ANTIOXIDANT PROPERTIES AND PHENOLIC CONSTITUENTS OF HERBAL TEAS FROM SABAH

CHIA KAH FEI

THESIS SUBMITTED IN FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE

SCHOOL OF FOOD SCIENCE AND NUTRITION
UNIVERSITI MALAYSIA SABAH
2012

PUMS 99:1

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN TESIS

JUDUL : _____
_____
_____

IJAZAH : _____
_____

SAYA : _____ **SESI PENGAJIAN** : _____
(HURUF BESAR)

Mengaku membenarkan tesis *(LPSM/Sarjana/Doktor Falsafah) ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis adalah hak milik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. Sila tandakan (/)

☐ SULIT (Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di AKTA RAHSIA RASMI 1972)

☐ TERHAD (Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)

☐ TIDAK TERHAD

Disahkan oleh:

_____ _____
(TANDATANGAN PENULIS) (TANDATANGAN PUSTAKAWAN)

Alamat Tetap: _____
_____
_____
_____

_____
(NAMA PENYELIA)

TARIKH: _____ TARIKH: _____

Catatan:
*Potong yang tidak berkenaan.
*Jika tesis ini SULIT dan TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.
*Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana Secara Penyelidikan atau disertai bagi pengajian secara kerja kursus dan Laporan Projek Sarjana Muda (LPSM).

DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

July 2012

_____

Chia Kah Fei
PN 2006 8598

CERTIFICATION

NAME	:	**CHIA KAH FEI**
MATRIC NO	:	**PN20068598**
TITLE	:	**ANTIOXIDANT PROPERTIES AND PHENOLIC CONSTITUENTS OF SELECTED HERBAL TEAS FROM SABAH**
DEGREE	:	**MASTER OF SCIENCE (FOOD SCIENCE)**
VIVA DATE	:	**14 NOVEMBER 2012**

DECLARED BY

1. **SUPERVISOR**
   Dr. Patricia Matanjun Signature
   .....

2. **CO-SUPERVISOR**
   Dr. Yasmin Beng Houi Ooi .....

ACKNOWLEDGEMENTS

I am very grateful to many people that have contributed direct or indirect ways to this thesis. First, I would like to acknowledge the support of the School of Food Science and Nutrition, Universiti Malaysia Sabah, and in particular Dean of SSMP, Associated Professor Dr. Sharifudin Md. Shaarani for giving me the opportunity to use lab facilities and instruments.

My deepest respect and gratitude go to main supervisor, Dr. Patricia Matanjun, for her expert suggestions and guidance, dedication and commitment, through the whole process of this thesis. I would also like to thank my co-supervisor, Dr. Yasmin Ooi B.H. for her remarkable idea and guidance for this thesis.

Many thanks are also owed to Centre of Postgraduate Studies, School of Sustainable Agricultural and Biotechnology Research Institute for their assistance throughout the years.

Finally, my indebtedness goes to those whose influence may not be evident but nevertheless provided the emotional foundation and mentally support without which this thesis would have never been possible: my parent and my future wife, Joyce Wong.

ABSTRACT

ANTIOXIDANT PROPERTIES AND PHENOLIC CONSTITUENTS OF SELECTED HERBAL TEAS FROM SABAH

Herbal teas, which are important source of bioactive compounds, are among the functional beverages that getting attention due to its health promoting properties. While the phenolic composition and their biological properties of the more common western herbal teas (e.g. chamomile) have been extensively investigated, herbal teas from Malaysian tropical herbs are relatively unexplored. The aim of this study was to determine the effects of different method of tea preparation (infusion, decoction, and cold infusion) and extraction condition (temperature and time) on antioxidant capacities and total phenolic content of selected herbs (*Rhodomyrtus tomentosa, Phyla nodiflora, Ficus deltoidea,* and *Limnophila aromatic*) from Sabah. The interaction effects of the herbs in combination with two commonly consume tea (green tea and black tea) in term antioxidant capacities and total phenolic content was investigated by comparing their experimental antioxidant capacity value of sample mixture with theoretical sum of individual sample antioxidant capacity. Four comprehensive in-vitro antioxidant assay (total phenolic content (TPC), ferric reducing antioxidant power (FRAP), ABTS• radical scavenging capacity (ABTS), and oxygen radical absorbance capacity (ORAC)) were used in this study. For phenolic constituents characterization, a reliable high-performance liquid chromatographic (HPLC) method coupled with diode array detector (DAD) was developed for simultaneous determination of 44 phenolic acids and flavonoids in herbal teas. All herbal teas decoction exhibited promising antioxidant activities ranged from 0.12 to 1.24 mg GAE/mL, 1.06 to 15.66 µmol TE/mL, 1.56 to 16.56 µmol TE/mL, 4.81 to 24.15 µmol TE/mL for TPC, FRAP, ABTS, and ORAC, respectively. Decoction of *R. tomentosa* exhibits the highest TPC (1.24 ± 0.08 mg GAE/L), FRAP (15.66±0.34 µmol TE/L), and ABTS (16.55±0.49 µmol TE/L). Total phenolics content and antioxidant activities of herbal teas are significantly ($p<0.05$) higher with the increase of water's temperature in tea preparation. Higher extraction water temperatures (80, 90°C and 100°C) yielded herbal tea rich in total phenolic content and antioxidant activities for all tested sample indicated that the antioxidant components are heat stable. There was no significant effect ($p>0.05$) of the extraction time on total phenolic content and antioxidant capacities when the herbal teas extracted at high temperature (80, 90 °C or 100°C). However, extraction time became a factor to influence the phenolics content and antioxidant activities at lower temperature extraction (60 °C or 70°C). Results for interaction study indicated the mixture of different combination of the herbal teas and teas produced additive, synergistic or antagonistic effects, with the antagonism predominated in all assays. Six compounds, namely catechin, epigallocathechin, epicatechin, orientin, vitexin, and isovitexin were successfully identified and quantified in the decoction of *F. deltoidea*. In conclusion, teas from *R. tomentosa* and *F. deltoidea* can emerge as excellent functional beverages as a source of antioxidant in daily diet.

ABSTRAK

Teh herba, merupakan sumber sebatian bioaktif yang penting, adalah salah satu jenis minuman berfungsi yang semakin menerima tumpuan kerana mempunyai ciri-ciri mengekalkan kesihatan. Komposisi fenolik dan ciri-ciri biologi bagi teh herba barat telah dikaji dengan teliti, teh herba dari herba tropika malaysia masih tidak diketahui. Objektif kajian ini adalah untuk menentukan kesan cara penyediaan teh yang berlainan (infusi panas, rebusan dan infusi sejuk) dan keadaan pemerahan (suhu dan masa) terhadap kapasiti antioxidant dan jumlah fenolik bagi herba terpilih di Sabah. Kesan interaksi campuran herba dengan dua teh yang biasa diminum(teh hijau dan teh hitam) terhadap kapasiti antioxidan dan jumlah fenolik telah dikaji dengan membandingkan nilai kapasiti antioxidan secara eksperimen dalam campuran dengan jumlah kapasiti antioxidan berasingan secara teori. Empat kaedah antioxidan secara in-vitro (jumlah kandungan fenolik (TPC), kuasa antioxidan penurunan ferik (FRAP), pemerangkapan radikal bebas ABTS (garam diamonium asid 2,2-azino-bis-(3-ethy6lbenzothiazolina-6-sulfonik), dan keupayaan penyerapan radikal oksigen (ORAC)) telah digunakan dalam kajian ini. Untuk pencirian komponen fenolik, kaedah Kromatografi Cecair Prestasi Tinggi (HPLC) yang dilengkapkan dengan pengesanan jajaran diod telah dihasilkan untuk penentuan 44 asid fenolik dan flavonoid dalam teh herba secara serentak. Semua teh herba menunjukkan aktiviti antioxidan yang tinggi dalam lingkungan 0.12 hingga 1.24 mg GAE/L, 1.06 hingga 15.66 µmol TE/L, 1.56 hingga 16.56 µmol TE/L, 4.81 hingga 24.15 µmol TE/mL, untuk TPC, FRAP, ABTS dan ORAC masing-masing. Rebusan dari Rhodomyrtus tomentosa menunjukkan TPC (1.24 ± 0.08 mg GAE/L), FRAP (15.66±0.34 µmol TE/L) dan ABTS (16.55±0.49 µmol TE/L) yang paling tinggi. Jumlah fenolik dan aktiviti antioxidan dari teh herba adalah signifikan ($p<0.05$) lebih tinggi dengan peningkatan suhu air semasa penyediaan. Suhu penyediaan tinggi (80, 90 dan 100°C) menghasilkan teh herba yang kaya dengan jumlah fenolik dan kapasiti antioxidan, ini menunjukkan komponen antioxidan adalah stabil kepanasan. Masa penyediaan teh herba tidak menunjukkan kesan yang signifikan ($p>0.05$) terhadap jumlah fenolik dan antioxidan pada suhu penyediaan tinggi (80, 90 dan 100°C), tetapi ia merupakan satu faktor pada suhu penyediaan rendah (60 dan 70°C). Keputusan dari kajian interaksi menunjukan bahawa campuran kombinasi herba dan teh yang berlainan menghasilkan kesan aditif, sinergistik dan antagonistik, dengan kesan antagonistic merupakan kesan utama dalam semua kaedah yang digunakan. Enam sebatian, katecin, epigalokatecin, epikatecin, orientin, vitexin dan isovitexin telah dikenalpasti dan dihitungkan dalam rebusan Ficus deltoidea. Secara kesimpulan, teh yang dihasilkan daripada Rhodomyrtus tomentosa and Ficus deltoidea dapat dijadikan minuman berfungsi yang baik dan sebagai satu sumber antioxidan dalam diet harian.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX

LIST OF ABBREVIATION

°C	Degree Celsius
g	Gram
%	Percent
mL	Milliliter
min	Minute
L	Liter
nm	Nanometer
mg	Milligram
µL	Micro liter
M	Molar
µg	microgram
mmol	Millimolar
µm	micrometer
i.d.	Internal diameter
ABTS	2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)
ORAC	Oxygen radical absorbance capacity
FRAP	Ferric reducing antioxidant power
DPPH	2,2-diphenyl-1-picrylhydrazyl
AAPH	2',2'-Azobis(2-amidinopropane) dihydrochloride
AUC	Area under curve
DHAA	Dehydroascorbic acid
GAE	Gallic acid equivalent
HAT	Hydrogen atom transfer
SET	Single electron transfer
FL	Fluorescein
PCA	Principal component analysis
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
TE	Trolox equivalent
TEAC	Trolox equivalent antioxidant capacity

TPTZ	2,4,6-tripyridyl-*s*-triazine
TRAP	Total radical trapping antioxidant parameter
trolox	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic
SOD	Superoxide dismutase
LDL	Low-density lipoproteins
L-AA	L- ascorbic acid
CAT	Catalase
CBG	Cytosolic b-glucosidase
LPH	Lactase phloridizin hydrolase
GST	Glutathione S-transferase
GPx	Glutathione peroxidase

CHAPTER 1

INTRODUCTION

1.1 Introduction

Growing interest in functional beverages has primarily come from a consumer shift toward healthier eating and lifestyle. These lead to the development of numerous novel functional beverages through incorporating one or more functional ingredient for example, natural antioxidant in beverages formulation by food scientist and manufacturer. Apart from their consumption as thirst quenchers, functional beverages may exert a specific health-beneficial effect beyond their nutritional properties when consumed regularly (Kaur and Das, 2011). Functional beverages which include soy drinks, juices or teas are claiming to possess energy boosting, anti-aging properties, relaxing effects and antioxidant potency (Gruenwald, 2009).

Tea is one of the most popular functional beverages consumed worldwide with the advantage of inherent health promoting attributes (Majchrzak *et al.*, 2004). While the term "tea" is very familiar to all public and professional, there is considerable variability in the meanings attributed to them. Usually, tea is defined as a beverage prepared by adding cured leaves of the *Camellia sinensis* plant to hot water. However, the term also refers to the plant (*Camellia sinensis*) itself (Ferruzzi, 2010). Green tea, oolong tea and black tea are tea that made from the leaves of the *Camellia sinensis* plant through a variety of manufacturing processes (Hakim *et al.*, 2000). A great number of plants and spices have been used to make infusions that are erroneously referred to as "teas" (du Toit *et al.*, 2001, Wanyo *et al.*, 2011). Therefore, in order to specify the use of this term, in this work, we refer tea as beverage made from leaves of plant from *Camellia sinensis*, and herbal tea as beverage prepared from roots, flower, leaves, seeds, or twigs of other plants.

Next to tea, herbal tea is one of the new emerging and fast growing segment on the functional beverage market because of its characteristic flavour, unique aroma, pleasant taste, and also their health benefit or therapeutic effect (Gruenwald, 2009). Traditionally, herbal teas have been used as folk remedies to

prevent or alleviate disorder, and thus reduce the risk of chronic disease (Guimarães *et al*., 2011). However, many of these anecdotal health claims have not been proven through scientific investigation (Kulip, 1996; Kulip, 2003). Although much work has been done to reveal their biological activities and attempt to link to its phytochemicals, more studies need to be conducted to ascertain the functional properties of various different types of herbal teas. A few mechanisms have been proposed for the health beneficial effects of herbal tea in different models of studies. The aspect of free radical scavenging and antioxidant properties of herbal tea are frequently cited as important contributions and has became the interest of many researchers (Wojcikowski *et al*., 2007; Tempest *et al*., 2008).

Antioxidants are of great interest because they may help to protect the body against damage by reactive oxygen species (ROS) (Fernandez-Panchon *et al*., 2008). Active oxygen in the form of superoxide anion ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), peroxyl radical ($ROO^{\cdot}$), and hydroperoxyl radical ($HOO^{\cdot}$) is a by-product of normal metabolism (Matés and Sánchez-Jiménez, 2000). Under oxidative stress condition, the ROS may attacks biological macromolecules consist of proteins, lipids, DNA, and RNA, leading to cell or tissue injury associated with degenerative diseases, such as cancer and cardiovascular diseases (Wang *et al*., 2011a; Wang *et al*., 2011b). Although human body possesses inherent defence mechanisms to neutralize them, continuous exposure to environmental factors including exposure to pollution, chemicals, and pesticides may lead to an increase in the amount of free radicals in the body beyond control, and cause irreversible oxidative damage (Ziech *et al*., 2011). Therefore, antioxidants with free radical scavenging activities could have great relevance as preventive agents in chronic diseases in which oxidants or free radicals are implicated. In fact, there are substantial epidemiological evidences that the consumption of fruits, vegetables, herbs, and tea is beneficial to health due to the protection provided by the antioxidant phytochemical contained in them (Stanner *et al*., 2003).

Antioxidative potentials of various types of teas (*Camellia sinensis*) have been studied extensively by many researchers around the world (Langley-Evans, 2000, Su *et al*., 2006, Anesini *et al*., 2008, Bancirova, 2010, Komes *et al*., 2010, Song *et al*., 2011). Numerous review papers have been published on health benefit

of tea in wide variety of aspect including cardiovascular protection (Stangl *et al.*, 2006), preventive and treatment of various cancer (Cooper *et al.*, 2005, Chacko *et al.*, 2010), improve neurological and psychological function (Weinreb *et al.,* 2004), control obesity via stimulating thermogenesis and fat oxidation (Wolfram *et al.*, 2006), and antioxidant functions (Higdon and Frei, 2003). The major phenolics compounds in green teas are tea catechins, which are flavonols with great antioxidant activity (Dufresne and Farnworth, 2001).

In recent years, the attention of researcher is shifting towards the study of herbs and their herbal tea products as sources of biologically active substances including antioxidants, anticarcinogens, anti-inflammatory, anticholinesterase, antimicrobial and anti-glycation (Atoui *et al.*, 2005, Leonard *et al.*, 2006, Chen *et al.*, 2008, Falé *et al.*, 2009, Rodríguez Vaquero *et al.*, 2010, Ho *et al.*, 2010). Many studies have been carried out on herbal tea made from common herbs such as rosemary (Aoshima *et al.*, 2007), sage (Atoui *et al.*, 2005; Zimmermann *et al.*, 2011), chamomile (Harbourne *et al.*, 2009b), mint (Katalinic *et al.*, 2006), lavender (Tsai *et al.*, 2007), lemon balm (Ferreira *et al.*, 2006), verbena (Ho *et al.*, 2010), and meadowsweet (Harbourne *et al.*, 2009a), which resulted in the development of natural antioxidant substances for food, beverage and other applications. The majority of these studies have focused on temperate or subtropical plants which being widely used in western medicine for millennia, comparatively little research has been done on herbal tea of tropical plants in term of antioxidant properties.

Several studies have been reported on tropical herbal tea including rooibos tea (Gadow *et al.*, 1997), mulberry leaf tea (Arabshahi-Delouee and Urooj 2007; Wanyo *et al.*, 2011), lemon grass tea (Cheel *et al.*, 2005), roselle tea (Aoshima *et al.*, 2007), bitter gourd tea (Chan *et al.*, 2010), fever tea (Shikanga *et al.*, 2010) and java tea (Akowuah *et al.*, 2005). Mate tea, an herbal tea derived from yerba mate plant (native to South America), has been the subject of an intensive study for its nutritional and phenolic antioxidant components in recent years (Bravo *et al.*, 2007). With its high nutritional profile and bioactivities, mate tea has now recognized worldwide as potential functional beverage (Heck and de Mejia, 2007). On the other hand, a few studies on herbal teas which derived from some uncommon herbs, such as *Phlomis lychnitis L.* (López *et al.*, 2010) and

Cyphostemma Digitatum (Al-Duais *et al*., 2009), have revealed new valorization potentialities as a source of natural antioxidant.

Local indigenous people have traditional medical practice as an integral part of their culture. A lot of herbs are available for the treatment of various diseases. A review of the literature indicates that some of the underexploited Malaysian tropical herbs possessed a good antioxidant potential (Abu Bakar *et al*., 2006; Omar *et al*., 2011). However, on the whole, scientific information on antioxidant properties of Malaysian herbal tea, particularly *Rhodomyrtus tomentosa*, *Limnophila aromatica*, *Ficus deltoidea* and *Phyla nodiflora* are relatively scarce.

R. tomentosa, a member of Myrtaceae family, is commonly known as Rose Myrtle (as Dundoruok in Sabah). *L. aromatic*, known as Rajo-rajo in Sabah, is an aquatic perennial herb widely used as spice and in traditional medicine. *F. deltoidea* is also one of the important medicinal plants known as Mas Cotek in Malaysia while *P. nodiflora*, common name Turkey Tangle Fogfruit, belongs to the family Verbenaceae. The herbs are selected based on their daily consumption by indigenous people as herbal tea in several district in Sabah after excluding those herbs that have been studied and therefore available in literatures. Therefore, it is necessary to continually uncover specific antioxidant components in local herbal tea and reveal their antioxidative potency to justify their use by the local community.

In Malaysia, traditionally, spontaneous wild herbs form secondary forest, as well as cultivated species from backyard are usually picked and subjected to air-drying under the sun or shade, then the dried herbs are ready for preparing into herbal tea. The reason of drying the herbs is to preserve the perishable post-harvested fresh material from spoilage and thereby extend their shelf life and increase the storage period (Lim and Murtijaya, 2007). In addition, the drying process also aids to develop and concentrate the flavour and aroma of herbs through the elimination of water (Fisher and Scott, 1997). During drying process, the removal of water from the herbs could retarded the microbial, chemical and enzyme-mediated reactions and thus reduce the metabolite decomposition (Kim and Verpoorte, 2010). Hence, the process of drying not only to preserve herbs for

longer shelf life, but also to inhibits the metabolic processes which can lead to a reduction in the active components (i.e. phenolic compounds) of the plants.

Although many advanced drying methods through new technology approaches have been developed and introduced, conventional hot air drying (oven drying) was reported as the most frequent drying method used in the food industry (Krokida *et al.*, 2003). Unfortunately, under certain circumstances hot air drying with the expose of high temperatures for a period of time may result in the loss of bioactive compounds and negatively affects the quality attributes (Korus, 2011). Freeze drying produces dehydrated products of superior quality but is often prohibitively expensive at the commercial scale (Claussen *et al.*, 2007). Despite of its high operation cost, the demand of superior quality products with natural colour and complement flavours and nutrients by consumer has driven the use of freeze dried foods particularly for high-value foods for example nutraceutical products, baby foods, seasonal foods and herbs (Ratti, 2001). Moreover, drying process with a minimum adverse effect on their quality and maximum retention of health beneficial chemical constituents is always the major concerns of the modern food industry (Claussen *et al.*, 2007). Therefore, selection of a suitable drying methods and conditions are of great important in the production of a good quality herbal tea.

The process of brewing of tea/herbal tea is one form of solid-liquid extraction which involved the extraction of organic compounds from solid plant leaves using hot water as the liquid. Water, ethanol, methanol, acetone are commonly used in extraction of plants (Tura and Robards, 2002). However, using hot water as an extraction medium is more relevant because it represent the actual way of tea/herbal tea was prepared for consumption in human daily diet. Moreover, water is non toxic and safe if compare to organic solvent. The preparation of a tea/herbal tea with water can be either performed by maceration, decoction, or infusion (D'Amelio, 1998) and the obtained tea/herbal tea was usually described as an aqueous extract in many studies (Chandra and de Mejia, 2004; Katalinic *et al.*, 2006; Ordóñez *et al.*, 2010).

Many factors such as water quality, the temperature and pH of the media, the duration of extraction, the form of tea, sample partical size, ratio water to sample, and presence of additive that affect the antioxidative activity and phenolic compounds have been discussed in Danrong *et al*. (2009), Zimmermann *et al*. (2011), Komes *et al*. (2010), Gião *et al*. (2009), and Dubeau *et al*. (2010). Among the various factors contributing to the extraction efficiency of antioxidants from natural materials, extraction time and extraction temperature are often investigated, especially in tea preparation. While the effect of extraction time and temperature on the phenolic content and antioxidant activities of green tea (Labbé *et al*., 2006; Rusak *et al*., 2008), black tea (Langley-Evans, 2000; Turkmen *et al*., 2007; Jung *et al*., 2007), white tea (Rusak *et al*., 2008; Horzic *et al*., 2009) and oolong tea (Su *et al*., 2006; Horzic *et al*., 2009) has widely been studied, only a few research have been done on herbal teas and it should be noted that there is unavailable data on how the extraction parameter mention above influence the antioxidant and content of phenolic in herbs species selected in present study. Therefore, taking into consideration of certain factors when conducting antioxidant study on tea/herbal tea, is clearly needed.

Lila and Raskin (2005) reviewed the interaction of phytochemical in detail and divided it into two types, endointeractions that occur between components within a plant species and exointeractions that occur between components from different plants or between plants and synthetic drugs, which may be ingested together. According to Wang *et al*. (2011c), there are three type of interaction effect, an additive effect refers to a mixture of food that provides a combined effect that is equal to the sum of the effects of the individual components; a synergistic effect occurs when the effect is greater than the sum of individual components, and antagonism occurs when the sum of the effects is less than the mathematical sum that would be predicted from individual components.

In the preparation of herbal tea, occasionally, herbs and tea leaves are combined in mixture in order to enhance the flavour and taste in the final herbal tea product (Samaniego-Sanchez *et al*., 2011) and it is believe to improve their health beneficial and therapeutic effect based on herbalist empirical observation (Guimarães *et al*., 2011). Owing to the great number of health claims are being