zn and Ca bioavailability contents in raw materials. The results indicated that, date syrup had the highest percent of Fe bioavailability (40.863%). While, pomegranate syrup had the lowest percent of Fe bioavailability (9.273%). The yoghurt sample had a high percent of Fe bioavailability (23.584%) compared to milk low fat and fermented milk (15.904% and 12.676%, respectively). The same table (3) showed the milk low fat was decrease when compared to fermented milk and yoghurt (31.38% and 21.48%, respectively). And, the red tahina had higher than white tahina of total Fe, Zn and Ca contents, but the white tahina had a higher percent of Fe, Zn and ca bioavailability than red tahina (about 52.5%, 25.17% and 41.19% for Fe, Zn and Ca, respectively).

Table (3): Bioavailability of Fe, Zn and Ca in vitro for ingredients (mg/100g):

Minerals / Ingredients	Fe			Zn			Ca		
	Total	Bioavailability		Total	Bioavailability		Total	Bioavailability	
	mg	mg	%	mg	mg	%	mg	mg	%
Milk low fat	0.486cd	0.0773b	15.904c	0.518e	0.311d	59.992a	209.907c	94.378c	44.962b
Fermented milk	0.628cd	0.0793b	12.676cd	0.515e	0.308d	59.780a	154.960e	91.517c	59.072a
Yoghurt	0.390d	0.092b	23.584b	1.034c	0.352d	34.403c	175.480d	95.844c	54.622a
Date syrup	0.633cd	0.259b	40.863a	0.568e	0.208e	36.798c	7.460f	3.254d	42.913b
Pomegranate syrup	0.881c	0.082b	9.273d	0.788d	0.426c	54.078b	13.795f	5.600d	40.596b
White tahini	5.875b	1.462a	24.896b	10.660b	1.294a	12.125d	309.657b	133.003a	42.952b
Red tahini	7.341a	1.195a	16.326c	11.816a	1.145b	9.687d	349.667a	106.333b	30.421c

*** Each value in a column followed by the same letter are not significantly different at (p≤0.05).**

The data in table (4) showed that, Fe, Zn and Ca bioavailability contents in some dairy products were study. Generally, the fortification by fruit syrups and tahina caused increase of Fe and Ca bioavailability compared to yoghurt only. The yoghurt which fortified by 5% date syrup had the highest percent of Fe and Zn bioavailability (38.454% and 44.444%, respectively). Whereas, fortified by 5% date to yoghurt increased of Fe bioavailability (63.05%) compared to yoghurt alone. Also, the white tahina had a higher percent of Fe, Zn and ca bioavailability than red tahina. While, fortification by tahina (both white and red) had the highest percent of Ca bioavailability (71.913% and 73.855%, respectively). Additional a flavored to fermented milk fortified by 15% date syrup were higher than fermented milk fortified by 15% date syrup only specially cinnamon bark powder (0.3%) for Fe and Zn bioavailability.

Table (4): Bioavailability of Fe, Zn and Ca in vitro for some dairy products (mg/100g):

Minerals / Ingredients	Fe			Zn			Ca		
	Total	Bioavailability		Total	Bioavailability		Total	Bioavailability	
	mg	mg	%	mg	mg	%	mg	mg	%
Yoghurt with 1% white tahini	0.870bc	0.291c	33.954a	0.966c	0.230d	24.244c	131.709abc	94.730a	71.913b
Yoghurt with 1% red tahini	1.049b	0.358b	34.722a	1.716a	0.374bc	21.692c	129.453cd	95.612a	73.855ab
Yoghurt with 5% pomegranate syrup	0.513e	0.182d	35.571a	0.580f	0.244d	42.011b	117.615e	69.360d	58.986de
Yoghurt with 5% date syrup	0.509e	0.195d	38.454a	0.700ef	0.311cd	44.444b	137.540a	76.452c	55.586e
Yoghurt drink with 1% white tahina	0.817cd	0.302c	37.097a	0.862cd	0.244d	28.361c	132.506abc	77.784c	58.709de
Yoghurt drink with 1% red tahini	1.238a	0.443a	35.774a	1.274b	0.552a	43.322b	136.649ab	76.267c	55.828e
Fermented milk with 15% date syrup	1.022b	0.190d	18.631b	0.835d	0.466ab	55.738a	131.203bc	79.599c	60.661d
Fermented milk with 15% date syrup and flavored by Strawberry	0.717cd	0.117e	16.309b	0.668f	0.395bc	59.170a	127.812cd	97.049a	75.957a
Fermented milk with 15% date syrup and flavored byCinnamon bark	0.655de	0.112e	17.138b	0.818de	0.497ab	60.654a	124.941d	84.468b	67.672c

*** Each value in a column followed by the same letter are not significantly different at (p≤0.05).**

These results are a line with (Salama, 2012) found that, the addition of pomegranate juice caused increase in Fe content and slightly increase in Zn content, but decrease in calcium content when compared to control.Salem et al. (2008) reported that, fermented milk which fortified by 15% date syrup caused increase in total Fe content and the additional of date syrup; replace sugar by date syrup (50%) had increase in total Fe content. Moreover, the rats fed on yoghurt fortified by 5% date syrup had increase in blood Fe and Ca contents (63.37% and 18.93%, respectively) compared to rats fed on control as described by (Mottwly et al., 2010), and (Arafa, 2013) who reported that feeding rats on different pomegranate products caused significant increase in the level of serum iron. On other hand, Ranjan et al. (2005) found that, the Ca bioavailability of buffalo milk without fortification by calcium was 50% of the total calcium content. Unal et al, (2005) reported that, the yogurt group was different from the other products and that the acidity affected calcium bioavailability.

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تدعيم بعض منتجات الألبان لزيادة القيمة الحيوية للعناصر و مضادات الأكسدة

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تهدف هذه الدراسة الى تقدير القيمة الحيوية المتاحة لكلا من الحديد والزنك والكاليسوم فى بعض منتجات الالبان المدعمة . تم اختيار مصادر طبيعية للحديد مثل دبس البلح والرمان ومعجون السمسم (الطحينة). تم تحليل محتوى العينات (المكونات ومنتجات الالبان) لتدعيم الزبادى و اللبن المتخمر (الرايب). تم تقدير الفينولات الكلية ونشاط مضادات الاكسدةالكلية.كما تم تقدير القيمة الحيوية المتاحة للحديد والزنك والكاليسوم معمليا للعينات كما يحدث داخل القناة الهضمية. أيضا تم تحليل النتائج احصائيا .اظهرت النتائج ان معجون السمسم (الطحينة)كان اعلىفى محتواه من الفينولات الكلية .أعطى اللبن المتخمر أعلىقيمة للنشاط المضاد للاكسدة (٩٦.٣٨٧) بطريقة DPPH وأعطى الزبادى أعلى قيمة للنشاط المضاد للاكسدة (٨٩.٩٠ %) بطريقة ABTS. اضافة دبس الفاكهة او الطحينة للزبادى أدى الى زيادة الفينولات الكلية ونشاط المضاد للاكسدة بالمقارنة بالزبادى فقط . واوضحت النتائج أيضا ان دبس البلح كانأعلىقيمة بالنسبة للحديد المتاح(٤٠.٨٦٠%) عن الطحينة البيضاء (٢٤.٨٦٩%) .وجدت أعلى نسبة للقيمة الحيوية المتاحة للزنك فى اللبن المنخفض الدهن واللبن المتخمر (٥٩.٩٩٢% و ٥٩.٧٨٠% على التوالى).بينماأعطى اللبن المتخمر أعلىقيمة حيوية متاحة للكاليسوم(٥٩.٠٧٨%) ثم الزبادى فقط (٥٤.٦٢٢%).وعموما التدعيم بدبس الفاكهة او الطحينة ادى الى زيادة القيمة الحيوية المتاحة معمليا من الحديد والزنك والكاليسوم. كما وجد أن الزبادى المدعم ب ٥% من دبس البلح كان اعلى فى النسبة المتاحة حيويا من الحديد والزنك (٣٨.٤٥٤% و ٤٤.٤٤٤%) على التوالى . بينما ادى التدعيم بالطحينة (البيضاء اوالحمراء) الى زيادة نسبة القيمة الحيوية المتاحة للكاليسوم (٧٣.٨٥٥% و ٧١.٩١٣%) على التوالى . أوضحت نتائج هذه الدراسة ان تدعيم منتجات اللبن المتخمرة أدى إلى زيادة القيمة الحيوية المتاحة من العناصر المعدنية مثل الحديد والزنك والكاليسوم و مضادات الأكسدة .