

**Kertas Asli/Original Article**

**Development and Validation of Food Frequency Questionnaire (FFQ)  
for Estimation of the Dietary Polyphenol Intake Among  
Elderly Individuals in Klang Valley**

(Pembentukan dan Penentuan Kesahihan Borang Soal Selidik Frekuensi Makanan bagi  
Anggaran Pengambilan Polifenol dalam Kalangan Warga Tua Sekitar Lembah Klang)

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ABSTRACT

*Polyphenol is a non-nutrient phytochemical compound existed abundantly in plant-based diet which has the properties to prevent age related oxidative damage induced diseases. However, there are difficulties in quantifying its intake and local food frequency questionnaire (FFQ) for the assessment is not available. Therefore, this study aimed to develop and validate FFQ for estimation of dietary polyphenol intake among 93 individuals aged 60 years and above recruited from several senior citizen clubs in Klang Valley. Phase I of the study involved the development of FFQ consisted of 117 items under 9 categories and formation of the database extracted from PHENOL-EXPLORER. In Phase II, the intake of polyphenol estimated using FFQ was compared with reference method consisted of 2-day diet records and diet history questionnaire (DHQ). The mean dietary polyphenol intake estimated from FFQ and the reference method was  $2770.7 \pm 1552.4$  mg/d and  $2171.4 \pm 898.8$  mg/d, respectively. Spearman's rho and Kendall's tau-b analysis indicated that there was a significant positive correlation between polyphenol intake estimated from FFQ and reference method ( $r = 0.41, p < 0.001$ ;  $r = 0.28, p < 0.001$ ). For Bland-Altman plot, 95.7% of scattered plot fell within  $\pm 1.96$  SD limits of agreement revealed that there was good agreement between the two methods used. Cross-classification analysis showed that 36.6% was categorized in the same quartile, 78.5% in identical and contiguous quartiles, with only 3.2% in the opposite quartiles. Regression analysis showed that all categories in FFQ significantly account for the inter-variance for dietary polyphenol intake after controlling for the other variables ( $R^2 = 1.000, p < 0.001$ ). In conclusion, the newly developed FFQ is considered valid and has the potential to be used as a tool to estimate polyphenol intake among elderly individuals in Malaysia.*

*Keywords: Polyphenol; FFQ; validation; elderly*

ABSTRAK

*Polifenol merupakan kompaun fitokimia jenis bukan nutrien yang wujud dengan banyak dalam diet yang berasaskan tumbuhan dan berpotensi dalam memerangi penyakit akibat kerosakan oksidatif berkaitan penuaan. Walau bagaimanapun, penentuan tahap pengambilannya adalah agak sukar dan masih tiada lagi borang soal selidik kekerapan (FFQ) tempatan yang sedia ada bagi tujuan penilaiannya. Oleh itu, kajian ini bertujuan untuk membentuk dan menentukan kesahihan FFQ bagi menganggar pengambilan polifenol dari diet di kalangan 93 individu yang berumur 60 tahun dan ke atas yang dicerap dari beberapa buah kelab warga emas sekitar Lembah Klang. Fasa I kajian ini melibatkan reka bentuk FFQ yang terdiri daripada 117 item dibahagi kepada sembilan kategori dan pembinaan pangkalan data yang diekstrak daripada PHENOL-EXPLORER. Dalam Fasa II, pengambilan polifenol yang dianggar oleh FFQ dibandingkan dengan kaedah rujukan yang terdiri dari rekod diet 2 hari dan borang soal selidik sejarah diet (DHQ). Min pengambilan polifenol diet yang dianggar oleh FFQ dan kaedah rujukan adalah  $2770.7 \pm 1552.4$  mg/hari dan  $2171.4 \pm 898.8$  mg/hari, masing-masing. Analisis Spearman's rho dan Kendall's tau-b menunjukkan korelasi positif yang signifikan antara pengambilan polifenol yang dianggar oleh FFQ dan kaedah rujukan ( $r = 0.41, p < 0.001$ ;  $\tau = 0.28, p < 0.001$ ). Bagi plot Bland-Altman, 95.7% plot bertabur dalam lingkungan had persetujuan  $\pm 1.96$  s.p. menunjukkan terdapat persetujuan yang baik antara dua kaedah. Klasifikasi bersilang menunjukkan 36.6% berada dalam kuartil yang sama, 78.5% dalam kuartil sama dan bersebelahan dan hanya 3.2% dalam kuartil yang bertentangan. Analisis regresi menunjukkan bahawa semua kategori dalam FFQ menyumbang secara signifikan kepada intervariasi pengambilan polifenol dari diet selepas pembolehubah lain dikawal ( $R^2 = 1.000, F(9,79) = 91433.67, p < 0.001$ ). Kesimpulannya, FFQ adalah sahih dan berpotensi digunakan sebagai kaedah penentuan pengambilan polifenol di kalangan warga emas di Malaysia.*

*Kata kunci: Polifenol; FFQ; kesahihan; warga tua*

## INTRODUCTION

Aging has been associated with an increased level of free radicals, with the free oxygen radical especially hydroxyl and hydroperoxyl which are the side products formed endogenously during metabolic process that utilize oxygen play vital roles in aging process, as hypothesized by Harman in the year 1955. Free radical and related molecules are classified as reactive oxygen species (ROS) to signify their ability in promoting oxidative changes to cells (Simonian & Cole 1996).

Fortunately, there are numbers of bioactive components in plant-based food and known as phytochemical that consists of redox active molecule that can be defined as antioxidants (Carlsen et al. 2010). Polyphenol is a type of naturally existed non-nutrient compound that can be classified into four classes which are phenolic acid, flavonoid, lignin and stilbene (Spencer et al. 2007).

According to Scalbert et al. (2005), polyphenol is a dietary antioxidant that existed abundantly in large quantity where its intake could reach 1g/day which is higher than the intake of all other classes of phytochemical and known antioxidant. The major sources of polyphenol from food are fruits, vegetables, cereals and nuts, cocoas and spices whereas the source from beverages included fruit juices, caffeinated drinks and soymilk (Chang et al. 2010).

Recognising the health benefits of polyphenol, several studies have been conducted to determine the polyphenol content in particular food and beverages and further enriched the food database for this compound. However, epidemiological studies evaluating dietary polyphenol intake among populations are rather limited of which most of the studies evaluated dietary polyphenol intake for certain classes only such as flavonoid (Li et al. 2013; Wang et al. 2011; Zamora-Ros et al. 2010; Zhang et al. 2010). A few other studies only focus on total polyphenol intake from food and beverages using non-specific spectrophotometric method (Brat et al. 2006; Ovaskainen et al. 2008).

As mentioned earlier although polyphenol has been recognized as powerful antioxidants, its intake among the population including the elderly is not known, probably due to the difficulties in estimating its consumption and the food database on polyphenol is rather limited as compared to other nutrients. A semiquantitative FFQ is able to estimate food consumption qualitative and quantitatively (Flegal and Larkin 1990). Thus, it is the most commonly used method to estimate subjects' habitual nutrient intake as it is relatively less expensive to implement FFQ especially for specific nutrients such as polyphenol and lesser burden to subjects as compared to diet record or dietary recall method (Cade et al. 2004). In Malaysia, few local FFQs have been developed and validated to assess particular nutrients intake among particular populations such as FFQ for Malaysian Chinese adults, to assess dietary patterns during pregnancy, sugar consumption and assessment of energy, total fat, fatty acids, vitamin A, C and E among Malaysian women

(Chong & Norimah 2002; Loy & Jan Mohamed 2013; Nik Shanita et al 2012; Shahril et al. 2008).

Thus, this study aimed to develop and validate a FFQ to estimate polyphenol intake among elderly individuals using a newly built food database extracted from PHENOL-EXPLORER.

## METHODS

This study was part of the Kuala Lumpur Aging Study (KLAS) and Longitudinal Research Grant Scheme TUA (LRGS TUA) that obtained ethics approvals from the Research Ethics Committee of Universiti Kebangsaan Malaysia. These projects are a large scale population based study to prospectively determine the magnitude of cognitive decline and the risk factors through a comprehensive multi-dimensional assessment that include physical health, psychosocial, nutritional and dietary pattern. This cross sectional study was conducted in two phases.

### PHASE I DEVELOPMENT OF THE FFQ AND DATABASE

The FFQ and the database were developed using MICROSOFT OFFICE EXCEL 2007 based on the database obtained from PHENOL-EXPLORER (<http://www.phenol-explorer.eu/>) and also several literatures that analyzed polyphenol contents of specific items of which the information was not available in PHENOL-EXPLORER. The flow of constructing items to be included in the newly developed FFQ was shown in Figure 1. A final version of semi-quantitative FFQ consisted of 117 items that comprises of 9 groups which are fruits, vegetables, cereals and nuts, cocoas, fruit juices, caffeinated beverages, soy milk, alcoholic beverages and spices (Appendix I). The list of items was divided into sections accordingly to facilitate the interview process. The polyphenol value was presented in frequently used unit (mg/day) and the items were marked with four different colors to signify the analytical method used to produce polyphenol value by the particular research article. A total of 47 items were produced by HPLC analysis, 19 items by means of HPLC after hydrolysis, 16 items through Folin Assay, 13 items from the combination of HPLC and HPLC after hydrolysis. The polyphenol values for the rest of the items were obtained from the literatures including meta-analysis. The serving size and weight of items in household measures were referred to the Nutrient Composition of Malaysian Foods (Tee et al. 1997).

### PHASE II VALIDATION OF THE DEVELOPED FFQ

*Sampling* A total of 93 elderly individuals aged 60 years old and above, who were apparently healthy, not terminally ill, consumed oral diet and also a permanent residence in the study areas for at least a year were recruited through flyers distributed to three Senior Citizen Clubs in Klang Valley.

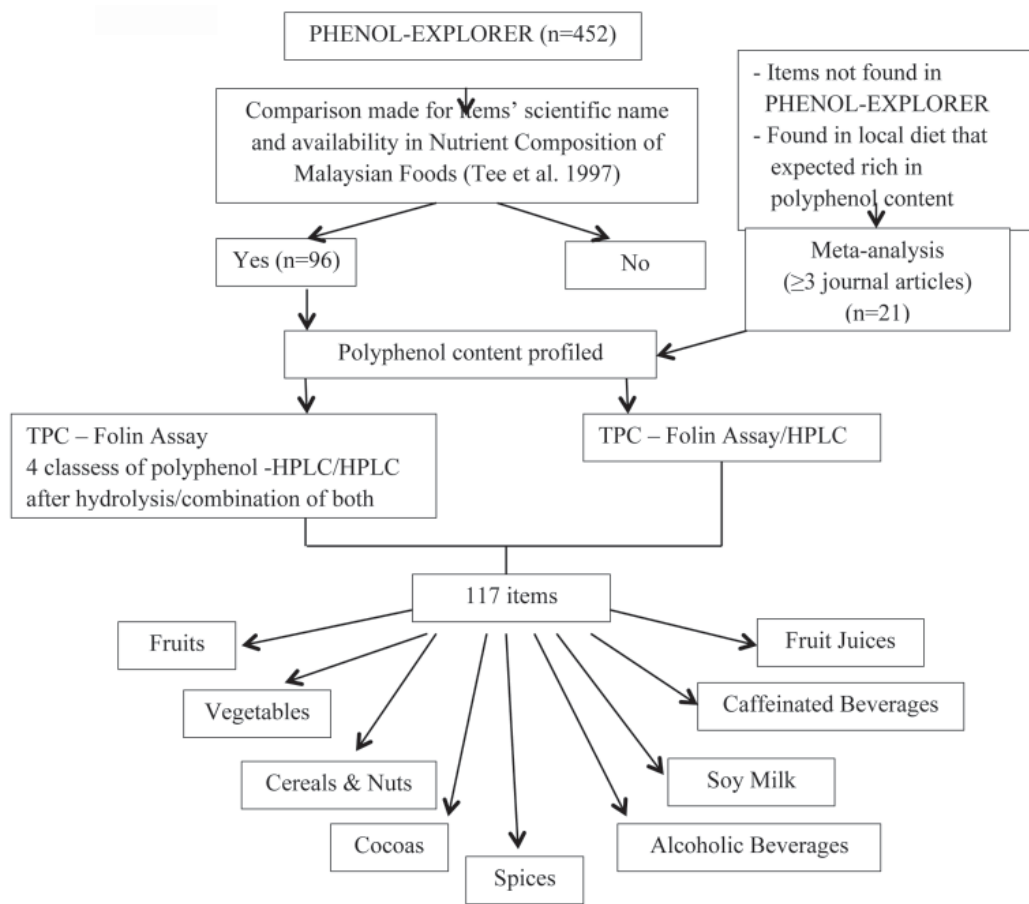


FIGURE 1. Flow chart of items' construction to be included in FFQ

Subjects voluntarily participated in the study at selected community halls and informed consents were obtained.

**Data collection** Subjects were interviewed for socio-demographic data by trained interviewers. Anthropometric measurements including weight and height were taken using TANITA HD-309 and Portable Leicester Stadiometer (CMS Weighing Equipment) according to standard protocol of NHANES (2007). Body mass index (BMI) was computed (WHO 2004).

A combination of two days diet record together with diet history questionnaire (Suzana et al. 2000) has been used as a reference method in this study. According to Streiner and Norman (1989), diet record is one of the most accurate methods in multiple measurements of nutrient intake. However, with the consideration of low literacy and possibility of poor compliance of elderly subjects to keep several days of records, thus DHQ that capable to describe a dietary pattern of subjects in a week was combined with the diet records, of which the mean value was taken.

With respect to the food intake data collection, subjects were randomly divided into two groups to avoid data bias. In the first group, subjects were interviewed with the newly developed FFQ, followed by DHQ and 2 days diet record (1 weekday and 1 weekend) two weeks after that and vice

versa for the second group. Household measures utensils and *Ipad* were used during the process of interviewing. The quantity of the items consumed were converted to wet weight before polyphenol value calculated by using modified formula from Wessex Institute of Public Health (1995) and Norimah et al. (2008) as the followings:

$$\text{Total Polyphenol Intake (mg/day)} = \frac{\text{Serving size (g)} \times \text{Intake frequency (conversion factor)} \times \text{Polyphenol content of the item (mg/100 g)}}{100}$$

The total polyphenol intake (TPC) for reported dishes from diet record and DHQ were calculated based on the ingredients found in standard recipes from 12 websites that offering local recipes that match with availability of items in the built database.

## STATISTICAL ANALYSIS

Statistical package for Sosial Sciences (SPSS) 16.0 and *MedCalc* 12.5.0 were used in data analysis. Spearman correlation and Intra-class correlation applied to test the relationship of TPC estimated from FFQ and reference

method. To determine the validity of the FFQ, Bland-Altman plot, cross-classification and Cohen's Kappa were used. Multiple hierarchical regression test done to determine the groups in FFQ that contribute to the inter-variation of dietary polyphenol intake estimated from the validated FFQ.

## RESULTS

As shown in Table 1, most of the subjects were Chinese (68.8%), aged 60-69 years old (72%), had primary (36.6%) and secondary (43.0%) education and were within the normal (59.1%) and overweight BMI categories (34.4%). The mean intake of dietary polyphenol estimated by FFQ ( $2770.7 \pm 1552.4$  mg/day) and reference method ( $2171.4 \pm 898.8$  mg/day) were not significant and highly correlated as assessed using Spearman correlation ( $r_s = 0.410$ ,  $p < 0.05$ ). The agreement between FFQ and reference method was further confirmed through graphical analysis (Bland

TABLE 1. Socio-demographic and antropometric profiles of subjects [Presented as n(%)]

Characteristics	Gender		Total (n + 93)
	Men (n = 47)	Women (n = 46)	
Age			
60-69 years old	31 (66.0)	36 (78.3)	67 (72.0)
70-79 years old	12 (25.5)	8 (17.4)	20 (21.5)
≥ 80 years old	4 (8.5)	2 (4.3)	6 (6.5)
Ethnics			
Malay	17 (36.2)	4 (8.7)	21 (22.6)
Chinese	24 (51.0)	40 (87.0)	64 (68.8)
India	6 (12.8)	2 (4.3)	8 (8.6)
Education level			
Not Educated	2 (4.3)	8 (17.4)	10 (10.7)
Primary	13 (27.7)	21 (45.7)	34 (36.6)
Secondary	23 (48.9)	17 (36.9)	40 (43.0)
Tertiary	9 (19.1)	0 (0.0)	9 (9.7)
Occupational Status			
Not Working	6 (12.8)	4 (8.7)	10 (10.8)
Housewife	0 (0)	27 (58.7)	27 (29.0)
Retired	27 (57.4)	12 (26.1)	39 (41.9)
Retired but Still Working	7 (14.9)	1 (2.2)	8 (8.6)
Working	7 (14.9)	2 (4.3)	9 (9.7)
Body Mass Index (BMI)			
Underweight (< 18.5 kg/m <sup>2</sup> )	2 (4.3)	1 (2.2)	3 (3.2)
Normal (18.5-24.99 kg/m <sup>2</sup> )	28 (59.6)	27 (58.7)	55 (59.1)
Overweight/Pre-obese (25.0-29.99 kg/m <sup>2</sup> )	15 (31.8)	17 (37.0)	32 (34.4)
Obese (≥ 30.0 kg/m <sup>2</sup> )	2 (4.3)	1 (2.1)	3 (3.2)

and Altman 1986), of which the difference between two methods (FFQ – reference method) was plotted against the mean intake from two methods (FFQ + reference method)/2). Figure 2 showed that the limits of agreement for these two methods fell between -82.7% to 114.7%, with 16% of mean difference.

Cross-classification was done based on the quartile categories of dietary polyphenol intake estimated by FFQ and reference method. A total of 36.6% subjects was correctly classified into same quartile which is not that good (lower than 50%) according to a reference suggested by Masson et al. (2003). However, identical & contiguously classified and also grossly misclassified subjects, the percentage were 78.5% and 3.2% respectively, higher than the percentage recommended by Erkkila et al. (2011) and Shatenstein et al. (2005).

Multiple hierarchical regression analysis showed that all groups in the validated FFQ significantly account for the inter-variance of dietary polyphenol intake after controlling for the variable gender, ethnics, education level and BMI. As seen in Table 2, cereals and nuts (17.6%), followed by caffeinated beverages (8.4%) and fruits (8.2%) were the major contributors of polyphenol intake variation.

## DISCUSSION

The study has successfully developed and validated a 117 items FFQ to estimate polyphenol intake among elderly individuals. Polyphenol intake estimated from FFQ ( $2770.7 \pm 1552.4$  mg/day) seems to be higher but not significant than those retrieved from a combination of 2 days diet record and DHQ, ie. the reference method used in this study. The ability of subjects to recall or memorising the foods they have consumed might have caused over or under-estimation whenever FFQ was used in assessing nutrient intake (Musgrave et al. 1989; Pietinen et al. 1988). FFQ has been reported to provide a higher estimation outcome than the reference method for most of the food categories and nutrients, especially if the FFQ consisted more than 100 items (Klipstein-Grobusch et al. 1998; Subar et al. 2001), as in present study.

The correlation coefficient of 0.410 (Spearman) found in present study was similar with previous research investigating the validity of FFQ consisted more than 100 items against diet record that applied for Korean Genome Epidemiologic Study (KoGES) and Shanghai Women's Health Study (Ahn et al. 2007; Shu et al. 2004). There was 95.7% of plots scattered within the Bland Altman standard deviation  $\pm 1.96$  and only 4 plots scattered out of the limits which represents good agreement. The pattern of scattered plots seen in present study indicated no significant changes could be seen in the differences of measurement for 2 methods and the agreement was consistent if the mean intake was not taking into consideration (Fernandez-Ballart et al. 2010).



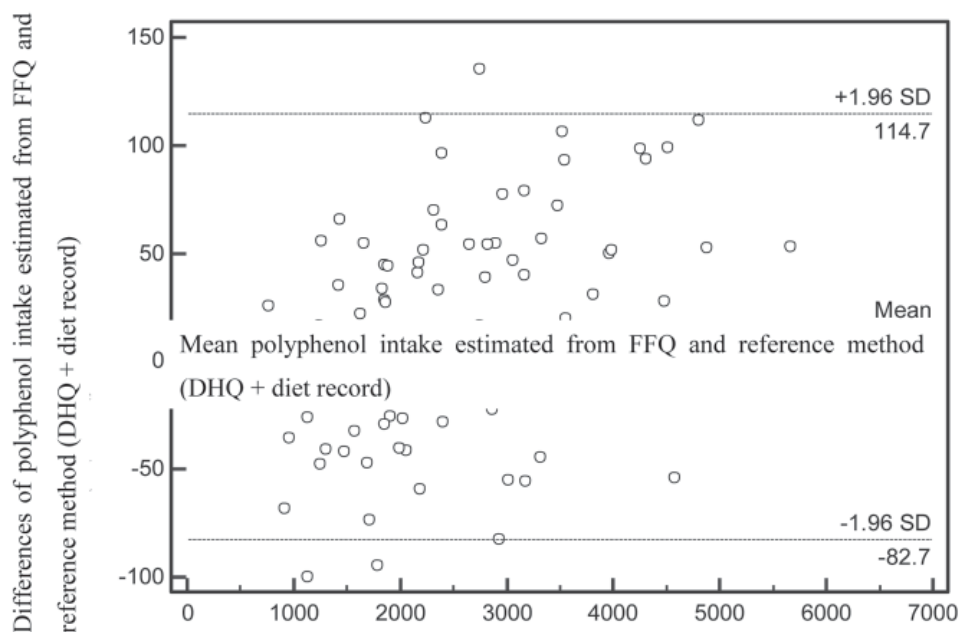


FIGURE 2. Bland Altman scattered plot showed agreement between FFQ and reference method in estimating dietary polyphenol intake

TABLE 2. Groups of food and beverage contributed to inter-variation of polyphenol intake based on FFQ

Groups	sr <sup>2</sup>	R <sup>2</sup>	R <sup>2</sup> changes	F
Fruits	0.082**	1.000	0.979	91433.67
Vegetables	0.029**			
Cereals & Nuts	0.176**			
Cocoas	0.001**			
Fruit Juices	0.005**			
Caffeinated Beverages	0.084**			
Soy Milk	0.001**			
Alcoholic Beverages	0.000**			
Spices	0.018**			

\*\* Partial correlation significant at  $p < 0.001$

Up to date, there are not many studies comprehensively evaluated the dietary polyphenol intake from a wide range and variety of food groups and beverages rich in polyphenol, thus the findings of the present study could only be compared with a few available studies that used different dietary assessment methods. A study conducted by Ovaskainen et al. (2008) considered as the first study with large sample size ( $n = 2007$ ) that estimated a wide range of polyphenol intake among adults population in Finland using 48 hour diet recall and data analysis were based on result of 143 samples sent to analytical lab. The study found that coffee and cereals were vital contributors to the total polyphenol intake, consistence with findings reported by Saura-Calixto et al. (2007) and also the present study.

Other studies with large sample sizes that utilized the same database PHENOL-EXPLORER conducted in recently revealed that caffeinated beverages and fruits were the main sources of polyphenol intake (Perez-Jimenez et al. 2011; Tresserra-Rimbau et al. 2013), as reported in the present study. Vegetables and spices were the least important contributors compared with the other food groups. This might be due to the polyphenol content ratio for a serving of vegetable was the least as compared to other groups. The others food groups such as cocoas, fruit juices, soy milk and alcoholic beverages also contributed significantly to the total dietary intake of polyphenol to a lesser content. The classes and concentration of the dominant polyphenol in each food groups and beverages were different. Furthermore, individual preferences may also cause high variability in diet intake at individual context (Ovaskainen et al. 2008).

It should be noted that although the mean intake of polyphenol from FFQ did not differ significantly from the reference method, only 40% of subjects were correctly classified and approximately 4% were grossly misclassified. These are probably due to the limitations of the Malaysian Food Composition Database and the PHENOL-EXPLORER, which are not comprehensive enough for polyphenol content. In this study, there are certain types of foods such as 'ulam' which is not listed in the database, thus the nutrients have to be estimated from other more or less similar foods within the same food groups. There is a need to include food analysis in future studies. The present study also faced difficulties in estimation of polyphenol intake from spices and the estimated polyphenol intake may not represent the actual value absorbed due to bioavailability variations. Future studies should further assess polyphenol

intake according to the four main classes (phenolic acid, flavonoid, lignin and stilbene) and to integrate biochemical marker along with dietary assessment. It should also be borne in mind that the study reflected the composition of older adults in urban settings, ie. comprised of 70% Chinese elderly individuals, thus there is a need to validate the newly developed FFQ among other populations such as the rural Malaysian elderly individuals.

## CONCLUSION

The developed FFQ is considered reasonably valid and has the potential to be used as a tool for application in future epidemiological studies aiming to determine the relationship between polyphenol intake and the risk of chronic diseases, of which an effective intake doses for exhibition of positive effects can be suggested. However, there is a need to further validate it among other populations such as rural Malaysian elderly individuals and improve the way that the intake of herbs and spices could be accurately estimated and an integration of biomarkers would be necessary.

## ACKNOWLEDGEMENT

The authors wish to thank the participants, field workers and also co-researchers of the project. The project was funded by Universiti Kebangsaan Malaysia Research University Grant (UKM-AP-2011-27) and Ministry of Education Long Term Research Grant (LRGS/BU/2012/UKM-UKM/K01).

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Received: September 2013  
Accepted for publication: May 2014

APPENDIX I

Kategori Buah-buahan	Item	Pengambilan Setiap Kali	Kekerapan Pengambilan		Berat (g)	Berat/hari (g/hari)
			1 Hari	1 Minggu		
	1. Anggur (Hijau)	biji				
	2. Anggur (Ungu)	biji				
	3. Aprikot	biji				
	4. Belimbing	biji				
	5. Betik	potong				
	6. Buah Ara (kering)	botol				
	7. Buah ceri	biji				
	8. Buah delima	biji				
	9. Buah jambu (Guava)	potong				
	10. Buah naga (Putih)	potong				
	11. Buah naga (Merah)	potong				
	12. Buah Pic (Buang kulit)	biji				
	13. Buah Pic (Dengan kulit)	biji				
	14. Buah Plum	biji				
	15. Epal (Buang kulit)	biji				
	16. Epal (Dengan kulit)	biji				
	17. Kesemak (pisang kaki)	biji				
	18. Kurma (kering)	biji				
	19. Kismis	sudu makan				
	20. Kiwi	biji				
	21. Laiici	biji				
	22. Limau Betawi/Bali	potong				
	23. Limau Gedang	biji				
	24. Limau Mandarin	biji				
	25. Longan	biji				
	26. Mangga	biji				
	27. Nenas	potong				