International Food Research Journal 22(2): 684-690 (2015)
Journal homepage: *http://www.ifrj.upm.edu.my*

Physicochemical properties of sweet potato cookies fortified with some nutrients

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Article history

Received: 8 January 2014
Received in revised form: 20 September 2014
Accepted: 25 September 2014

Keywords

Sweet potato
Fortification
Micronutrient
Retention
Cookies characteristic

Abstract

Micronutrient deficiencies have become a chronic health problem especially in the developing countries over the world. An attempt to overcome some micronutrient deficiencies was performed in this study by providing a fortification of sweet potato cookies. Fortification of 1.25 g vitamin C, 75 mg iron, 42 mcg vitamin B_{12}, 2000 mcg folic acid and 10.5 mg vitamin A per 100 g cookies was conducted. Physicochemical properties of the fortified cookies, as well as the evaluation of micronutrients retention in the cookies were investigated. Vitamins A, C and folic acid in the cookies were analyzed by HPLC method, whilst for iron by AAS method. Fortified sweet potato cookies had different characteristics from the control (unfortified) either in the chemical, physical or sensory properties. The fortification significantly increased the hardness of the cookies. However, the fortified cookies had a lower consumer preference in terms of texture and overall acceptability. The evaluation of micronutrients retention showed that the retention of vitamins in the cookies differed from each other. The amounts of vitamins A and C in the cookies could be retained more than 60% from the initial amount. In contrast, folic acid and vitamin B_{12} had a much lower retention (below 15%). Iron, as predicted, had 100% retention. Hence, the fortified cookies had a potential as a source of vitamin A, vitamin C and iron to overcome the micronutrient deficiencies.

Introduction

Currently, it is known that micronutrient deficiencies threat 2 billion people in the world (FAO, 2013). Among them, iron deficiency related-anemia is the most chronic since its prevalence worldwide reached 24% (Benoist *et al*., 2008). The last national survey in Indonesia (2007) showed that anemia prevalence reached 14.8% of the population, and the highest was found in Southeast Sulawesi (31.2%) and the lowest was in Jambi (6.6%) (Indonesian Ministry of Health, 2008). The anemia could reduce mental and physical performance, because anemia patients tend to feel headache, dizziness, and loss of appetite; moreover the severe anemia in pregnant women has been known to give a significant contribution to the maternal death, prematurely born and low birth weight (Sarin, 1995; Galloway *et al*., 2002). Other micronutrient deficiencies (vitamin A, vitamin B_{12}, folic acid, vitamin C deficiencies) also cause some specific symptoms (Canfield *et al*., 2006, Lee-Guzman *et al.*, 2011; Talaulikar and Arlkumaran, 2013). This problem threats the people in some parts of the world.

Food base approaches, by means of food diversification or fortification, are considered as more strategic program for sustainable prevention of micronutrient deficiencies in general population than by supplementation (Benoist *et al.*, 2008). Furthermore, the food fortification will become a promising alternative to reduce the micronutrient deficiencies when food diversification is not affordable for many people (Akhtar *et al*., 2011). The effective food fortification program is using daily consumed food products and ingredients, such as wheat flour, bread, noodles, rice, vermicelli or biscuits (cookies and wafers), to have a significant effect on the micronutrients intake (Shikany *et al.*, 2004; Sadighia *et al.*, 2008; Cheung *et al.*, 2009; Akhtar *et al*., 2011; Kam *et al*., 2012).

The monitoring of micronutrients retention and their effect on the physicochemical changes of the fortified product is important in order to evaluate the fortification effectiveness (Akhtar *et al.*, 2011; Kyritsi *et al*., 2011; Kam *et al*., 2012). Vitamins A and C are well known to be unstable under heating, light and some of minerals (i.e. iron) exposure (Lee and Kader, 2000; Hal *et al.*, 2012). Retention of folic

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acid and other vitamins B have also to be considered for food fortification due to their readily degraded by low pH and common food processing condition (Gregory III, 2008). Although iron is quite stable in food processing, the undesirable effect may occur due to the mineral interaction with other cookies components.

In this study, sweet potato cookies were chosen to be fortified by adding vitamin C, iron, vitamin B_{12}, folic acid and vitamin A. Sweet potato is one of important indigenous carbohydrate source and it is more common and affordable to be consumed by Indonesian people besides staple food, rice. Sweet potato is suitable for cookies raw material because it can be processed into flour with high carbohydrate content to replace wheat flour. Moreover, its productions that reach more than 2 million tons annually (Indonesian Center Agency of Statistics, 2014) make it possible to be a raw material for the mass production of commercial fortified cookies. The micronutrients fortification in sweet potato cookies has never been reported. This study may give important information on the possibility of sweet potato cookies as an appropriate way for micronutrients fortification. Therefore, the aims of this study were to determine the effect of those micronutrients fortification on the cookies characteristics, and to evaluate the retention of micronutrients fortified in sweet potato cookies.

Materials and Methods

Materials

Sweet potato flour and other ingredients (butter, margarine, sugar, skim milk, vanilla, eggs, baking powder, and baking soda) for cookies formulation were purchased from local market in Bogor, West Java Indonesia. Vitamin C (Aland Jiangsu Jiangshan, China), vitamin A (retinil acetate 325 GFP, from BASF, Germany), Vitamin B_{12} (BASF, Germany) and folic acid (Hebei Jiheng, China) were provided by Global Chemindo Megatrading Co., Indonesia, the materials contained active components 99.8%, 359000 IU/g, 0.1%, 97%, respectively. Ferrous fumarate (containing 32.87 % of iron) was purchased from Nila Merkindo Utama Co., Indonesia.

Micronutrients fortification and cookies production

Sweet potato flour (42.98%) was dry-mixed with baking soda (0.42%) and baking powder (0.17%). Butter (15.74%), palm oil margarine (5.25%), sugar (18.89%), skim milk (5.46%), vanilla (0.34%), and egg yolks (6.72%) were stirred by a hand mixer (Phillips, Netherland) in high speed for 10 minutes to form creamy texture. Dry material and cream were mixed to make a dough. Chocolate chips (5.04%) were added into the dough, and the dough was shaped (round shape with 4 cm in diameter and 0.5 cm in thickness), and baked in the baking oven (Mahyih, MY-736, Taiwan) at 160°C for 27 minutes. Cookies with fortification treatment include the addition of micronutrients premix at the first step of dry-mixing. The amounts of micronutrients addition were determined base on the previous study by Briawan *et al.* 2008 that give a good result for increasing the nutritional status of teenage girls (1250 mg of vitamin C, 75 mg of iron, 42 mcg of vitamin B_{12}, 2000 mcg of folic acid and 10.5 mg of vitamin A in 100 g of cookies). To achieve this level, 11.65 g vitamin C, 2.20 g ferrous fumarate, 691.1 mg vitamin A, 357.4 mg vitamin B_{12}, and 19.6 mg folic acid were added in 1000 g of cookies dough.

Micronutrients retention

Micronutrients retention was calculated based on the percentage of micronutrient concentration existing in fortified sweet potato cookies divided by the sum of micronutrient addition and initial micronutrient content (concentration level in the unfortified cookies) (Kyristi, *et al*., 2011), as follow:

$$M_r = \frac{M_f}{M_a + M_i} x\ 100$$

M_r, M_f, M_a and M_i were representing micronutrient retention (%), micronutrient concentration in fortified sweet potato cookies, added micronutrient and initial micronutrient concentration in the unfortified ones, respectively.

Vitamin C analysis

Vitamin C analysis was conducted by HPLC (Waters e2695, USA) equipped with UV-VIS detector (Waters corporation, 2004 with some modifications). Approximately 250 mg samples were weighed in 25 mL volumetric flask, mixed with 10 mL TFA 0.1% using ultrasonic for 30 minutes and then tared to volume by TFA 0.1%. Sample solution was filtered through a filter paper (Whatman No.42) and 0.45 µm Millipore membrane. The final sample solution (10 µL) and a series of standard solutions were injected into HPLC instrument. Separation was conducted by using C18 (ODS) column at room temperature. Trifluoroacetic acid (TFA) 0.1% as a mobile phase was delivered at a flow rate of 0.8 mL/minute. The targeted compound was detected at UV 245 nm.

Folic acid analysis

Folic acid was analyzed using UPLC (Waters H Class) linked to UV-Vis detector (Rahimi and

Goodarzi, 2011). Sample (5 g) was weighed and transferred into 25 mL volumetric flask, then added by 10 mL phosphate buffer 0.1 M pH 7.00 and 5 mL acetonitrile, mixed for 30 minutes, and finally added by phosphate buffer 0.1 M pH 7.00 to volume. The sample solution was then centrifuged at 8500 rpm for 15 minutes. The solution was then filtered using a filter paper (Whatman No. 42), activated SPE Sep-Pax C18 and 0.20 µm Millipore membrane. Sample solution (2 µL) and a series of standard solutions were injected to UPLC instrument. This separation used Acquity UPLC BEH Shield RP-18 column. Solvent A (2% acetic acid pH 2.8) and Solvent B (acetonitrile) were used as mobile phases and delivered in gradient mode at a rate of 0.3 mL/min. Gradient condition was set as follows: 0 to 4 min from 5% to 15% solvent B, 5 to 6 min from 5% 15% to 5% solvent B. Folic acid was detected at UV 283 nm.

Vitamin A analysis

Vitamin A was determined by HPLC (Waters e2695, USA) completed with UV-VIS detector (Ministry of Health of the People's Republic of China, 2010). Sample (2 g) was weighed in 50 mL centrifuge tube, and added by 5 mL ethanol-ascorbic acid 0.1% and 4 mL KOH solution (12.5%), then heated at 70°C in a water bath for 30 min, vortex, and cooled. The targeted compound in the sample solution was extracted by liquid-liquid extraction using 5 mL n-hexane. Hexane fraction was collected. Further separation was conducted by adding 1 mL methanol-ascorbic acid 0.1% and 2 times of 10 mL n-hexane. Hexane fraction was pooled into one fraction and then evaporated until dry, re-diluted by HPLC grade methanol, transferred to 50 mL volumetric flask and added by methanol to volume. Sample was filtered using a 0.45 µm Millipore membrane prior to HPLC injection. Sample or standard solutions (each 20 µL) were separately injected into HPLC. The separation was conducted using RP-18 column. Methanol as a mobile phase was delivered at 0.7 mL/min. Vitamin A was detected at UV 325 nm.

Vitamin B_{12} analysis

HPLC (Waters e2695, USA) with UV-Vis detector was used for Vitamin B12 determination (Heudi *et al*., 2006 with some modifications). Sample (1 g) was weighed in a tube, then added by 5 mL H_2SO_4 1%, heated at 100°C in a water bath for 30 min, vortex every 10 min and cooled. Sample solution was added by 1.4 mL sodium acetate 2 M and quantitatively transferred to 10 mL volumetric flask. Sample solution was added by 2 mL papain 0.1%, added by distilled water to volume and filtered through a filter paper Whatman No. 42 and a 0.45 µm Millipore membrane before HPLC injection. Sample or standard solution (each 20 µL) was injected into HPLC. The targeted compound was separated by RP-18 column. Water, acetonitrile and TFA 0.025% as a mobile phase was distributed at a flow rate of 0.7 mL/min. Vitamin B_{12} was detected at UV 361 nm.

Iron analysis

Iron measurement was performed by Flame-Atomic Absorption Spectrophotometer (AAS) (AOAC, 2005). Sample was prepared using wet ashing method. AAS- flame was applied using the Fe cathode lamp with a wavelength of 248.3 nm. Standard curve was established using a series of Fe standard solutions prepared from a Fe stock solution (1000 mg/L).

Chemical composition

Unfortified and fortified sweet potato cookies were analyzed in terms of proximate: moisture content, ash content, crude fat content and crude protein content (AOAC, 2005). Carbohydrate content was calculated using by-difference method. The difference of chemical composition between unfortified and fortified cookies was evaluated using paired two samples for means t-test.

Color determination

The color of cookies was determined using a colorimeter (Minolta CR 300, Japan). This instrument measured 3 color parameters, L^*, a^* and b^*. L^* represented the brightness from black (0) to white (100); a^* represented the color of green (-) and red (+); and b^* represented the color of yellow (+) and blue (-). The value of °Hue was calculated by arc tan (b^*/a^*) to describe the overall color of the sample.

Hardness

Cookies hardness was analyzed using Stevens LRFA Texture Analyzer (Škrbić and Cvejanov, 2011 with some modifications). Hardness was defined as the peak force of the first compression of the product. The setting for instrument was as follows: pre test speed at 2 mm/s, test speed at 0.5 mm/s, post test speed at 10 mm/s, rupture test distance at 1.0 mm, distance at 2.0 mm, force at 100 g, load cell at 25 kg, time at 5 sec and count at 2. Paired two samples for means t-test was used to verify the difference of hardness between fortified and unfortified cookies.

Acceptability test

Acceptability test was conducted by rating hedonic test (Meilgard *et al*., 2007). Both fortified

Table 1. Sweet potato cookies composition

Chemical composition	Resulted cookies		Other cookies (Wheat flour cookies)	
	Unfortified Cookies	*Fortified Cookies*	*Cookies 1**	*Cookies 2***
Moisture content (g/100g)	3.37±0.28[b]	2.18±0.11[a]	3.57 ± 0.03	2.320 ± 0.055
Ash content (g/100g)	2.69±0.06[a]	2.80±0.16[a]	0.59 ± 0.02	0.530 ± 0.015
Crude fat content (g/100 g)	16.73±1.36[a]	14.91±2.13[a]	17.7 ± 0.2	22.710 ± 0.193
Protein content (g/100g)	4.47±0.23[a]	5.15±0.41[b]	7.45 ± 0.12	6.670 ± 0.174
Carbohydrate (g/100g)	72.74±0.69[a]	74.97±2.76[a]	65.1 ± 0.3	NA
Vitamin C (mg/100g)	0.89±0.91[a]	814.28±7.06[b]	NA	NA
Vitamin B_{12} (mcg/100g)	2.72±0.41[a]	2.73±0.42[a]	NA	NA
Vitamin A (mcg/100g)	102.30±3.11[a]	10530.35±14.64[b]	NA	0.160 ± 0.003
Folic acid (mcg/100g)	213.71±32.87[a]	248.38±49.67[a]	NA	NA
Mineral Fe (mg/100g)	5.00±0.09[a]	94.90±1.74[b]	1.24 ± 0.07	NA

Value with different superscript in the same row is significantly different ($P < 0.05$)
*(Škrbić B., and Cvejanov. 2011)
**(Butt *et al.*, 2007)
NA: Data are not available

and unfortified samples were given to 70 schoolgirls of a junior high school in Bogor, Indonesia. The panelists tested two pieces (4 g each for fortified and unfortified) of three digit coded sample served with random permutation. The test was conducted individually in a classroom. The panelists answered the questions regarding the preference attributes on color, aroma, texture, taste and overall. For this question, 7-point hedonic scales (extremely like (7), like (6), like slightly (5), neither like nor dislike (4), dislike slightly (3), dislike (2) and extremely dislike (1)) were given as an expression of panelist acceptability. The panelist response was analyzed using a statistical analysis of paired two samples for means t-test.

Result and Discussion

Chemical composition

The chemical composition of fortified and unfortified sweet potato cookies is presented in Table 1. Statistical analysis results using paired two samples for means t-test showed that moisture content in fortified sweet potato cookies was lower than in the unfortified one, while vitamin C, vitamin A and iron concentrations in fortified sweet potato cookies were much higher. In contrast to those parameters, micronutrients fortification did not affect the concentration of ash, crude fat, protein, carbohydrate, vitamin B_{12} and folic acid.

Based on the proximate data (moisture, ash, protein and carbohydrate content), micronutrient fortification significantly affected moisture content, due to the increase of solid content with the fortification treatment. Vitamin C, vitamin A and iron concentrations in fortified cookies were higher than those in unfortified ones, due to the condition described above. However, folic acid and vitamin B_{12} concentrations relatively unchanged by the fortification treatment. When fortified cookies was compared to other cookies, it show that fortified cookies had much higher vitamin A and Iron level than standard cookies from wheat flour.

Physical characteristic

Physical characteristic of fortified and unfortified sweet potato cookies is shown in Table 2.

Table 2. Physical characteristic of sweet potato cookies

	Unfortified cookies	*Fortified cookies*
Color		
L*	48.48 ± 0.76[a]	47.56 ± 1.23[a]
a*	9.38 ± 0.29[a]	9.42 ± 0.31[a]
b*	29.54 ± 0.35[a]	29.15 ± 0.37[a]
°Hue	72.38 ± 0.68[b] (yellow red)	72.09 ± 0.70[a] (yellow red)
Hardness (gf)	2,642.3 ± 492.48[a]	3,521.8± 135.29[b]

Value with different superscript in the same row is significantly different ($P < 0.05$)

Sweet potato cookies had a specific color and texture profile. They had yellow red color and tended to have low brightness. Micronutrients fortification only gave slight effect on their color, where the affected color parameter was only °Hue value. However, based on their °Hue values, the color of unfortified and fortified cookies was categorized as the same color, i.e. yellow red. It means that micronutrients fortification did not give significant effect on color.

Table 4. Micronutrients retention in sweet potato cookies

Micronutrients	Initial micronutrients	Added micronutrients in fortified cookies	Micronutrient content in fortified cookies	Retention
Vitamin C	0.88 ± 0.91 mg/100g	1252.44 ± 3.61 mg/100g	814.28±7.06mg/100g	64.94 ± 0.61 %
Folic acid	213.71 ± 32.87 mcg/100g	2047.59 ± 9.79 mcg/100g	248.38±49.67 mcg/100g	10.99 ± 2.24 %
Vitamin B_{12}	2.72 ± 0.41 mcg/100g	42.34 ± 0.08 mcg/100g	2.73±0.42 mcg/100g	6.05 ± 0.92 %
Vitamin A	102.30 ± 3.11 mcg/100g	8024.38 ± 35.98 mcg/100g	10530.35±14.64 mcg/100g	129.58 ± 0.75 %
Mineral Fe	5.00 ± 0.08 mg/100g	75.22 ± 0.06 mg/100g	94.90±1.74 mg/100g	118.30 ± 2.15%

The other physical parameter, hardness was affected by micronutrient addition. Fortified sweet potato cookies were harder than the unfortified one, in which the treatment increased the hardness of sweet potato cookies. This was probably caused by changes in the rheological properties of the dough of sweet potato cookies due to the presence of additional iron. Rheological properties such as water absorption capacity, dough development time and dough stability time, have been reported to change in whole wheat dough as a result of iron and zinc fortification (Akhtar *et al*., 2008).

Sensory characteristic

Based on the sensory analysis results, fortified cookies had a preference score 4.1 – 4.7 using 1 – 7 scales for all attributes, while the unfortified one had a preference score 4.5 – 5.6 (Table 3).

Table 3. Acceptance level of sweet potato cookies rated by 1 – 7 scoring test

	Unfortified cookies	*Fortified cookies*
Color	4.5±1.2[a]	4.7±1.0[a]
Aroma	4.6±1.4[a]	4.4±1.4[a]
Taste	5.4±1.3[a]	4.1±1.2[a]
Texture	5.2±1.3[b]	4.2±1.2[a]
Overall	5.6±1.2[b]	4.7±1.2[a]

Value with different superscript in the same row is significantly different ($P < 0.05$)

The panelists acceptance on sweet potato cookies were in the range of between neither like nor dislike (score 4) and like slightly (score 5) for the color and aroma, whereas the acceptance on taste, texture and overall was between like slightly (score 5) and like (score 6).

The acceptance for color, aroma and taste of fortified cookies didn't change due to the micronutrients addition. However, in terms of texture and overall parameter, the fortification treatment reduced the panelists acceptance, perhaps due to the increase of fortified sweet potato cookies hardness.

Many studies explained that micronutrients fortification in a lower level did not give significant preference on fortified food. Prom-u-thai (2008) proved that iron fortification in form of $FeSO_4$ did not lead significant changes in color and flavor of parboiled rice. Other study reported that iron fortification in form of ferrous fumarate did not give an adverse effect on overall acceptability of product prepared from fortified millet and sorghum flours (Tripathi *et al*., 2012). The acceptability of school meal was not affected by the addition of micronutrients (iron, vitamin A, zinc, folic acid, iodine, vitamin C, thiamin, riboflavin, niacin, vitamin B_{12}, vitamin B_6, vitamin D, vitamin E and copper) in the meal (Osei *et al*., 2008).

Retention of micronutrients

Table 4 shows the retention of micronutrients added in the fortified sweet potato cookies.Vitamin C, vitamin A, and iron could be retained for more than 60% in the fortified cookies, while the retention of vitamin B_{12} and folic acid was very low. Different micronutrients had different retention levels in fortified sweet potato cookies. It depended on the stability of each micronutrient during processing of sweet potato cookies which involved several steps including mixing, forming and baking. However some micronutrients used in this study are found to be stable in the fortified cookies, such as vitamin C, vitamin A and iron, even though among the micronutrients, vitamin C could be 35% reduced in the fortified cookies due to those processing steps. Besides cookies processing condition, the reduction of vitamin C could be affected by the presence of iron. A study on the retention of vitamin C in iron fortified raw milk showed the same result (Hegenauer *et al*., 1979).

Vitamin A (retinyl acetate) was found very stabile during baking. Butt *et al.* (2007) also reported that vitamin A has an excellent retention during wheat cookies baking with the losses of 8.69–11.11%. Furthermore, the report revealed that this reduction could be decreased as the dose of retinyl acetate increased. In this study, the concentration of vitamin A in the fortified cookies, 8000 mcg/100 g, is relatively high compared to levels used in the wheat cookies study. Beside the stability reason, the recovery of

analysis that could reach more than 100% caused the level of vitamin A was higher than the true level. Previous study showed that the recovery of vitamin A analysis by LC-MS was 91 – 114% (Plozza *et al*, 2012).

Like other minerals, iron is not sensitive on heat, light, oxidizing agents, extreme pH or other factors that affect organic compounds (Miller, 2008). Therefore, as predicted, iron in the fortified cookies could be fully retained. The lost of folic acid was as high as 89% during sweet potato processing. The very high lost might be affected by the condition present in this study. It has been known that the mechanism of folic acid degradation is affected by the form of folate and its environment (Gregory III, 2008). Folic acid is susceptible to oxidative degradation to form inactive product. Moreover, the substance can be degraded under low pH and high temperature (Arcot and Shrestha, 2005). The low pH condition was found in this study due to the presence of vitamin C at high concentration in the fortified cookies. This fact has also been occurred for vitamin B_{12}, therefore the retention of vitamin B_{12} was also very low.

Conclusion

In this study, micronutrients fortification in sweet potato cookies was proper for vitamin C, vitamin A and iron. Meanwhile, folic acid and vitamin B_{12} was not recommended for fortification in cookies. The fortified sweet potato cookies could still be accepted by the panelists according to their physical and sensory characteristics.

Acknowledgements

The authors thank to Directorate General of Higher Education, Indonesian Ministry of Education and Culture for financial support.

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