Assessment of a portable device to quantify vitamin A in fortified foods (flour, sugar, and milk) for quality control

Arnaud Laillou, Cécile Renaud, Jacques Berger, Regina Moench-Pfanner, Laura Fontan, and Sylvie Avallone

Abstract

Background. *Simple-to-use quantitative methods are needed to check the adequacy of vitamin A fortification levels.*

Objective. *To assess the capacity of a portable fluorometer (iCheck FLUORO) and its test kit vials (iEx Mila) to quantify retinyl palmitate in fortified milks, flours (wheat, maize), and sugar.*

Methods. *The portable fluorometer was assessed in a three-step procedure to determine its working range and linearity, intra-assay precision, and interperson precision. Measurements were compared with the results obtained by high-performance liquid chromatography (HPLC), commonly regarded as the standard method for vitamin A analysis.*

Results. *The portable fluorometer (iCheck FLUORO) and its test kit vials (iEx Mila) precisely determined the vitamin A contents in fortified flours, sugar, and milks. Its working range was 1 to 10, 0.5 to 3.0, and 5 to 15 mg retinol equivalents (RE) kg^{-1} for flours (wheat and corn), milks, and sugar, respectively; these values are in accordance with the World Health Organization recommendations for food fortification in least developed countries. The limits of detection are higher than those of HPLC but are all satisfactory (< 1.46 mg RE kg^{-1}). The coefficients of variation within and between observers were satisfactory, especially for sugar and milk.*

Conclusions. *The linear relationship between the data from the portable fluorometer and the HPLC data confirms that the portable fluorometer provides a good determination of the vitamin A content of the fortified products in the tested range.*

Key words: quality control, retinyl palmitate, fortified food, flour, sugar, milk

Background

Addressing vitamin A deficiency has been agreed as a global priority since the World Summit for Children in 1990 established the goal of "virtual elimination of vitamin A deficiency and its consequences, including blindness" [1]. In 2009, the World Health Organization (WHO) estimated that approximately 190 million preschool children, representing roughly 35% of this age group globally, were affected by vitamin A deficiency between 1995 and 2005. For pregnant women, the estimate was around 20 million individuals (15% of this population globally) [2].

The most common cause of vitamin A deficiency is insufficient dietary intake of vitamin A, which is normally found in animal products as preformed vitamin A (or retinol) and in plant-source foods as provitamin A. The proven success of fortification programs has been evidenced by dramatic reductions in national indicators of mortality and clinical signs of micronutrient deficiency disease in several countries. Several studies have demonstrated the efficacy and/or effectiveness of the addition of vitamin A to staples and condiments consumed daily [3–14]. Industries have been voluntarily or mandatorily fortifying staple foods with vitamin A since the 1970s [15]. For 10 years, increasing the amount of large-scale food fortification has been the goal of several national and international organizations. The focus on fortification reflected a growing consensus that food fortification was one of the most cost-effective nutrition interventions [16]. Unfortunately, failure to ensure proper quality control

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monitoring will jeopardize the expected outcome of programs to prevent micronutrient deficiencies among the general population. For example, in Nigeria, mandatory fortification of wheat and maize flour, edible vegetable oil, and sugar was enacted in February 2000 and enforcement began in September 2002. However, the fortification levels are not consistent. A survey conducted in October 2003 by the Nigerian National Agency for Food and Drug Administration and Control (NAFDAC) that sampled flour and vegetable oil from the distribution chain for laboratory assessment of vitamin A levels indicated only 5% compliance [17]. In South Africa, a recent analysis of the national fortification of maize and wheat flour with vitamin A showed that there was evidence of insufficient addition of vitamin A, as most of the samples were below the requirements [18]. Compliance is not only an issue in developing countries; in the late 1990s in Ontario, Canada, 22 of the 39 fortified milk samples (56%) tested for vitamin A content were in compliance, whereas 17 (44%) were below the required level [19].

Various factors may lead to degradation of the quality of fortified food as it moves along the chain from producer to consumer. In order to ensure that the targeted individuals receive appropriate amounts of vitamin A to achieve the nutritional goal, continuous monitoring of quality at each stage of the chain is required; it is vital that the quality of the fortified foods remain at the expected level during the whole distribution process. An important challenge in assessing micronutrient concentrations is the fact that current methods of quantitative analysis are technically quite demanding, time-consuming, and expensive, whereas qualitative methods do not measure the adequacy of fortification but only provide information on the presence or absence of a fortificant.

To check the adequacy of fortification levels, simple-to-use quantitative methods that yield immediate and reliable results are needed at the site of production (internal quality control), for coverage surveys, and for external and regulatory monitoring. The objective of the present study was to assess a portable fluorometer (iCheck FLUORO) and its test kit (iEx Mila) for measuring vitamin A content in fortified milks, flours (wheat, maize), and white sugar. The portable fluorometer was assessed in a three-step procedure to determine its working range and linearity, intra-assay precision, and interperson precision. Measurements were compared with the results obtained by high-performance liquid chromatography (HPLC), commonly regarded as the standard method for vitamin A analysis. The portable fluorometer was used and partially evaluated for vitamin A quantification in biological fluids and fortified foods [19–22], but no assessment was made in comparison with the standard method (HPLC).

Methods

Materials and reagents

Food products were purchased from a supermarket in Montpellier, France. The white sugar was in crystallized form. Wheat flours were chosen with a low (T_{55}) and high (T_{110}) level of extraction. The maize flour was a nixtamalized corn flour, and the liquid milks were either whole (3.0% fat) or low-fat (1.5% fat) milk. Experiments were performed on each food for 1 week.

HPLC standards (retinol, retinyl acetate, and retinyl palmitate), solvents (acetone, methanol, methyl tert-butyl-ether, and ethyl acetate), and chemicals (ethylenediaminetetraacetate [EDTA] and KOH pellets) were obtained from Sigma-Aldrich. The saponification solution was prepared by dissolving 50 g of KOH in 100 mL of distilled water.

The fortificants were delivered in March 2012 by DSM (Basel, Switzerland) and stored at +4°C during the study. The following fortificants were used. For flour and milk fortification: dry vitamin A palmitate 250 S/N-B (250,000 IU/g). The retinyl palmitate is dispersed in a cornstarch-coated matrix of modified food starch and sucrose. Butylated hydroxytoluene (BHT) and sodium ascorbate are added as stabilizers. For sugar fortification: dry vitamin A palmitate 50 (50,000 IU/g). The retinyl palmitate is dispersed in a matrix of modified food starch, sucrose, and fractionated coconut oil. BHT, sodium ascorbate, sorbic acid, and sucrose are added as preservatives and processing aids.

The vitamin A contents of the fortificants were analyzed independently by dissolving them in ethanol. The absorption was measured by spectrophotometry (Perkin-Elmer) at a wavelength of 325 nm (all-*trans*-retinyl palmitate), and the samples were diluted until an extinction value of approximately 0.5 was reached. Retinyl palmitate concentration was calculated according to Lambert–Beer's law using the molar extinction coefficient of vitamin A ($\varepsilon = 49{,}260$ L cm^{-1} mol^{-1}) [23].

Sugar, flour, and milk fortification

The foods were fortified on the day of analysis, and the level of fortification was chosen according to international guidelines [24], as follows: for flour, 0, 1.0, 2.5, 5.0, 7.5, and 10 mg retinol equivalents (RE) kg^{-1} (150 g was prepared per level); for milk, 0, 0.5, 1.0, 1.5, 2.0, and 3.0 mg RE kg^{-1} (100 g was prepared per level); for sugar, 0, 5.0, 7.5, 10.0, 12.5, and 15 mg RE kg^{-1} (250 g was prepared per level). Dispersion of the fortificant in the sample was performed by two successive dilutions in the product. The fortified foods were protected from light to prevent vitamin A isomerization. After 10 minutes of gentle stirring, sampling was performed.

Quantification of vitamin A in fortified food by HPLC

To quantify the vitamin A in the solid samples (flours and white sugar), extraction was necessary. The powders (1 g of flours or sugars) were homogenized in 20 mL of distilled water. After 10 minutes of stirring, 16 mL of ethanol/hexane (4:3, v/v) was added and the samples were stirred (Turrax) for 30 seconds in a glass tube [25]. The extracts were centrifuged (5 minutes, 10,000*g*, ambient temperature) (Heraeus Multifuge X1R) and the organic extracts were dried under nitrogen. The residues were dissolved in 4 mL of acetone and the extracts were filtered prior to HPLC analysis (0.2 µm, polytetrafluoroethylene minisart SRP4 membranes, Sartorius).

To produce a good recovery of the vitamin A from the fortified milk, saponification was necessary [26]. Ethanol (4 mL) was added to 1 g of low-heated milk (~ 40°C). Nitrogen was flushed to prevent oxidation. After 10 minutes, 0.5 mL of saturated EDTA and 0.5 mL of KOH 9 M were added. The samples were heated for 10 minutes at 100°C, vortexed, and boiled again for 10 minutes. After cooling in a cold water bath (10 minutes), retinyl palmitate was extracted with 2 mL of hexane/ethyl acetate (8:2, v/v). The samples were stirred for 10 minutes and centrifuged (2 minutes, 10,000*g*, 4°C), and the upper phases were transferred to amber tubes. The samples were extracted again with 2 mL of hexane/ethyl acetate (8:2, v/v), stirred for 5 minutes, and centrifuged (2 minutes, 10,000*g*, 4°C). The upper phases were pooled and dried under nitrogen. The residues were dissolved in 1 mL of acetone and the extracts were filtered prior to HPLC analysis (0.2 µm, polytetrafluoroethylene minisart SRP4 membranes, Sartorius).

Vitamin A was quantified with an Agilent 1100 series chromatograph. The column was a polymeric YMC_{30} (4.6 mm i.d. × 250 mm, 5 µm particle size). The mobile phase comprised two mixtures (methanol and milli-Q water, 60:40, v/v, and methanol, methyl tert-butyl-ether and milli-Q water, 28.5:67.5:4, v/v/v) at a flow rate of 1 mL min^{-1}. The gradient used went from 100% to 0% over a period of 35 minutes. A UV-visible photodiode array detector (Dionex UVD 340U) was used, and the chromatograms were analyzed at the wavelength of maximum absorption of vitamin A in the mobile phase (λ_{max} = 325 nm). Vitamin A was identified by comparing its retention time and spectra with commercial standards. External calibration was performed by the use of stock standard solutions by dissolving the pure chemical in acetone. These calibration solutions were prepared weekly and analyzed with HPLC.

Quantification of vitamin A in fortified food by the portable fluorometer

The iCheck FLUORO with iEx Mila test kit was developed to determine the vitamin A content in fortified foods such as flour, milk, sugar, and premix, as well as in biological fluids. A one-step extraction from the sample is performed by the iEx Mila vial, which serves thereafter for a direct measurement on the iCheck FLUORO.

Prior to analysis, the portable fluorometer and the reagents were stored at room temperature (20°–25°C). According to the provider, the fluorometer has a working range of 50 to 3,000 µg RE L^{-1}. Since flours and sugars are usually fortified in the range of 1.0 to 9.0 and 5 to 15 mg RE kg^{-1}, the fortified products have to be diluted with distilled water at ratios of 1:10 and 1:20, respectively.

The diluted sample was then injected with a syringe (0.5 mL) into the extraction vial. After shaking for 10 seconds, the vial was left for 5 minutes at ambient temperature for the phase separation and complete extraction of the vitamin A from the sample. Afterwards it was inserted in the fluorometer measurement chamber and the portable fluorometer was covered with a metal cap. Four measurements were performed on each sample. In 5 minutes, the portable fluorometer indicated the results on the screen in mg RE kg^{-1}.

Procedure for the assessment of the portable fluorometer

To determine the *calibration curves* of the portable fluorometer and HPLC, measurements were performed on four levels of concentration with five repetitions for each one. Five calibration curves were determined by linear regression. The concentration of retinyl palmitate [RP] in the fortified product was related to the response R (obtained by the fluorometer or HPLC):

$$R = a_0 \times [RP] + b_0 \quad (1)$$

where *a* is the slope (or sensitivity) and *b* is the intercept of the straight line. The mean sensitivity was determined on the five repetitions.

The *linear range* of both methods was determined by the use of five levels of concentration with three repetitions for each one. Therefore, 15 samples were analyzed per fortified product.

The *intra-assay precision* is the variability in repeated measurements on the same item performed by one person with the same instrument and under similar conditions. Measurements were carried out for two concentrations with eight repetitions: 1 and 5 mg RE kg^{-1} for flours, 0.5 and 1.5 mg RE kg^{-1} for milks, and 5 and 10 mg RE kg^{-1} for sugar. The coefficient of variation (CV) of the results was calculated.

The *interperson precision* refers to the possibility for another operator working independently to reproduce an entire experiment and obtain similar results. Measurements were carried out by two technicians for one medium concentration with three repetitions on the

same day: 5 mg RE kg^{-1} for flours, 1.5 mg RE kg^{-1} for milks, and 10 mg RE kg^{-1} for white sugar. Thus, six measurements for each product could be statistically compared.

The limits of detection and quantification were calculated using the parameters a_0 and b_0 determined by linear regression with the calibration curves (equation 1). The *limit of detection* (LOD) is the lowest quantity of a compound that can be distinguished from the total absence of that substance in a blank [27]. The standard deviation (SD) of 10 blanks, sb_0, was determined, and LOD was calculated as follows:

$$\text{LOD} = (b_0 + 3\ sb_0)/a_0 \quad (2)$$

The *limit of quantification* (LOQ) is the limit at which we can reasonably admit that there is a difference between two values and is calculated as:

$$\text{LOQ} = (b_0 + 10\ sb_0)/a_0 \quad (3)$$

The LOQ is the lowest concentration of an analyte that can be determined with acceptable precision and accuracy.

Statistical analysis

The data were processed with Excel 2010, and statistics were computed with Statgraphics Plus 5.1. The results obtained with the portable fluorometer and with HPLC were compared using a linear regression by the least squares method. To evaluate the linearity of the calibration curve, an analysis of variance associated with a lack of fit tests was conducted on the data to guarantee that the model was linear and predictive. The coefficient of determination (R^2) was calculated to evaluate the goodness of fit of the linear model to the data. If the value obtained was greater than 0.9, the linearity was considered satisfactory.

To determine the interperson precision, the results obtained with the six measurements for each fortified product were compared by analysis of variance, Fisher's test, and Fisher's LSD multiple range test. Significance was accepted at $p < .05$.

A Bland–Altman plot was used to represent the difference between the results obtained with the two methods when measuring the same chemical [28]. This method can determine whether there is any bias between the measurements.

Results

Calibration functions and linear range

For each product and method, five calibration curves were obtained (**table 1**). The mean sensibility and intercept were determined. Parameters (the slope a_0 and the intercept b_0 of the straight line) of these calibration curves were used to calculate the limits of detection and quantification. Linearity of the model previously determined by the least squares method and the coefficients of determination R^2 revealed a good linearity of both methods for all the tested products (always > 0.9)

TABLE 1. Calibration curves for the determination of vitamin A in flours, sugar, and milks

Food	HPLC	Portable fluorometer
Wheat flour T_{55}	$R_{HPLC} = 1.0320\ [RP] + 0.0063$	$R_{iCheck} = 0.9442\ [RP] + 0.4733$
Wheat flour T_{110}	$R_{HPLC} = 1.1393\ [RP] + 0.0953$	$R_{iCheck} = 0.8836\ [RP] + 0.2580$
Corn flour	$R_{HPLC} = 1.0766\ [RP] + 0.0972$	$R_{iCheck} = 0.6596\ [RP] + 0.5143$
White sugar	$R_{HPLC} = 1.0028\ [RP] - 0.1427$	$R_{iCheck} = 0.9331\ [RP] + 0.5537$
Milk 1.5% fat	$R_{HPLC} = 0.9002\ [RP] + 0.3100$	$R_{iCheck} = 1.0847\ [RP] + 0.0149$
Milk 3.0% fat	$R_{HPLC} = 0.8078\ [RP] + 0.5459$	$R_{iCheck} = 0.8158\ [RP] + 0.6861$

HPLC, high-performance liquid chromatography; [RP], retinyl palmitate concentration

TABLE 2. Linearity, limits of detection, and quantification of the portable fluorometer versus high-performance liquid chromatography (HPLC)[a]

		HPLC			Portable fluorometer		
Food	[RP]	R^2	LOD	LOQ	R^2	LOD	LOQ
Wheat (T_{55})	1–10	0.9576	0.14	0.34	0.9477	1.46	1.86
Wheat (T_{110})	1–10	0.9567	0.25	0.42	0.9072	0.59	0.75
Corn	1–10	0.9663	0.48	0.76	0.9191	0.97	1.42
Milk 1.5% fat	0.5–3	0.9851	0.60	1.00	0.9455	–0.08	–0.02
Milk 3.0% fat	0.5–3	0.9372	0.89	1.30	0.9436	0.88	1.01
Sugar	5–15	0.9802	0.30	0.36	0.9667	0.97	2.28

a. Retinyl palmitate concentration [RP], limit of detection (LOD), and limit of quantification (LOQ) are expressed in mg RE kg^{-1}.

TABLE 3. Interperson and intra-assay precision between the portable fluorometer versus high-performance liquid chromatography (HPLC)

	Interperson precision		Intra-assay precision			
	HPLC	Fluorometer	HPLC	Fluorometer	HPLC	Fluorometer
Food	*p*	*p*	CV (%)	CV (%)	CV (%)	CV (%)
[RP] (mg RE kg^{-1})	5	5	1	1	5	5
Wheat (T_{55})	0.0128*	0.9331	30.08	11.26	7.82	17.42
Wheat (T_{110})	0.4603	0.8732	8.03	11.69	9.50	17.52
Corn	0.6039	0.4886	15.13	8.35	12.70	8.66
[RP] (mg RE kg^{-1})	10	10	5	5	10	10
Sugar	0.2455	0.5611	11.06	9.77	5.72	9.35
[RP] (mg RE kg^{-1})	1.5	1.5	0.5	0.5	1.5	1.5
Milk (1.5% fat)	0.0735	0.8964	6.73	5.25	16.80	5.56
Milk (3.0% fat)	0.1040	0.5826	12.37	4.87	8.38	2.87

CV, coefficient of variation; RE, retinol equivalent; [RP], retinyl palmitate concentration; p statistic test performed (*$p < .05$ indicates a significant difference between data.)

(**table 2**). Even if R^2 for the fluorometer values was generally smaller than R^2 for the HPLC values, the linearity was always very satisfactory ($R^2 > 0.907$).

Limits of detection and quantification

The limit of detection (LOD) is defined as "the lowest concentration of analyte that gives a signal significantly different from the blank or background signal." The limit of quantification (LOQ) is the lowest concentration of an analyte that can be determined with acceptable precision and accuracy. The HPLC method can detect small quantities of vitamin A with an LOD between 0.14 and 0.89 mg RE kg^{-1} and an LOQ between 0.34 and 1.30 mg RE kg^{-1} (**table 2**). The portable fluorometer could quantify vitamin A in wheat flour with an LOQ until 0.59 and 1.46 mg RE kg^{-1} depending on the characteristic of the flour.

In **table 2**, LOQ is always superior to LOD, whichever method is used. This indicates that the analytical methods seek to detect analyte in low quantities, but higher concentrations have to be analyzed to produce reliable results. Globally, the minimum of vitamin A detected (LOD) and quantified (LOQ) by HPLC are lower in comparison to the portable fluorometer. The fluorometer is slightly less reliable than the HPLC.

Interperson and intra-assay precision

The interperson precision was satisfactory, and no significant differences were observed between the results obtained by both technicians the same day with the portable fluorometer (except for wheat T_{55} data obtained by HPLC). During the assessment of intra-assay precision, the CV of the eight values was determined; it represents the disparity of the measurements at one concentration. The CV for intra-assay precision ranged from 2.87% to 17.52% with the portable fluorometer and from 5.72% to 30.08% with HPLC (**table 3**). The intra-assay precision was sometimes better with the fluorometer than with HPLC. For wheat T_{55}, the HPLC analysis of vitamin A was not satisfactory, because interference with another biochemical compound did not allow a good integration on the chromatograms, even with manual integration.

Comparison between HPLC and the portable fluorometer

A rather good linearity was observed between the results obtained with the fluorometer and the results obtained with HPLC (**fig. 1**). The coefficients of determination (R^2) were greater than 0.91 (except for corn flour, with approximately 0.89). The linear relationship between the two methods in the range of concentrations tested confirms that the portable fluorometer provides a good determination of the vitamin A content in the fortified products. According to the Bland–Altman plot, there was no systematic and observable bias between the data obtained with the fluorometer and the HPLC, and adequacy was good (**fig. 2**).

Discussion

The portable fluorometer (iCheck FLUORO) and its test kit vials (iEx Mila) precisely determined the vitamin A contents in fortified flours, sugar, and milks. The working range was 1 to 10, 0.5 to 3.0, and 5 to 15 mg RE kg^{-1} for flours (wheat and corn), milks, and white sugar, respectively. Its limits of detection are higher than those of HPLC but are always low (< 1.3 mg RE kg^{-1}). This range is in accordance with the WHO recommendations for food fortification in least developed countries. The low coefficient of variation and limits of detection indicate that the portable fluorometer is

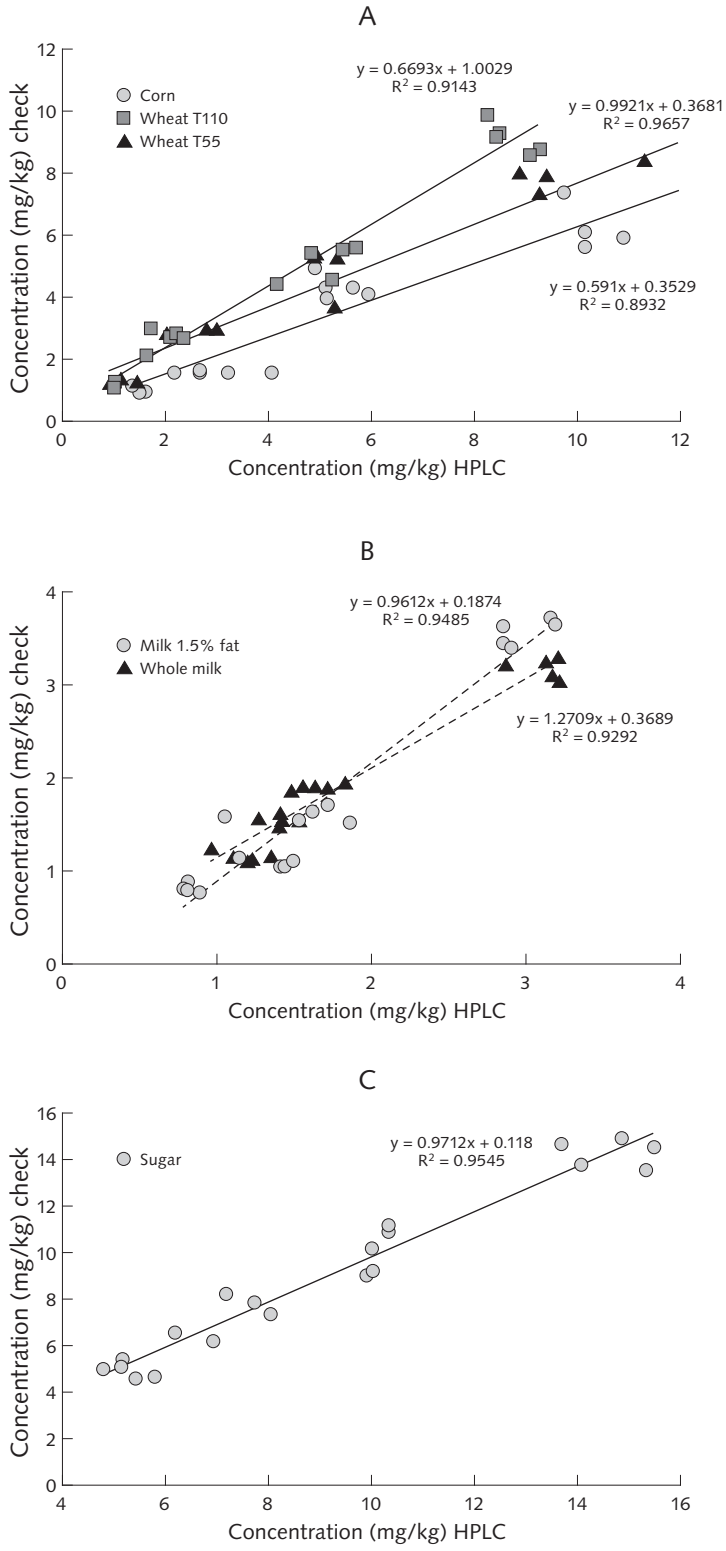

FIG. 1. Linear regression between vitamin A quantification in flours (A), milks (B), and sugar (C) obtained with high-performance liquid chromatography (HPLC) and the portable fluorometer iCheck FLUORO

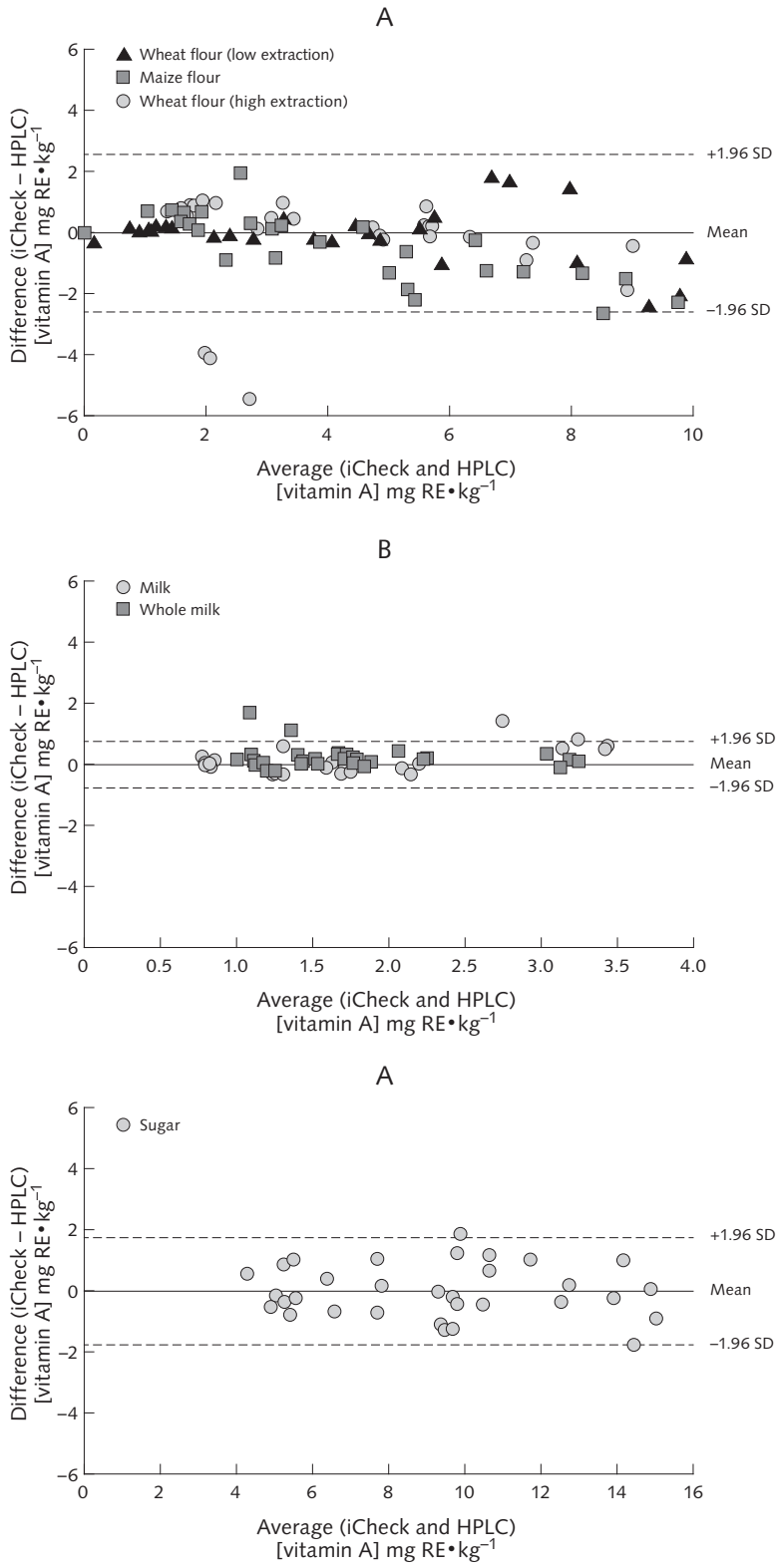

FIG. 2. Bland-Altman plot comparing results obtained with the portable fluorometer iCheck FLUORO and high-performance liquid chromatography (HPLC). The ± 1.96 SD lines represent the 95% limits of agreement

as sensitive as HPLC and sometimes more sensitive. Therefore the fluorometer can be considered a good tool to enforce large-scale fortification legislation.

The product and technical information given by the providers indicates that no additional equipment is required to perform the analysis. After injection of the flour or sugar extracts into the vial, it must be shaken and left for 5 minutes to obtain two clear distinct phases. During our experiments, a single decantation (even over a long time) was not enough to separate the two phases; a slow centrifugation was always necessary, even at low speed (manual equipment can be used).

One of the main hurdles for many stakeholders and international organizations in charge of fortification projects is to analyze fortified food products in suitable, reliable, and cost-transparent laboratories. Lack of quality control capacity and systems for inspection represents a risk for governmental investment in fortification (to ensure efficient use of public funds), but it is also a significant impediment to private-sector companies exporting their products to neighboring countries with specific fortification standards. The laboratories need to have experience in analyzing vitamins and minerals in the food matrix and to be accredited by an international standard laboratory in order to guarantee their capabilities. In some countries, in addition to the lack of technical capacity, there are also issues of transparency in the analytical results. This rapid test kit will allow fieldworkers to perform first-line analyses of the quality of the products at the various stages of the distribution chain. Additionally, it could allow food inspectors to perform tests on site (at the industry level), enabling immediate actions on vitamin A incorporation. Finally, as has been already highlighted by several articles, it is urgent for the manufacturer of the portable fluorometer to support the management of the chemical waste due to their solvent composition. Even if the hazardous reagents are reduced with this device, once the vials are used, they still must be handled by a specialized company, and unfortunately such facilities are not available in most developing countries.

Conclusions

The portable fluorometer assessed in our study offers a viable solution to the challenge of analyzing vitamin A contents simply, reliably, and safely in several foods (milk, wheat and maize flour, and sugar). Further research and field use are warranted to obtain more experience with possible drawbacks (such as the process of recycling the chemicals) and to extend the assessment to local conditions, such as high temperature and high humidity.

Conflicts of interest

The authors declare that they have no conflicts of interest and are not directly or indirectly affiliated with any profit-making units that might create a conflict of interest.

Authors' contributions

Arnaud Laillou, Cécile Renaud, Jacques Berger, Regina Moench-Pfanner, and Sylvie Avallone conceived and designed the experiments. Cécile Renaud, Laura Fontan, and Sylvie Avallone performed the experiments. Arnaud Laillou, Cécile Renaud, Jacques Berger, and Sylvie Avallone analyzed the data. Arnaud Laillou, Cécile Renaud, Jacques Berger, Regina Moench-Pfanner, and Sylvie Avallone wrote the paper.

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References

1. UNICEF. Goals for children and development in the 1990s. New York: UNICEF, 1990
2. World Health Organization. Global prevalence of vitamin A deficiency in populations at risk, 1995–2005. WHO Global Database on Vitamin A Deficiency. Geneva: WHO, 2009.
3. Aykroyd W. Medical resurvey of nutrition in Newfoundland 1948. Can Med Assoc J 1949:60:329–52.
4. Candelaria LV, Magsadia CR, Velasco RE, Pedro MR, Barba CV, Tanchoco CC. The effect of vitamin A-fortified coconut cooking oil on the serum retinol concentration of Filipino children 4–7 years old. Asia Pac J Clin Nutr 2005;14:43–53.
5. Muhilal, Murdiana A, Azis I, Saidin S, Jahari AB, Karyadi D. Vitamin A-fortified monosodium glutamate and vitamin A status: a controlled field trial. Am J Clin Nutr 1988;48:1265–70.
6. Solon FS, Klemm RD, Sanchez L, Darnton-Hill I, Craft NE, Christian P, West KP Jr. Efficacy of a vitamin A-fortified wheat-flour bun on the vitamin A status of Filipino schoolchildren. Am J Clin Nutr 2000;72:738–44.
7. Van Stuijvenberg ME, Kvalsvig JD, Faber M, Kruger M, Kenoyer DG, Benadé AS. Effect of iron-, iodine-, and β-carotene–fortified biscuits on the micronutrient status

of primary school children: a randomized controlled trial. Am J Clin Nutr 1999;69:497–503.
8. Latham MC, Ash DM, Makola D, Tatala SR, Ndossi GD, Mehansho H. Efficacy trials of a micronutrient dietary supplement in schoolchildren and pregnant women in Tanzania. Food Nutr Bull 2003;24:120–8.
9. Vinodkumar M, Erhardt JG, Rajagopalan S. Impact of a multiple-micronutrient fortified salt on the nutritional status and memory of schoolchildren. Int J Vitam Nutr Res 2009;79:348–61.
10. Chen K, Li TY, Chen L, Qu P, Liu YX. Effects of vitamin A, vitamin A plus iron and multiple micronutrient-fortified seasoning powder on preschool children in a suburb of Chongqing, China. J Nutr Sci Vitaminol 2008;54:440–7.
11. Aaron GJ, Kariger P, Aliyu R, Flach M, Iya D, Obadiah M, Baker SK. A multi-micronutrient beverage enhances the vitamin A and zinc status of Nigerian primary schoolchildren. J Nutr 2011;141:1565–72.
12. Huo J, Sun J, Huang J, Li W, Wang L, Selenje L, Gleason GR, Yu X. The effectiveness of fortified flour on micronutrient status in rural female adults in China. Asia Pac J Clin Nutr 2011;20:118–24.
13. Lien do TK, Nhung BT, Khan NC, Hop le T, Nga NT, Hung NT, Kiers J, Shigeru Y, Biesebeke R. Impact of milk consumption on performance and health of primary school children in rural Vietnam. Asia Pac J Clin Nutr 2009;18:326–34.
14. Arroyave G, Mejia LA. Five decades of vitamin A studies in the region of Central America and Panama. Food Nutr Bull 2010;31:118–29.
15. Moench-Pfanner R, Van Ameringen M. The Global Alliance for Improved Nutrition (GAIN): a decade of partnerships to increase access to and affordability of nutritious foods for the poor. Food Nutr Bull 2012;33:S373–80.
16. Akinyele IO. Ensuring food and nutrition security in rural Nigeria: an assessment of the challenges, information needs, and analytical capacity. Background Paper No. NSSP 007. Garki, Abuja, Nigeria: International Food Policy Research Institute, 2009.
17. Yusufali R, Sunley N, de Hoop M, Panagides D. Flour fortification in South Africa: post-implementation survey of micronutrient levels at point of retail. Food Nutr Bull 2012;33:S321–9.
18. Faulkner H, Hussein A, Foran M, Szijarto L. Survey of vitamin A and D contents of fortified fluid milk in Ontario. J Dairy Sci 2000;83:1210–6.
19. Bechir M, Schelling E, Kraemer K, Schweigert F, Bonfoh B, Crump L, Tanner M, Zinsstag J. Retinol assessment among women and children in Sahelian mobile pastoralists. EcoHealth 2012;9:113–21.
20. Schweigert F, Frey S, Mothes R, Dary O, Juarez P, Lascano V. A new test kit's potential for the rapid analysis of vitamin A in human and cow milk. Sight and Life 2011; 3:18–22.
21. Sanchez-Mena JD, Zambo Z, Orozco M, Solomons NW. Vitamin A content in sugar determined by a rapid assay device (Icheck® FLUORO). Center for studies of Sensory Impairment, Aging and Metabolism. Bulletin of Research Abstracts. Special Issue on Vitamin A 2012; 23:1–5.
22. Zambo Z, Sanchez-Mena JD, Orozco M, Solomons NW. Mounting and adaptation of a fluorescent rapid-assay device (Icheck® FLUORO) for vitamin A in sugar and biological fluids. Center for studies of Sensory Impairment, Aging And Metabolism. Bulletin of Research Abstracts. Special Issue on Vitamin A 2012;23:1–4.
23. Barua AB, Furr HC, Olson JA, van Breemen RB. In: de Leenheer AP, Lambert WE, Van Bocxlaer JF, eds. Modern chromatographic analysis of vitamins, 3rd ed. New York: Marcel Dekker, 2000:1–74.
24. Allen L, de Benoist B, Dary O, Hurell R. Guidelines on food fortification with micronutrients. Geneva: World Health Organization/Food and Agriculture Organization, 2006.
25. Taungbodhitham AK, Jones GP, Wahlqvist ML, Briggs DR. Evaluation of extraction method for the analysis of carotenoids in fruits and vegetables. Food Chem 1998;63:577–84.
26. Salo-Väänänen P, Ollilainen V, Mattila P, Lehikoinen K Salmela-Mölsä E, Piironen V. Simultaneous HPLC analysis of fat-soluble vitamins in selected animal products after small-scale extraction. Food Chem 2000;71:535–43.
27. Pénicaud C, Peyron S, Bohuon P, Gontard N, Guillard V. Ascorbic acid in food: development of a rapid analysis technique and application to diffusivity determination. Food Res Int 2010;43:838–47.
28. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1:307–10.