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ORIGINAL CONTRIBUTION

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Orange juice is a good folate source in respect to folate content and stability during storage and simulated digestion

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Abstract *Background* Estimated average folate intake in Sweden is less than 55% of the recommended daily intake (RDI) for women of childbearing age (Becker and Pearson in Riksmaten 1997–1998 Kostvanor och näringsintag i Sverige. National Food Administration, Uppsala, pp 34, 44, 121, 2002). Because a good folate status reduces the risk of neural tube defects, mandatory folic acid fortification is discussed in some European countries. This however, could lead to exposure to unintentionally high amounts of folic acid for some population groups, therefore targeted folic acid fortification could be an alternative. *Aims* To (1) determine natural folate content in three popular brands of orange juice sold in Sweden, (2) determine stability of natural folate and folic acid fortificant during shelf life in a folic acid/iron fortified orange juice, (3) determine folate stability in four juices during simulated household consumption for one week and (4) determine the in vitro bioaccessibility of natural folate in one brand of orange juice using the TNO gastroIntestinal Model (TIM). *Methods* Natural folate content in juices was determined using RP-HPLC-FL. To determine folic acid content and confirm RP-HPLC-FL values LCMS was used. Stability during shelf life was determined in unopened bottles of a folic acid/iron fortified juice and for one week in four popular juices under household consumption conditions with reopening of bottles daily. For an in vitro folate bioaccessibility experiment in orange juice the TNO TIM Model was used. *Results* 5-CH_3-H_4folate was the dominant natural folate form in the juices with contents ranging from 16–30 μg/100 g. Shelf life losses of folic acid fortificant were 1–4%. During one week simulated household consumption 5-CH_3-H_4folate content decreased by up to 7% (n.s). Bioaccessibility of natural folate in orange juice was almost 100%. Most folate was released for absorption in jejunum between 60–120 min after trial start. *Conclusion* Orange juice may be considered a good source of natural folate in respect to content and stability during storage and simulated digestion. Moreover, added folic acid fortificant in a folic acid/iron fortified orange juice was stable during shelf life.

Key words folate – folic acid – fortification – orange juice – in vitro bioaccessibility

Introduction

Folate is a B-vitamin essential for cellular replication. Estimated average folate intake is less than 55% of the recommended daily intake (RDI) for women of childbearing age (17–44 years) in Sweden [3]. RDI of folate for women of childbearing age (400 μg) [26] is higher than other adults because of enhanced requirements during increased cell multiplication during pregnancy. A good folate status reduces the risk of neural tube defects [4] and spontaneous abortions [8].

To reduce the gap between recommended and actual daily intake introduction of mandatory fortification is discussed in for example Ireland and the UK. Concerns that folic acid fortification may mask symptoms of vitamin B_{12} deficiency [24] and increase dizygotic twinning [12] have caused European authorities to hesitate about introducing mandatory fortification. However, since 1998 when mandatory fortification was introduced in the United States, no increased twinning [2] but a 15–50% reduction in rate of neural tube defects [14] has been observed. On the other hand, reports that folic acid supplementation may promote existing tumors have raised concern that populations not at risk of neural tube defects could be exposed to high intakes of folic acid from fortification [14]. An alternative to mandatory fortification of staple foods could be folic acid fortification of foods consumed by a specific target group, i.e. women of childbearing age. Because consumption of juices is highest among the younger adults [3], orange juice could be a possible food source for targeted folic acid fortification. Orange juices contain 15–44 μg folate/100 g according to food tables, which is high compared with other beverages [18].

Another limiting micronutrient among women of childbearing age in European countries is iron. In UK about 40% of women aged 19–34 years consume less than the lower reference intake [6]. In Sweden iron fortification of flour was carried out until 1995; thereafter, women have only reached two thirds of the recommendation while men still exceed the RDI [3]. Juice fortified with folic acid and iron could be suitable to improve both iron and folic acid intake in women of childbearing age.

To estimate folate absorption from a specific food in human trials is difficult and expensive. As alternative, the amount of folate available for absorption from various food products has been studied using in vitro models [1, 25, 30]. Using the computer-controlled TNO gastroIntestinal Model (TIM), which mimics the gastric small-intestinal tract, information can be gained about a nutrient's bioaccessibilty (the amount available for absorption) [17]. This model has been optimised for the estimation of bioaccessible folate [30].

Our objectives were to (1) determine natural folate content in three popular Swedish orange juice brands using a validated HPLC method, (2) determine stability of natural folate and folic acid fortificant during shelf life in a folic acid/iron fortified orange juice, (3) determine folate stability in four brands of orange juice during one week simulated household consumption and (4) determine in vitro bioaccessibility of natural folate in one brand of orange juice using the dynamic gastrointestinal model TIM.

Materials

Food samples

Content of natural folate was determined in three popular brands (based on turnover) of orange juice and concentrate (Table 1, A–C). Juices and juice concentrate were purchased in Uppsala (in autumn) and stored as recommended (below 8°C) in original packages until analysis the day after.

Shelf life stability of natural folate and folic acid was determined in a folic acid/iron fortified orange juice. At the industrial test plant of JO-Bolaget (Stockholm, Sweden) two fortification levels of folic acid (40 and 60 μg/100 g) were added to a ready-to-drink orange juice fortified with ferrous lactate (3 mg/100 g). The juices were pasteurised (90°C, 15 s), packed into aseptic glass bottles (1 L) and stored sheltered from light at below 8°C. Natural folate and folic acid content was determined from unopened bottles using LCMS at day 1, 21 and 35 (best before date).

Stability of natural folate during simulated household consumption was tested in three orange juice brands and one folic acid/iron fortified juice available on the market (Table 1). Juices were bought in Uppsala (in spring) and stored at below 8°C in original packages with screw caps. Orange juice concentrate was diluted as recommended with drinking water (1 + 4) and stored in a porcelain jar without lid. To simulate breakfast conditions 100 mL of each juice was removed every morning and the jar and bottles left in room temperature and light for 15 min. Duplicate samples for analysis were taken at day 1, 3 and 7, poured into 5-mL Sarstedt tubes (Nürmbrecht, Germany) and stored at −20°C until analysis day 8.

For the bioaccessibility study an orange juice concentrate (Table 1, C) was donated by the producer and kept at −20°C for transport and storage. The concentrate was diluted with drinking water (1 + 4) prior to the TIM experiment.

As in-house control sample, a different batch of the juice concentrate (Table 1, C) was received directly from the producer and stored in −20°C until analysis.

Table 1 Characteristics of the different types of purchased orange juices. Folate concentrations at purchase day and during household consumption

	A, Freshly pressed	B, Ready-to-drink	C, Concentrate (1 + 4)	D, Folic acid/iron fortified ready-to-drink
Orange cultivar[a]	*Valencia late, Salustianas, Baladi*	*Pera, Valencia, Hamlin*	*Pera, Valencia, Hamlin*	89% as B (11% juice from passion fruit, cactus fig)
Treatment[a]	72°C, short time	90°C, 15 s	90°C, 15 s	90°C, 15 s
Packaging[a]	High density polyeten	Elopak ™	Aseptic tetrapak™	Elopak ™
BRIX[a]	10.5–12.0	11.3	48.6	11.3
Organic acids[a]	0.8–0.9%	0.7%	4.15%	0.7%
Ascorbic acid[a]	45 mg/100 g	30 mg/100 g	186 mg/100 g	30 mg/100 g
pH	3.8	3.5	3.4	3.5
5-CH_3-H_4folate content (μg/100 g)				
Purchased autumn[b]	15.7 (15.4–16.0) (n = 3)	23.4 (22.8–23.6) (n = 3)	102.4 (90.3–105.7) (n = 5)[d]	Not available on market
Purchased spring[c]	22.15, 23.76	23.74, 24.06	22.16, 24.78[e]	29.2, 29.9
5-CH_3-H_4folate losses				
Day 3[f]	−1%	0	+2%[e]	−5%
Day 7[f]	−2%	−2%	+4%[e]	−7%

[a]Producers information
[b]Median (min–max)
[c]Duplicates as individual results
[d]Analysed as concentrate
[e]Analysed after dilution with drinking water (1 + 4)
[f]Losses of 5-CH_3-H_4folate during household consumption (n.s.)

Solvents and reagents

The chemicals 2, 3-dimercapto-1-propanol (BAL); DL-dithiothreitol (DTT); piperazin; (+)-sodium-L-ascorbate and trifluoroacetic acid (TFA) were purchased from Sigma Aldrich (Steinheim, Germany). Other chemicals were purchased from Merck (Darmstadt, Germany) unless otherwise stated. A Milli-Q system (Millipore, USA) was used to purify water.

Calibrants

Folate standards, 5-CH_3-H_4folate ((6S)-5-methyl-5,6,7,8-H_4folate, sodium salt), H_4folate ((6S)-5,6,7,8-H_4folate, sodium salt) and folic acid, were a gift from Merck Eprova AG (Schaffhausen, Switzerland). Folate standard stock solutions with concentrations about 200 μg/mL were prepared according to Jastrebova et al. [11], stored under nitrogen atmosphere at −80°C and used within three months.

Methods

TIM experiment

For the in vitro study on folate bioaccessibility, the TNO's dynamic computer controlled model (TIM) consisting of compartments for stomach, duodenum, jejunum and ileum was used [17]. Because in vitro models contain no enterocytes, instead of actual absorption folate bioaccessibility is assessed.

Diluted orange juice concentrate (300 g) was mixed with a buffer solution (60 g) containing amylase to a so-called gastric intake. Gastric intakes (300 g) were put in the gastric compartment of TIM at two different days (TIM A and TIM B) as described by Verwei et al. [30]. Dialysates were collected from the jejunum and ileum compartments during four time intervals (0–60, 60–120, 120–240, 240–360 min) of the transit through TIM. Moreover a pooled dialysate sample (0–360 min) was collected from both compartments. As non-bioaccessible fraction, the ileal delivery was collected. After each TIM run, residues from all compartments were collected to calculate folate mass balance. Sodium ascorbate (1% w/v) was added to all TIM samples (dialysates, residues and gastric intakes). Samples were stored under nitrogen atmosphere at −20°C until analysis.

Sample preparation

Frozen TIM samples, gastric intakes, orange juices and the in-house control sample were thawed at room temperature in the dark. All samples (thawed and stored at below 8°C) were shaken vigorously. Duplicate TIM samples (7.5–15.0 g) were mixed with 2 and replicate (n = 3–6) juice samples (0.8–3.0 g) with 8–30 volumes of 0.1 M phosphate buffer, pH 6.1, containing 2% sodium ascorbate (w/v) and 0.1% BAL (v/v). After adjustment to a pH of 6–6.5 with 4 M KOH, samples were heat extracted as described by Patring and Jastrebova [20]. TIM samples were filtered; dialysates through 0.2 μm cellulose acetate filter (Sun International, Uppsala, Sweden) and gastric intakes, ileal

deliveries and residues through 0.45 μm filter (Whatman, Dassel, Germany). Folate deconjugation was done according to Patring et al. [21] by incubation of 3–12 mL sample extract with 100–200 μL of rat serum (Scanbur, Sollentuna, Sweden) dialysate. Results were corrected for endogenous folate content in rat serum dialysate.

Affinity chromatography columns were prepared by immobilising 0.5 mg folate binding protein (Scripps, San Diego, USA) onto 2-mL agarose gel (Affigel 10, Bio-Rad, Sundbyberg, Sweden) as described by Konings [15]. TIM extracts (3–12 mL) were loaded onto preconditioned cartridges as described by Kariluoto et al. [13]. After washing folate was eluted with 7 mL elution buffer [13], adding BAL (0.1%) instead of mercaptoethanol to the elution tubes. Binding capacity of the columns was tested at each occasion by alternated application of 1.5 mL 5-CH_3-H_4folate solution (concentration 300 ng/mL) to one of the columns. For sample clean-up columns were only loaded to 25% (100 ng) of the folate binding capacity.

Juice extracts were purified by solid phase extraction as described by Nilsson et al. [19] with minor modifications. Preconditioned strong anion exchange cartridges (500 mg, Isolute, International Sorbent Technology, UK) were loaded with 2.5 mL sample extract and after washing, folate was eluted with 4 mL elution buffer containing 0.1% BAL instead of mercaptoethanol. Purified samples were stored in the autosampler at 8°C under nitrogen atmosphere until HPLC analysis within 15 h.

Folate quantitation

Folate in orange juices and TIM samples was quantified using an HPLC system consisting of a gradient quaternary pump, a thermostated autosampler (8°C) and column compartment (23°C), a fluorescence detector (ex/em at 290/360 nm) and a multiwavelength detector (290 nm) (Agilent 1100, Agilent Technologies, USA; software Chemstation Rev A 10.02 [1757]). Folate forms were separated on a Zorbax SB C_8 (Scantec lab, Partille, Sweden) or an Aquasil C_{18} column (Chromtech, Stockholm, Sweden) under linear gradient elution conditions using phosphate buffer (30 mM, pH 2.3) and acetonitrile at flow rate 0.4 mL/min according to Jastrebova [11]. 5-CH_3-H_4folate was identified by retention time, and peak identity was confirmed by comparison of relative peak areas in both detectors. Quantitation was based on a multilevel (n = 7) external calibration curve with a linear range over 0.3–94.8 ng/mL using fluorescence detection. Intra-assay CV for 5-CH_3-H_4folate in juice samples was 2% (n = 12), inter-assay of the in-house control was 6% (n = 9) and relative recoveries 103 and 97% (dotation of 50 and 100% of initial content). For TIM samples, 5-CH_3-H_4folate recovery was 94 and 97% (100%) including all analytical steps.

Folic acid in folic acid/iron fortified juice samples was quantified using LCMS. The system consisted of a gradient quaternary pump, an autosampler (8°C), a column compartment (23°C), a UV detector (290 nm) and an LCQ ion-trap mass spectrometer (Agilent 1100; software LC/MSD Chemstation Rev B 01.01 [164]). H_4folate, 5-CH_3-H_4folate and folic acid were separated on an Ace 3 C_{18} column (Scantec lab, Partille, Sweden) under linear gradient elution conditions using acetic acid (10 mM, pH 2.3), acetonitrile and methanol at a flow rate of 0.3 mL/min according to Patring and Jastrebova [20]. The spectrometer was operated in positive electrospray mode using selected ion monitoring (SIM) and retention times for peak identification. Quantitation was based on a multilevel (n = 7) external calibration curve with a linear range over 1.3–107.0 ng/mL for H_4folate, 1.1–94.8 ng/mL for 5-CH_3-H_4folate and 10.7–214.5 ng/mL for folic acid. Intra-assay CV for 5-CH_3-H_4folate in orange juice were 3% (n = 12) and for folic acid 1% (n = 3). Relative recoveries for 5-CH_3-H_4folate were 103 and 105% and for folic acid 109 and 111% (dotation 50 and 100%). Inter-assay CV for 5-CH_3-H_4folate in the in-house control sample was 2% (n = 9). RP-HPLC-FL results were verified by LCMS using the RP-HPLC-FL analysed extracts of folic acid/iron fortified orange juice (n = 6). 5-CH_3-H_4folate content did not differ between the methods; RP-HPLC-FL 24.8 μg/100 g (23.8–25.5, n = 6) and LCMS 24.3 μg/100 g (22.3–25.4, n = 6).

Calculations and statistics

To compare the folate content of the various juice types and the storage effects on folate content, non-parametric Mann-Whitney test, Minitab software release 14 (Minitab, Coventry, UK), was used. The level of significance was set at P-values < 0.05.

Results

Mean 5-CH_3-H_4folate concentrations ranged from 15.7–29.5 μg/100 g in the leading orange juice brands in Sweden purchased on two occasions (Table 1). 5-CH_3-H_4folate concentrations did not differ significantly (P = 1.0) between the purchased batch of juice concentrate (Table 1, C: 102.4 μg/100 g, n = 5) and the in-house control batch directly received from the producer (98.0 μg/100 g, n = 3).

Shelf life retention of 5-CH_3-H_4folate and folic acid in the folic acid/iron fortified orange juice was com-

plete, no significant reduction in folate retention was observed between day 1 and day 35 (Table 2). The folic acid/iron fortified juice directly delivered from the test plant contained small quantities of H_4folate ranging from 3.9 to 4.5 μg/100 g, which decreased with 35% ($P < 0.05$) during storage until best before. However, due to the initially low endogenous H_4folate content total folate loss was only 1–4% and not significant (data not shown).

After one week of simulated household juice consumption, losses of 5-CH_3-H_4folate in the four juices were below 7% and not significant (Table 1).

Folate bioaccessibility from orange juice made from concentrate (Table 1, C) was high, 97 and 99% (Fig. 1). Table 3 shows the 5-CH_3-H_4folate content of gastric intake and individual TIM fractions. Non-bioaccessible folate was estimated from ileal deliveries (2.1 and 2.7 μg in TIM A compared with 2.2 and 2.8 μg in TIM B) and residues (1.2–1.7 μg in TIM A compared with 2.4–3.6 μg in TIM B). The folate mass balance calculated for each TIM trial from individual TIM fractions were 109 and 117% (not taken into account for calculations).

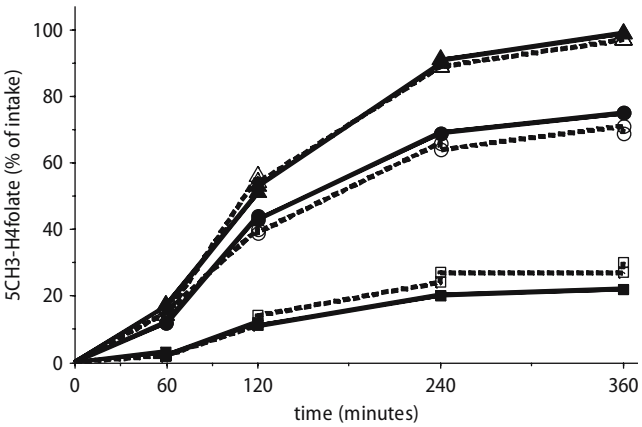

Fig. 1 Cumulative bioaccessible 5-CH_3-H_4folate from orange juice made of concentrate (as percent of intake) assessed in two TIM trials (each analysed as duplicates). TIM A solid line and TIM B dotted line. Bioaccessible folate (as duplicates) found in ileum dialysates (■/□), jejunum dialysates (●/○) and as sum of dialysates (▲/△).

Table 3 5-CH_3-H_4folate content (μg) in gastric intake and bioaccessible fractions (jejunum and ileum dialysates) from two TIM trials with orange juice made from concentrate (*C*)

5-CH_3-H_4folate	Collection Interval (min)	TIM A		TIM B	
		Jejunum	Ileum	Jejunum	Ileum
Intake		40.4 ± 2.3 ($n = 6$)		41.6 ± 5.2 ($n = 8$)	
Bioaccessible (dialysate)	0–60	5.9, 5.9	0.8, 0.9	4.9, 5.0	1.0, 1.1
	60–120	9.6, 10.4	3.5, 4.8	13.1, 13.2	3.5, 4.0
	120–240	10.2, 10.4	5.3, 5.3	10.6, 10.7	3.2, 3.8
	240–360	1.8, 2.0	1.2, 1.3	2.4, 2.4	0.8, 1.0
	Pooled 0–360	29.6, 29.9	9.5, 10.3	30.3, 30.5	8.9, 10.2

Discussion

Aim of this study was to determine in orange juice the content and stability of natural folate as well as folic acid fortificant during shelf life and simulated household consumption. In addition stability of natural folate was assessed during simulated digestion in order to evaluate if orange juice is a good folate source.

The natural folate content in the analysed orange juice samples was high (Table 1); e.g. about four times higher than milk and three times higher than beer on average [10]. Folate content in analysed orange juices ranged from 16 to 30 μg/100 g. This is in accordance with values reported by others using various detection methods ranging from 13 to 30 μg/100 g [16, 23, 27] with large variation between juices from different brands, freshly pressed juice and juice made from concentrate [16, 27]. However, a large number of representative samples would be required to determine whether e.g. orange cultivars, harvest time, ascorbic acid content or packaging and storage may affect folate content.

Orange juice can be considered a suitable matrix for providing both natural folate and folic acid fortificant as indicated by data on storage stability. Neither during shelf life studies with a folic acid/iron fortified orange juice nor in conditions simulating household consumption in four orange juices were significant losses of 5-CH_3-H_4folate found (Tables 1 and 2). The increased 5-CH_3-H_4folate concentration in the orange juice made from concentrate stored in a jar without lid may be due to evaporation of water, which did not occur in the other juices stored in the original containers with screw caps. The folic acid/iron fortified juice from the pilot plant was stored sheltered from light in glass bottles, which is less optimal compared to usual storage in tetrapack™ with minimized headspace. The high endogenous ascorbic acid content in the juice (30.0 mg/100 g) might have prevented

Table 2 Concentrations of folic acid (individual results) and 5-CH_3-H_4folate (median (min–max)) during shelf life in unopened bottles of folic acid/iron fortified orange juice (μg/100 g)

	Folic acid ($n = 2$)		5-CH_3-H_4folate ($n = 4$)
Day 1	39.4, 42.4	61.6, 62.6	23.6 (22.3–24.3)
Day 21	41.9, 42.3	63.2, 58.5	23.6 (23.5–23.8)
Day 35	38.3, 40.3	62.8, 61.4	24.2 (23.7–24.6)

Concentrations over time (at day 1, 21, and 35) no significant differences

oxidation of 5-CH_3-H_4folate and possibly oxidative cleavage of folic acid. Previous studies also report good stability of 5-CH_3-H_4folate in orange juice during heat treatment (30 min at 120°C) [9] and pasteurisation (losses <2.5%) [16]. This is mainly attributed to ascorbic acid [9, 16]. Moreover, ferrous iron might have improved stability. In a model food system Day et al. [5] found ferrous sulphate (6.7 mg/100 mL) more efficient to retain 5-CH_3-H_4folate and folic acid during heat treatment (2 h at 120°C) than sodium ascorbate (6.4 mg/100 mL).

Natural folate occurs in orange juice mainly as polyglutamate [25], which is converted into its monoglutamate form by the intestinal enzyme pteroylpolyglutamate hydrolase (PPH) during absorption. Wei et al. [31] found PPH activity was inhibited by orange juice in vitro. This pH independent competitive inhibition is mainly caused by the low-molecular-mass anions citrate and malate [31]. Wei et al. [32] also report incomplete bioavailability of a single dose of (stable-isotope) labelled polyglutamate folate added to orange juice and given to seven men, suggesting incomplete deconjugation of folate polyglutamates by PPH during absorption. However, because folic acid is added as a fortificant in monoglutamate form, the orange juice matrix with a high ascorbic acid concentration protects folic acid but does not inhibit the absorption. A glass of a folic acid/iron fortified orange juice (200 mL) would contribute 40–50% of the Swedish RDI for folate for women [26] of childbearing age. Considering that the actual folate intake of this group of the Swedish population is 55% of RDI [3], an additional glass of fortified orange juice would almost be sufficient to reach recommendations.

In a simplified in vitro model, Seyoum and Selhub found the retention of folate in orange juice to be 86% when mimicking conditions of the gastrointestinal tract [25]. In the more advanced dynamic in vitro model (TIM), 5-CH_3-H_4folate in orange juice was stable during simulated digestion despite the change of pH from acidic to neutral, presence of enzymes and 6-h-long gut passage. Furthermore, 5-CH_3-H_4folate release from the orange juice matrix seemed high, more than 95% was bioaccessible. Most of the orange juice 5-CH_3-H_4folate was available for absorption in the "jejunum", during 60–240 min; a similar observation was made for yoghurt [1]. Verwei et al. also reported high folate bioaccessibility (91%) from a sample of pasteurised orange juice using the TIM model [29].

A high folate mass balance during a TIM experiment with orange juice was expected because entrapment of the juice in the TIM system was not likely. Folate recoveries of 88–136% have been reported by others for TIM studies with other foods [1, 29, 30]. In this study data for folate mass balance were not corrected for folate content in the enzyme solutions (amylase, lipase, pepsin pancreatic acid and bile) used in TIM. However, Verwei [30] reported endogenous folate content from bile and pancreatic solution to be less than 2% of the added food folate content in the test food milk and correction would therefore only slightly affect results. Correction of 5-CH_3-H_4folate overestimation due to our calculated 5-CH_3-H_4folate mass balance (109 and 117%) would result in bioaccessibility of natural folate from orange juice of 85–89%. Data regarding high folate bioaccessibility from orange juice as found in this in vitro trial are supported by an in vivo study [7] in which a 44% increase of plasma folate concentrations was observed after a one-week intervention with a large portion of orange juice (708 mL/day).

High exposure to folic acid fortificant is discussed as a potential risk of mandatory folic acid fortification of a staple food. Concerns about presence of unmetabolised folic acid in peripheral plasma [22], masking of vitamin B_{12} deficiency in the elderly and promotion of existing tumors are raised as reviewed among others by Kim [14] and Ulrich and Potter [28]. Because of these concerns Swedish authorities recently decided against introduction of mandatory folic acid fortification. The Swedish Council on Technology Assessment in Health [26] estimated that women by mandatory flour fortification would receive 125 μg additional folic acid/day. In countries without mandatory folic acid fortification a glass of folic acid fortified orange juice could provide women with a similar amount folic acid fortificant.

Our results suggest that orange juice may be considered a good source of natural folate in respect to content and stability during storage and a suitable vehicle for targeted folic acid fortification.

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References

1. Arkbåge K, Verwei M, Havenaar R, Witthöft C (2003) Bioaccessibility of folic acid and (6S)-5-methyltetrahydrofolate decreases after the addition of folate-binding protein to yoghurt as studied in a dynamic in vitro gastrointestinal model. J Nutr 133:3678–3683
2. Bailey L, Berry R (2005) Folic acid supplementation and the occurrence of congenital heart defects, orofacial clefts, multiple births, and miscarriage. Am J Clin Nutr 81:1213S–1217S
3. Becker M, Pearson M (2002) Riksmaten 1997–1998 Kostvanor och näringsintag i Sverige. National Food Administration, Uppsala, pp 34, 44, 121
4. Czeizel AE, Dudás I (1992) Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. N Engl J Med 327:1832–1835
5. Day BPF, Gregory JF (1983) Thermal stability of folic acid and 5-methyltetrahydrofolic acid in liquid model food systems. J Food Sci 48: 581–587
6. Fletcher RJ, Bell IP, Lambert JP (2004) Public health aspects of food fortification: a question of balance. Proc Nutr Soc 63:605–614
7. Franke AA, Cooney RV, Henning SM, Custer LJ (2005) Bioavailability and antioxidant effects of orange juice components in humans. J Agr Food Chem 53: 5170–5178
8. George L, Mills JL, Johansson ALV, Nordmark A, Olander B, Granath F, Cnattingius S (2002) Plasma folate levels and risk of spontaneous abortion. J Am Med Assoc 288:1867–1873
9. Indrawati, Arroqui C, Messagie I, Nguyen MT, Van Loey A, Hendrickx M (2004) Comparative study on pressure and temperature stability of 5-methyltetrahydrofolic acid in model systems and in food products. J Agr Food Chem 52:485–492
10. Jägerstad M, Piironen V, Walker C, Ros G, Carnovale E, Holasova M, Nau H (2005) Increasing natural food folates through bioprocessing and biotechnology. Trends Food Sci Tech 16:298–306
11. Jastrebova J, Witthöft C, Grahn A, Svensson U, Jägerstad M (2003) HPLC determination of folates in raw and processed beetroots. J Food Chem 80:579–588
12. Källen B (2004) Use of folic acid supplementation and risk for dizygotic twinning. Early Hum Dev 80:143–151
13. Kariluoto S, Vahteristo L, Piironen V (2001) Applicability of microbiological assay and affinity chromatography purification followed by high-performance liquid chromatography (HPLC) in studying folate contents in rye. J Sci Food Agric 81:938–942
14. Kim Y (2004) Will mandatory fortification prevent or promote cancer? Am J Clin Nutr 80:1123–1128
15. Konings EJ (1999) A validated liquid chromatographic method for determining folates in vegetables, milk powder, liver and flour. J AOAC Int 82:119–125
16. Matella NJ, Braddock RJ, Gregory JF, Goodrich RM (2005) Capillary electrophoresis and high-performance liquid chromatography determination of polyglutamyl 5-methyltetrahydrofolate forms in citrus products. J Agr Food Chem 53:2268–2274
17. Minekus M, Marteau P, Havenaar R, Huis in´t Veld JHJ (1995) A multicompartimental dynamic computer-controlled model simulating the stomach and small intestine. Altern Lab Anim 23:197–209
18. National Food Administration (NFA). Swedish Database version 04.1.1. http://www.192.121.81.11/livsmedelsok/. Accessed 26 April 2007
19. Nilsson C, Johansson M, Yazynina E, Strålsjö L, Jastrebova J (2004) Solid-phase extraction for HPLC analysis of dietary folates. Eur Food Res Technol 219:199–204
20. Patring J, Jastrebova J (2007) Application of liquid chromatography-electrospray ionisation mass spectrometry for determination of dietary folates: effects of buffer nature and mobile phase composition on sensitivity and selectivity. J Chromatogr A 1143:72–82
21. Patring J, Jastrebova J, Hjortmo S, Andlid T, Jägerstad M (2005) Development of a simplified method for the determination of folates in baker's yeast by HPLC with ultraviolet and fluorescence detection. J Agr Food Chem 53:2406–2411
22. Pfeiffer CM, Fazili CMZ, McCoy L, Zhang M, Gunter EW (2004) Determination of folate vitamers in human serum by stable-isotope-dilution tandem mass spectrometry and comparision with radioassay and microbiologic assay. Clin Chem 50: 423–432
23. Rychlik M (2004) Revised folate content of foods determined by stable isotope dilution assays. J Food Comp Anal 17:475–483
24. Scott JM, Rébeillé F, Fletcher J (2000) Folic acid and folates: the feasibility for nutritional enhancement in plant foods. J Sci Food Agric 80:795–824
25. Seyoum E, Selhub J (1998) Properties of food folates determined by stability and susceptibility to intestinal pteroylpolyglutamate hydrolase action. J Nutr 128:1956–1960
26. The Swedish Council on Technology Assessment in Health (2007) Nyttan att berika mjöl med folsyra i syfte att minska risken för neuralrörsdefekter. En systematisk litteraturöversikt. (Benefits of fortifying flour with folic acid to reduce risk of neural tube defects.) Stockholm, Sweden, SBU
27. Thomas PM, Flanagan VP, Pawlosky RJ (2003) Determination of 5-methyltetrahydrofolic acid and folic acid in citrus juices using stable isotope dilution-mass spectrometry. J Agr Food Chem 51:1293–1296
28. Ulrich C, Potter J (2006) Folate supplementation: too much of a good thing? Cancer Epidemiol Biomarkers Prev 15:189–193
29. Verwei M (2004) Bioaccessibility of folate from several liquid and solid food products. Dissertation, Wageningen University, Wageningen, pp 62–64
30. Verwei M, Arkbåge K, Havenaar R, van den Berg H, Witthöft C, Schaafsma G (2003) Folic acid and 5-methyltetrahydrofolate in fortified milk are bioaccessible as determined in a dynamic in vitro gastrointestinal model. J Nutr 133:2377–2383
31. Wei MM, Gregory JF III (1998) Organic acids in selected foods inhibit intestinal brush border pteroylpolyglutamate hydrolase in vitro: potential mechanism affecting the bioavailability of dietary polyglutamate folate. J Agr Food Chem 46:211–219
32. Wei MM, Bailey LB, Toth JP, Gregory JF (1996) Bioavailability for humans of deuterium-labeled monoglutamyl and polyglutamyl folates is affected by selected foods. J Nutr 126:3100–3108