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Vitamin A Activity of Rice-based Weaning Foods Enriched with Germinated Cowpea Flour, Banana, Pumpkin and Milk Powder

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ABSTRACT

The objective of this study was to identify the effect of different drying methods on vitamin A activity of formulated weaning food. Weaned foods on vitamin A activity of formulated using treated cowpea flour, locally available rice flour, banana-pumpkin, skim milk powder and sugar in the ratio 35:35:15:15:5. Treated cowpea flour consisted of original cowpea flour, 24 h germinated cowpea flour. Each treated flour was mixed separately with the other ingredients and cooked into a slurry. Each mixture was either oven-dried or freeze-dried to produce a dry flaky mixture. The carotenoid composition of the product was determined by HPLC. Vitamin A activity of oven-dried weaning food was significantly reduced ($p<0.05$) compared to freeze-dried weaning food. The freeze-dried weaning foods showed a higher retinol equivalent than oven-dried weaning foods for all treatments. The results of the study found that an intake of 100 g of freeze-dried weaning foods enriched with banana-pumpkin and cowpea flour provided an adequate amount of the recommended daily allowance (RDA) of vitamin A for infants.

INTRODUCTION

Preschool children often suffer from vitamin A deficiency and symptoms show up after liver reserves of the vitamin have been depleted. Vitamin A deficiency most commonly and prominently affects the eye in children. Internally, vitamin A deficiency affects the eye by impairing dark adaptation, leading to night blindness and externally by disrupting the epithelia of the cornea leading to a condition known as xeropthalmia (WHO, 1982). Young children with vitamin A deficiency are usually more susceptible to severe infection, particularly dehydrating diarrhea, complications from measles, and respiratory infection. An infection usually precedes xerophthalmia, making the child more susceptible to vitamin A deficiency which eventually leads to blindness (Guthrie & Picciano, 1995).

Vitamin A in the human diet is derived from preformed vitamin A (retinol) and provitamin carotenoids from plants. The carotenoids ranging from red to yellow in color are found in many plants especially dark green leafy vegetables and orange colored fruits and vegetables (Lanchance and Fisher, 1990). Among the carotenoids, β-carotene has the highest vitamin A activity and is also the most widely distributed in nature (Mercandante and Rodriguez, 1990) It

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constitutes more than 90% of the total carotene in fruits and vegetables. Fruits and vegetables are among the cheapest sources of vitamin A for developing countries. A fruits and vegetable found in abundant supply are the banana and pumpkin. Banana plays an important role in the nutrition of people in poor regions of the world. It is a high yielding source of dietary carbohydrate and is also rich in a number of vitamins and important minerals (INIBAP, 1994). Pumpkin is yellow color fruit and is a good source of pro-vitamin carotenoids. It is one of the most reliable and cheapest sources of dietary vitamin A that is affordable by low-income families.

One way to increase vitamin A intake of infants is through the incorporation of high carotenoid food in their diet. Weaning foods are used during the transition period between breastfeeding and total oral intake. Weaning foods should be nutritionally balanced and easily digestible. Rice is often used as the main ingredient in weaning foods because it is highly digestible, low in anti-nutritive factor, and seldom causes allergic reactions. Rice is almost invariably devoid of carotene and adding pumpkin will increase the amount of vitamin A in rice. The level of protein in rice is also below the requirement needed for child growth (Hansen *et al*., 1981) but the nutritional value of rice can be greatly enhanced when mixed with legumes such as cowpea. Cowpea is a readily available grain legume, which is rich in protein and is often incorporated into glutinous rice preparations (ketupat) of traditional Malay food.

In Malaysia, commercial preparation of baby foods by freeze-drying is available. Another technique of drying is oven-drying and this technique is also common for most foods. Freeze-drying which involves high cost equipment can only be done commercially, while the simpler and less expensive technique of oven-drying can be carried out at home. As part of this study, the effects of freeze-drying and oven-drying on vitamin A activity of weaning food enriched with cowpea flour, banana and pumpkin were assessed.

MATERIALS AND METHOD

Rice flour (*Oryza sativa*), cowpea (*Vigna unguiculata*), banana (*Musa sapientum*), pumpkin (*Cucurbita moshata*) and milk powder were purchased in bulk from a local market in Serdang.

Preparation of ingredients

Cowpea seeds were steeped for 12 h and then germinated for 24 and 48 h. The sprouts were washed, derooted and dried at 60ºC for 12 h in an electric oven (Contherm, Quantherm 200L, Contherm Scientific, Lower Hutt, New Zealand). Each treatment was ground separately in a hammer mill (Cullati, Model: JKA Werk, Type: DCFH, Germany) and sieved through a 60-mesh screen (Model BS 410, Endecotts, London, England). Each treatment was stored separately in airtight container and kept at 7ºC till use. The three treated cowpea flour were (i) control-cowpea flour (CCF), (ii) 24 h germinated cowpea flour (48 h GCF). Pumpkin was cleaned with water, the skin removed and steamed for 30 min. The cooked pumpkin was then combined with peeled ripe banana to a 1:1 ratio by weight. The micture was blended in a food processor (Waring: Model 34BL47, Dynamic Corporation, New Hartford, Connecticut.) resulting in a smooth slurry.

Preparation of weaning food

Three formulations were prepared using the different treatments of cowpea flour. Each formulation consisted of rice flour, cowpea flour, banana-pumpkin mixture, skim milk powder and sucrose mixed in a 35:35:15:15:5 ratio to provide not less than 15% protein. Slurry (15% w/v) of each formulation was pre-cooked at 95ºC for 15 min. An amount (100 g) of the precooked slurry was spread onto a plastic tray and pressed with a spatula to produce a 1-2 mm thick layer. Each preparation was then freeze-dried or oven dried. For freeze-drying, the sample was frozen at –40ºC and free-dried at a temperature of –53ºC, under low vacuum at 200 microns Hg for 16 h in a pilot plant-sized equipment (Virtis Co. Inc. New York, USA). For oven-drying, the sample was dried for 2 h at 90ºC. The flakes were then scraped onto a stainless steel tray and cooled to room temperature. The flakes were ground and sieved through a 60 mesh screen. The finished product containing approximately 5% moisture was stored at 5ºC in a polyethylene bag for vitamin A analysis.

Vitamin A analysis

Vitamin A composition was determined using HPLC according to the procedure of Tee, Goh & Khor, (1995). Retinol, cryptoxanthin, lutein, β and α-carotenes and lycopene standards were purchased from Sigma Chemical Company. Peak areas were quantitated with a Gilson 714 System Controller Software (Gilson Manufacturer Electronics, Villiers-le-Bel, France) operating in an IBM-compatible microcomputer, comparing with reference standards similarly chromatographed. Vitamin A activity of the vegetables was calculated as (g Retinol Equivalents (RE) according to FAO/WHO (1998) procedure. The activities in μg RE/100 g sample were calculated from the relationship:

$$\mu g\ RE/100\ g = \frac{\mu g\ \beta\text{ - carotene}/100g}{6} + \frac{\mu g\text{ of active carotenoids}/100g}{12}$$

Statistical analysis

A one-way analysis of variance (ANOVA) of the Statistical Analysis System Institute (1990) was used to evaluate the mean values of vitamin A composition in the treatments. Differences in treatments were compared using Duncan New Multiple Range Test.

RESULTS AND DISCUSSION

Vitamin A composition of raw ingredients of weaning food

Carotenoids composition

The concentration of major carotenoids quantified in control-cowpea flour (CCF), 24 h germinated-cowpea flour (24 h GCF), 48 h germinated-cowpea flour (48 h GCF), and banana-pumpkin mixture are shown in Table 1. For all flour types, the carotenoids detected were β-carotene, lutein and crytoxanthin. For CCF, 24 h GCF and 48 h GCF products studied, lutein made up over 70% of the sum total of carotenoids quantified. The mean values were 48.45

μg/100 g of samples, respectively. β-carotene and cryptoxanthin were detected in treated cowpea flour but in low proportions comprising 10% of the sum of total carotenoids. α-carotene and lycopene were not detected under the same conditions. Other carotenoids in treated cowpea flour were encountered infrequently and at low levels (13% of total carotenoids).

The carotenoid composition of banana-pumpkin flour was different from that obtained for the cowpea flour and germinated cowpea flour. The highest carotenoid detected was lutein which contributed 38% of the total carotenoids at 1,200.30 μg/100g of the sample. The other carotenoids made up over 30% of the total carotenoids at 1,185.61 μg/100g of sample. β-carotene and α-carotene which were detected contributed over 25% of the total carotenoids at 579.56 μg and 347.47 μg/100 g of the sample respectively. Crytoxanthin and lycopene were found in a low proportion of less than 5%. The mean values were 108.19 μg/100 g of the sample, respectively.

A different vitamin A compositionwas found in skim milk powder compared to those obtained for the CCF, 24 h GCF, 48 h GCF and banana-pumpkin. Major vitamin A detected were retinol and β-carotene. The mean values were 145.33 ± 2.96 μg and 21 ± 1.54 μg /100 g of the sample, respectively. The other carotenoids in skim milk powder were 42.41 ± 1.30 μg/100 g of the samples.

Table 1. Composition of vitamin A and carotenoids of ingredients used for weaning food formulas, μg/100 g

Vitamin A	**CCF**	**24 h GCF**	**48 h GCH**	**B-P**	**SMP**
Retinol	0*	0	0	0	145.33±2.96
Lutein	48.54±2.4	51.89±1.9	54.80±0.8	1,200.30±12.1	0
Cryptoxanthin	3.21±1.24	4.12±2.1	5.46±1.3	108.19±14.70	0
Lycopene	0	0	0	6.88±5.60	0.47
α-Carotene	0	0	0	347.47±7.13	0
β-Carotene	5.69±1.30	5.07±2.7	5.85±2.6	579.56±14.10	21.00±1.54
Others**	8.5±1.70	9.37±1.6	7.59±2.1	1,185.61±4.70	42.41±1.30
Sum***	65.85±2.34	70.45±3.4	73.70±3.7	3,311.1±10.15	209.7±2.25

Note : Means of duplicate analysis following GILSON HPLC System, expressed as μg per 100 g of samples
* : Not detected under the present HPLC conditions
** : Unidentified carotenoids
*** : Summation of all carotenoids tabulated
CCF : Control Cowpea Flour
GCF : Germinated Cowpea Flour
B-P : Banana Pumpkin
SMP : Skim Milk Powder

Sum of carotenoids

The sum of all carotenoids for treated cowpea flour, banana pumpkin and skim milk powder is tabulated in the last row of Table 1. Carotenoid concentration for banana-pumpkin powder was several times higher compared to the 48 h GCF, 24 h GCF and CCF. The mean values were

3,311.01 µg, 73.7 µg, 70.45 µg and 65.85 µg/100 g edible portion of the sample respectively. Though the value of vitamin A is not high in cowpea flour, it does provide some vitamin A activity if banana-pumpkin is not added to the weaning food. The high protein content in cowpea flour provides the necessary amount of protein and amino acids for infants 0-12 months as required by Codex Alimentarius Commission (1976).

Vitamin A activity (retinol equivalent)

Vitamin A activity of µ-carotene, expressed as µg retinol equivalent (RE), was calculated as follow: RE = (µg β-carotene) ÷ 6. As α-carotene, ϒ-carotene and cryptoxanthin possess only unsubstituted µ-ionine ring, they may be expected to have about 50% of the biological activity of β-carotene. The formula used for these pro-vitamin used for these pro-vitamin A carotenoids was therefore RE = (µg carotenoids) ÷ 12. The RE values are tabulated in Table 2. Vitamin A of the CCF, 24 h GCF and 48 h GCF studied were classified as "low" vitamin A activity, (<100 µg RE per 100 g samples; Tee & Lim, 1991). The mean values were 1.21 µg, 1.18 µg and 1.43 µg RE per 100 g of the samples respectively. In contrast, banana pumpkin powder was found to have a "medium" RE (100-499 µg RE per 100 g of the samples), with mean values of 134.46 µg RE per 100 g of the samples. Vitamin A activity was found to be highest in skim milk powder; however, it also had a RE classified as medium, with mean values of 148.83±2.25 µg RE per 100 g of the samples.

Table 2. Vitamin A activity (retinol equivalent) of ingredients used for production of weaning food formulas

Treatment	Retinol Equivalent (RE)*			
	Retinol	β-Carotene	Other pro-vitamin A	Total RE
CCF	-	0.94±0.83	0.26±0.75	1.21±0.58
24 h GCF	-	0.84±0.50	0.34±0.33	1.18±0.83
48 h GCF	-	0.97±0.50	0.45±0.33	1.43±0.33
B-P	-	96.50±0.67	37.96±0.50	134.46±1.70
SMP	145.33±2.96	3.5±1.54	-	148.83±2.25

Note : Derived values from the conversion of µg of β-carotene and 12 µg of other active carotenoids equivalent to 1 µg RE
* : Retinol equivalent expressed as µg per 100 g samples
CCF : Control Cowpea Flour
GCF : Germinated Cowpea Flour
B-P : Banana Pumpkin
SMP : Skim Milk Powder

The effects of dehydration on vitamin a content of weaning foods

Vitamin A in weaning foods is provided by two main sources: preformed vitamin A and pro-vitamin A carotenoids. Preformed vitamin A retinol was from skim milk powder, while the sources of pro-vitamin A carotenoids were banana-pumpkin and treated cowpea flour. The results of the effect of dehydration (freeze-dried and oven-dried) on the retention of pro-vitamin A composition are summarized in table 3. the data show the concentrations of retinol, β-carotene, α-carotene, lycopene, cryptoxanthin, lutein and other carotenoids of the different weaning food formulations. The total amount of vitamin A freeze-dried (FD) weaning foods was higher than in the oven-dried (OD) counterpart weaning food. The mean values of freeze-dried weaning foods were 1,773.82 μg, 1,760.04 μg and 1420.41 μg/100 g of the sample for CCF, 24 CCF and 48 h GCF, respectively. The mean values of oven-dried weaning foods were 1707.83 μg, 1651.00 μg and 1594.50 μg/100 g of the sample for CCF, 24 h GCF and 48 h GCF, respectively. The concentrations of β-carotene, α-carotene, lycopene, cryptoxanthin, lutein and other carotenoids were reduced significantly ($p<0.005$) in the oven-dried weaning food. The highest reduction (19.44%) in retinol was recorded for OD-CCF followed by OD-48 h GCF (17.33%) and OD-24 h GCF (15.54%). The decrease in the concentration of β-carotene was higher ($p<0.05$) in OD-224 h GCF weaning food than in other treatments. The retention rates of β-carotene content after drying were 2.88%, 3.48% and 6.45% for OD-CCF, OD-24h GCF and OD-48 h GCF weaning foods, respectively. Oven-dried weaning food showed a significant ($p<0.05$) reduction in α-carotene. The extent of reduction in an α-carotene was 21.55%, 22.25% and 19.84% for OD-CCF, OD-24 h GCF and OD-48 h GCF, respectively.

Table 3. The effects of dehydration on vitamin A composition of weaning foods.

Vitamin A content	Freeze-dried weaning foods			Oven-dried weaning foods			
	CCF	24hGCF	48hGCF	CCF	24hGCF	48hGCF	MSD
Retinol	115.60$^{b1/}$	113.41^{c}	116.70^{a}	93.12^{e} (19.44)$^{2/}$	85.77^{d} (15.54)	96.47^{d} (17.33)	4.1796
Lutein	248.38^{b}	251.48^{a}	252.04^{a}	220.02^{d} (11.33)	231.49^{c} (7.94)	250.79^{b} (0.50)	7.4534
Cryptoxanthin	26.38^{a}	18.08^{c}	18.03^{c}	22.03^{b} (14.46)	17.63^{c} (2.48)	17.32^{c} (3.93)	1.6259
Lycopene	15.45^{a}	15.49^{a}	15.86^{a}	14.19^{b} (8.15)	13.74^{c} (13.80)	15.13 (3.46)	1.9264
α-Carotene	264.67^{b}	285.86^{a}	261.85^{b}	207.63^{d} (21.52)	222.24^{c} (22.25)	209.89^{d} (19.84)	13.6389
β-Carotene	279.67^{c}	288.86^{b}	299.19^{a}	271.61^{cd} (2.88)	278.79^{d} (3.48)	280.59^{ab} (6.45)	9.5138
Others$^{3/}$	983.89^{a}	894.20^{e}	956.95^{c}	970.82^{a} (1.24)	801.75^{f} (10.34)	910.13^{d} (4.89)	7.2273
Sum$^{4/}$	1773.82^{b}	1707.83^{c}	1760.04^{a}	1651.00^{c} (7.33)	1420.41^{e} (11.67)	1594.50^{d} (8.41)	15.5679

Note : Means of duplicate analysis following GILSON. HPLC System, expresses as (g per 100 g edible portion of samples.
1/ : Means in the same row with different superscripts denote a significant difference ($p<0.05$).
2/ : Figures in parentheses indicate percent decrease in vitamin A content.
3/ : Unidentified carotenoids.
4/ : Summation of all vitamins A tabulated.
MSD : Minimum significant difference
CCF : Control Cowpea Flour
GCF : Germinated Cowpea Flour
B-P : Banana Pumpkin
SMP : Skim Milk Powder

The high losses in β- and α-carotene in oven-dried compared to freeze-dried were probably due to the high sensitivity of vitamin A to light and heat during processing. Oven temperature could have also contributed to the reduction of vitamin A (Badifu, Akpapunam & Mgbemere, 1995). Moreover, the oven-drying system which involves heat and light, could damage carotenoids through oxidation, isomerization and free radical formation (Chandler & Schwartz, 1998; Speek *et al*., 1998).

The effects of dehydration on vitamin A activity in weaning foods

Table 4 shows vitamin A activity of freeze-dried and oven-dried weaning foods. Oven-dried weaning food showed a decrease in vitamin A activity for both the CCF and GCF weaning foods. Vitamin A activity (189.88 μg RE) which was highest in freeze-dried 48 h GCF weaning food ($p<0.05$) was reduced in an oven-drying process by as much as 14.59%. As can be seen from the RE values tabulated in Table 4, freeze-dried weaning foods (186.46-189.88 μg RE) met the vitamin A requirement by FAO/WHO (1988) for young infants (180-350 μg RE per day). Oven-drying resulted in decreased vitamin A activity by as much as 13.19% to 15.53% compared to freeze-dried weaning foods.

Table 4. Dehydration effects on vitamin A activity (retinol equivalent) of weaning foods

Vitamin A content	Freeze-dried weaning foods			Oven-dried weaning foods			
	CCF	24hGCF	48hGCF	CCF	24hGCF	48hGCF	MSD
RE of retinol	115.60$^{b1/}$	113.41^{c}	116.70^{a}	93.12^{e} (19.44)$^{2/}$	95.77^{d} (15.54)	96.47^{d} (17.33)	4.1796
RE of β-carotene	46.61^{c}	48.14^{b}	49.86^{a}	45.25^{d} (3.37)	46.46^{c} (5.33)	46.76^{c} (14.41)	2.6063
RE of α-carotene + cryptoxanthin	24.25^{b}	25.32^{a}	23.32^{c}	19.13^{d} (20.88)	19.98^{d} (21.09)	18.93^{e} (18.82)	1.2190
Total RE	186.46^{b}	186.87^{b}	189.88^{a}	157.50^{d} (15.53)	162.21^{c} (13.19)	162.16^{c} (14.59)	8.8110

Note : Retinol Equivqlent (RE) expressed as μg per 100 g sample, RE derived values from the conversion of 6 μg of β-carotene and 12 μg of other active carotenoid equivalent to 1 μg RE.
1/ : Means in the same row with different superscripts denote significant difference ($p<0.05$).
2/ : Figures in parentheses indicate percent decrease in vitamin A activity.
MSD : Minimum significant difference
CCF : Control Cowpea Flour
GCF : Germinated Cowpea Flour
B-P : Banana Pumpkin
SMP : Skim Milk Powder

CONCLUSION

The results of this study indicate that a rice-based mixture enriched with banana-pumpkin blend yields a high concentration of pro-vitamin A carotenoids. The blend when incorporated with cowpea flour, either control or germinated, into a weaning food provides the required daily intake of vitamin A/100 g (FAO, 1988) for children. Skim milk powder was found to provide the major retinol in the weaning foods, while the banana-pumpkin mixture and the cowpea flour provided β-carotene, as well as other phytochemicals such as lutein, cryptoxanthin, lycopene and α-carotene. The sum total ranged from 1420 μg/100 g to 165 μg/100 g for oven dried weaning food and 1707 μg/100 g to 1773 μg/100 g for freeze-dried weaning foods. Freeze-drying had minimum effects on vitamin A activity. However, weaning foods subjected to oven-drying still maintained a significant amount of vitamin A activity/100 g . Further research on the effects of drying at different oven temperatures on vitamin A activity is recommended.

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