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Roselle (*Hibiscus sabdariffa* L.) Seeds – Nutritional Composition, Protein Quality and Health Benefits

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ABSTRACT

Roselle (*Hibiscus sabdariffa* Linn) is a herbal shrub plant reported native to tropical Africa and grown in warm countries including Malaysia. This plant is grown for the calyces or petals of the flower which are mainly used to prepare herbal drink, beverages, jam and jellies. After removing the calyces, the velvety capsules containing the seeds are disposed as a by-product. In addition, it is a waste if the seeds are left untouched, without effort to exploit its usefulness and benefits. Moreover, with limited research on the usefulness of roselle seeds, no review paper on this topic has ever published. Thus, we took the initiative to prepare a review on this subject based on findings from most of our work from 2003-2007. In this paper, the most important aspects namely, nutritional composition, including antioxidant properties, protein quality and the effect of seeds on lipid profiles of hypercholesterolemic rats have been reviewed. Based on the compositional analysis, roselle seeds are rich in nutritional components especially proteins, oil and dietary fiber. The seeds are rich in lysine, arginine, leucine, phenylalanine and glutamic acid. The antioxidative effect of roselle seed extract increases when combined with other antioxidant compounds compared to when used alone. The protein quality of roselle seed powder prepared from dried and boiled seeds is similar. Roselle seeds were also reported to possess anti-hypercholesterolemia effects tested using an animal model. To exploit the seeds which contain high value health-promoting components as functional ingredients, their possible application in food products should be studied.

Keywords: anti-hypercholesterolemia effect, nutrient components, protein quality, toxicity

CONTENTS

INTRODUCTION

The importance and health benefits of grain and bean consumption in the prevention of chronic diseases have been documented. Even though nutritional guidelines put grains and grain products at the base of the food guide pyramid to emphasize their importance for optimal health, little attention has been paid to grains, beans and also to seed consumption compared to fruits and vegetables. Seeds are one of the cheap sources of nutritious food. Researchers have confirmed their nutritional usefulness, and many different interpretations and view have been discussed about its nutritional benefits (Salleh 1992; Barampama and Simard 1993). Chau *et al.* (1998) reported that protein concentrates from legume seeds (*Phaseolus angularis*, *P. calcaratus* and *Dolichos lablab*) had a very pronounced hypocholesterolemic effect compared to casein. Furthermore, Dabai *et al.* (1996) showed that seeds of different legume species contribute different hypocholesterolemic effects. However, limited research exists on the exploitation and utilization of seeds as a potential candidate for human alternative food source.

Hibiscus is one of the most common flower plants grown worldwide. There are more than 300 species of hibiscus around the world. One of them is roselle (*Hibiscus sabdariffa* Linn.), which is a member of the plant family, Mal-

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Invited Review

Table 1 Different names of *Hibiscus sabdariffa* L.

Regions	Vernacular names	Source
English-speaking	rozelle, sorrel, sour-sour, Queensland jelly plant, jelly okra, lemon bush, Florida cranberry	Morton 1987
French-speaking	*oseille rouge*, *oseille de Guinée*	Morton 1987
Spanish-speaking	*quimbombó chino*, *sereni*, *rosa de Jamaica*, *flor de Jamaica*, *Jamaica*, *agria*, *agrio de Guinea*, *quetmia àcida*, *viña* and *vinuela*	Morton 1987
Portuguese-speaking	*vinagreira azeda de Guiné*, *cururú azédo*, and *quiabeiro azéd*	Morton 1987
Dutch-speaking	*zuring*	Morton 1987
Africa	sorrel	Omobuwajo *et al.* 2000
North Africa, Near East	*karkadé* or *carcadé*	Morton 1987; Abu-Tarboush 1997
Senegal	*bissap*	Morton 1987
Indian subcontinent	mesta or *meśta*/*meshta*	Rao 1996; Wikipedia® 2008
Malaysia	asam paya, asam susur, asam kumbang	Mat Isa *et al.* 1985
Australia	rosella	Wikipedia® 2008
Myanmar	*chin baung*	Wikipedia® 2008
Thailand	*krajeab*	Wikipedia® 2008
Mali	*dah* or *dah bleni*	Wikipedia® 2008
Gambia	*wonjo*	Wikipedia® 2008
Nigeria	*zobo*	Wikipedia® 2008
Namibia	*omutete*	Wikipedia® 2008
Panama	*saril*	Wikipedia® 2008
Indonesia	*rosela*	Wikipedia® 2008

Wikipedia® (2008) Roselle (plant). Available online: http://en.wikipedia.org/wiki/Hibiscus_sabdariffa

vaceae. There are two main types of *H. sabdariffa* var. *altissima* and var. *sabdariffa*. *Altissima* is nearly branchless and can grow up to 3-5 m in height. Its flowers are yellow, and calyces are red or green with high fiber but not used for food. The other distinct type of hibiscus, *sabdariffa* grows in a bush with many branches. The flowers are axillaries or in terminal racemes, the petals are white with reddish center at the base of the staminal column. The calyx enlarges at maturity. The more economically important is var. *altissima*, which is cultivated for its jute-like fiber in India, the East Indies, Nigeria and South America, whereas var. *sabdariffa* is another distinct type of roselle and is also widely exploited for its calyces (Abu-Tarboush *et al.* 1997) and fiber (Morton 1987; Abu-Tarboush *et al.* 1997).

ROSELLE (*HISBISCUS SABDARIFFA* L.)

Description and ecology

Roselle is an annual erect, bushy, herbaceous sub shrub, with smooth or nearly smooth, cylindrical, typically red stems (**Fig. 1**). It can grow to 0.5-3 m in height with a green or red coloured stalk, and a red or pale yellow calyx that is edible (Brouk 1975; Purseglove 1986; Morton 1987). The plant takes about 3-4 months to reach the commercial stage of maturity before the flowers are harvested. Roselle plants are suitable for tropical climates with well-distributed rainfall of 1500-2000 mm/year, from sea-level to about 600 m in altitude. The plant tolerates a warmer and more humid climate with night time temperature not below 21C, and is most susceptible to damage from frost and fog. In addition, it requires 13 hours of sunlight during the first months of growth to prevent premature flowering (Robert 2005). Roselle plants also require a permeable soil, preferably a friable sandy loam with humus; however, it will adapt to a variety of soils. It can tolerate floods and heavy winds and is not shade tolerant, and must be kept weed-free (Duke 1983).

Roselle is a very versatile plant similar to the coconut tree (Quezon 2005). Roselle can be found in almost all warm countries such as India, Saudi Arabia, Malaysia, Indonesia, Thailand, Philippines, Vietnam, Sudan, Egypt and Mexico (Mat Isa *et al.* 1985; Rao 1996; Abu-Tarboush *et al.* 1997; Chewonarin *et al.* 1999; Quezon 2005). The origin of roselle is uncertain, while others believe that its home country is India (Mat Isa *et al.* 1985) and Saudi Arabia (Abu-Tarboush *et al.* 1997). Roselle is also known by different synonyms and vernacular names (**Table 1**). It is a new commercial crop of Malaysia, where it was natively brought from India to Malaysia.

Calyces

The most exploited part of a roselle plant is its calyces (**Fig. 2**). Calyces are obtained by removing the calyces or petals of the flower (**Fig. 3**) from its capsules containing the seeds (**Fig. 4**). Calyces are used for the preparation of a herbal drink, cold and warm beverages, jams and jellies (Rao 1996; Abu-Tarboush *et al.* 1997; Tsai *et al.* 2002). The brilliant red colour and unique flavour make these valuable food products (Tsai *et al.* 2002). In Mexico, a red beverage known as Jamaica is a popular product made from roselle. In the West Indies, the calyces are freshly used for making wine, jelly, syrup, gelatin, beverages, pudding, and cakes, and dried roselle calyces are used for tea, jelly, marmalade, ices, ice cream, sherbets, butter, pies, sauces, tarts, and other desserts. Calyces are also used in the West Indies as colour and flavour ingredients in rum. For non-food applications, the flower and fleshy fruit are used in pharmaceutical industry to relieve symptoms of bronchitis and coughs. In addition, calyces are used for the treatment of hypertension, diarhea, Ceylon mouth and many other diseases (Chewonarin *et al.* 1999; Faraji and Tarkhani 1999).

Calyces from the roselle flower, which are very high in vitamin C, are processed to produce juices. Duke and Atchley (1984) reported that every 100 g of fresh calyx contains 2.85 μg vitamin D, 0.04 mg vitamin B1, 0.6 mg vitamin B2, and 0.5 mg vitamin B complex. Furthermore, other studies have reported that it is good in reducing hypertension (Adegunloye *et al.* 1996; Onyenekwe *et al.* 1999).

Roselle contains three times more vitamin C than blackcurrant (*Ribes nigrum* L.) and nine times more than citrus (*Citrus sinensis* L.) fruit. In Sudan, the leaves are eaten green or dried, cooked with onions and ground nuts. In Malaysia, the leaves are also eaten as vegetables after cooking. The calyces are used for tea and as a base for jam (Mat Isa *et al.* 1985; Emmy 2006).

Nutritional properties of roselle calyces were previously reported. Duke (1983) and Mat Isa *et al.* (1985) found that 100 g of fresh roselle calyces contain 84.5% of moisture content. The calyces contain 49 calories, 1.9 g protein, 0.1 g fat, 12.3 g total carbohydrate, 2.3 g fiber and 1.2 g ash (Duke and Atchley 1984). Different values were observed by another study conducted in Sudan, it which calyces contained 15% moisture content, 5% protein, 15% total carbohydrate, 12% fiber and 7% ash (Gabb 1997). Nutritional data from U.S. Department of Agriculture also reported different value than previous findings, namely 1% protein, 11.2% carbohydrate and 0.6% fat. The results differ probably because of the different varieties, genetic, environment,

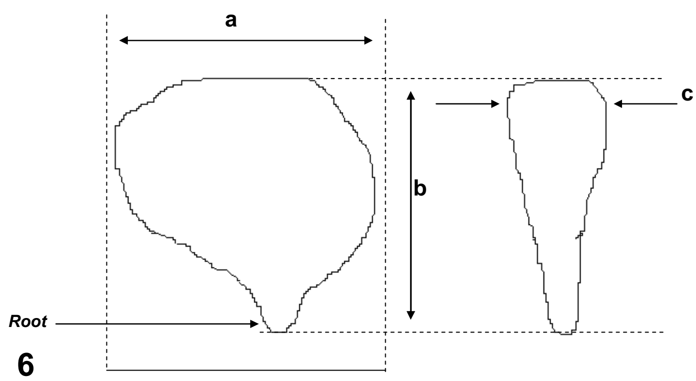

Fig. 1 Roselle (*Hibiscus sabdariffa* L.) farm at Sungkai Perak, Malaysia. The roselle plantation was held on dry season from May to September. There are two main varieties planted in Malaysia. Growers usually call these varieties as 'Terengganu' and 'Arab'. Shown in this picture is roselle var. Terengganu. The plant takes about 3-4 months to the reach commercial stage of maturity before the flowers are harvested. **Fig. 2** Four month-old Roselle (*Hibiscus sabdariffa* L) var. 'Terengganu' calyces. The calyces were collected or plucked manually from its plant by the farmers. The size of the calyx is varied, but ranges from 25.4 to 38.2 mm in diameter. **Fig. 3** Separating calyces from capsules by a decoder, a simple hand-held gadget to obtain its calyx. The capsule was separated from the flower by placing the decoder at the receptacle part of the flower and push upward. **Fig. 4** Four month-old Roselle var. 'Terengganu' capsules (containing the seeds). **Fig. 5** Roselle var. 'Terengganu' seeds. Shown in the picture are dried roselle seeds. The seeds were obtained by removing them manually from their dried capsule. **Fig. 6** Characteristic shape of roselle seeds and designation of the characteristic dimensions: (a) 5.58 mm (major diameter); (b) 5.21 mm (intermediate diameter); (c) 2.81 mm (minor diameter). Source of **Fig. 6**: Based on Omobuwajo *et al.* (2000).

ecology and harvesting conditions of the plant (Atta 2003).

Fresh roselle calyces contain 1.72 mg Ca, 57 mg Fe, 300 μg β-carotene equivalent, and 14 mg ascorbic acid/100 g (Duke 1983). In contrast, dried roselle calyces contain a high amount of ascorbic acid between 360-280 mg/100 g. Mat Isa (1985) indicated that roselle calyces contain a high amount of ascorbic acid, 260-280 mg/100 g. This indicated that roselle has a higher content of ascorbic acid than guava and orange (Tee *et al.* 1997). According to Morton (1987), the dried calyces contain the flavonoids gosypetine and sabdaretine. The major pigment, formerly reported as hibiscin, had been re-defined as daphniphylline. Despite that, delphinidin 3-monoglucoside, cyanidin 3-monoglucoside (chrysanthenin) and delphinidin are also types of anthocyanins present in the calyces. Anthocyanin is a water-soluble pigment responsible for the orangey-red colour of the calyces and their juice (Tsai and Ou 1996). High in anthocyanin, roselle calyces are a good colorant and also a potentially good source of antioxidant components.

Seeds

Roselle seeds (**Fig. 5**) are bigger than the pearl millet varieties having average principal dimensions of 2.98-3.36, 1.86-2.24, and 1.70-2.01 mm (Jain and Bal 1997). Omobuwajo *et al.* (2000) found that the average of roselle seeds' 3 principal diameters were 5.58, 5.21, and 2.81 mm, respectively (**Fig. 6**). The seeds were reported to be smaller than

Table 2 Comparison of chemical composition of different varieties of seeds.

Varieties of seeds	Chemical composition					References
	Moisture (%)	Protein (g)	Total Dietary Fiber (g)	Carbohydrate (g)	Fat (g)	
Hibiscus sabdariffa	9.9	33.5	18.3	13.0	22.1	Hainida *et al.* 2008
Amaranthus spp.	11.3	14.5	ND	63.8	7.5	Elias 1977
Nigella sativa L.	4.6	20.9	7.9	31.9	38.2	Salleh 1992
Pisum sativum	0.3	20.8	25.1	4.7	0.4	Black *et al.* 1998
Achyrantes bidentata	9.0	22.0	ND	53.0	11.5	Marcon *et al.* 2003
Acacia colei	8.0	23.4	39.9	12.4	ND	Adewusi *et al.* 2003
Simmondsia chinensis	4.3	14.9	ND	ND	53.2	Cappillino *et al.* 2003
Passiflora edulis	6.6	8.3	64.8	1.1	24.5	Chau and Huang 2004

ND: not determined

the African breadfruit (*Treculia Africana*) seed with an average principal diameter of 11.91, 5.69 and 4.64 mm (Dutta *et al.* 1988). They are much smaller than the oil bean (*Pentaclethra macrophylla* Benth) seeds with corresponding dimensions of 65.4, 41.3, and 13.7 mm (Oje and Ugbor 1991).

The nutritional compositions of roselle seeds as well as their functional properties are rarely studied compared to the calyces. Studies on nutritional composition of roselle seeds are scarce compared to studies on other seeds such as black cumin seed (*Nigella sativa* L.) and jojoba seed (*Simmondsia chinensis*). As shown in **Table 2**, roselle seed is among the highest protein-containing seeds when compared with other seeds like passion fruit (*Passiflora edulis*), *Amaranthus* seeds, black seeds (*Nigella sativa* L.) and *Pisum sativum* seeds.

Previous studies had significantly shown that roselle seeds contained high amounts of protein, dietary fiber, and minerals such as phosphorus, magnesium and calcium. The seeds from Egypt contain 7.6% moisture, 34.0% protein, 22.3% fat, 15.3% fiber, 23.8% nitrogen-free extract, 7.0% ash and 0.3% Ca (Samy 1980). Another study from India found that the seeds contain 6-8% moisture, 18-22% crude protein, 19-22% fat, 5.4% ash, 39-42% total dietary fiber, 119-128 mg Ca, 596-672 mg P, 4.0 mg Zn, 3.1 mg Cu, 393-396 mg Mg, 0.08-0.18 Cr, 0.36-0.51 mg riboflavin and 0.9 mg nicotinic acid (Rao 1996). Latest findings by Hainida *et al.* (2008) found that the seeds from Malaysia are composed of 9.9% moisture, 33.5% protein, 22.1% lipids, 13.0% available carbohydrate, 18.3% total dietary fibers and 7.5% ash. Samy (1980), El-Adawy and Khalil (1994), Rao (1996) and Hainida *et al.* (2008) reported quite similar results for moisture, lipid and ash contents.

In Africa, dried roselle calyces are pressed into solid cakes or balls for food preparation. The seeds are pressed for oil and the remaining cake is cooked seasoned with *kambo*, a local condiment (Gabb 1997). The kernels and seeds are roasted and consumed, though slightly bitter (Morton 1987). In some countries, the seeds have been exploited as a substitute for coffee for human consumption and are considered an excellent feed for chicken (Morton 1987). The seeds are also used for their oil in China and eaten in West Africa (Robert 1996). In Malaysia, the seeds of roselle plant are used to produce scrubs and soaps. However, most of the seeds are merely discarded as by-products by the manufacturers.

However, in Africa, roselle seeds which are somewhat bitter, are roasted and ground into a powder then used in oily soup and sauces as a meal for human consumption. Roasted seeds have been used as a coffee substitute that is said to have aphrodisiac properties (Duke 1983). According to Omobuwajo *et al.* (2000), in northern Nigeria, the seeds are fermented into a condiment known as *mungza ntusa*. Meanwhile, Al-Wandawi *et al.* (1984) reported that in Sudan, the seeds are used for edible oil production and the by-products of this process were used for poultry feeding. Besides that, roselle seeds are used for medical purpose in some communities. For instance, in Myanmar, the seeds are used to treat debility (Perry 1980). Moreover, Taiwanese regard the seeds as diuretic, laxative and tonic (Duke 1983).

Heat treatment through processing methods like cooking and boiling is known to improve the availability of some nutrients, inactivate enzymes that speed up nutrient damage, and destroy undesirable microorganisms and food contaminants (Lawal 1986). Cooking generally inactivates heat-sensitive antinutrients such as protein inhibitors (e.g. L-Dopa (L-3,4-dihydroxyphenylalanine) from *Mucuma pruriens* seeds (Pugalenthi and Vadivel 2007). On the other hand, the content of tannins and catechins in lentils (*Lens culinaris*) and broad beans (*Vicia faba* L.) seems to increase after these treatments. Heat-treatment of seeds not only causes a number of desirable modifications to their chemical composition, nutritional and organoleptic properties, but can also induce losses of essential amino acids and vitamins (Emmy 2006). Heat denaturation also has a marked influence on the functional properties of proteins, which are strictly related to their modified physicochemical characteristics, namely dissociation into constituent subunits, unfolding and surface exposure of the hydrophobic side groups. Heat denaturation is usually accompanied by a decrease of solubility, which results from the aggregation of unfolded molecules. Lawal (1986) found that dry heat treatment (roasting) decreased the mineral content and amino acid contents of *Treculia africana* seeds. In addition, a study conducted by Lawal and Bassir (1986) showed that parboiled and cooked seeds also decreased the dietary constituents such as Fe, P and Ca.

Other parts

Leaves of the roselle plant are reported to contain 43 calories, 85.6% moisture, 3.3 g protein, 0.3 g fat, 9.2 g total carbohydrate, 1.6 g fiber, 1.6 g ash, 214 mg P, 4.8 mg Fe, 4135 μg β-carotene equivalent, 0.17 mg thiamine, 0.45 mg riboflavin, 0.5 mg niacin and 54 mg ascorbic acid (Duke and Atchley 1984).

NUTRITIONAL COMPOSITION OF ROSELLE SEEDS

Proteins and amino acids

Proteins are formed by amino acids linked by peptide bonds. All amino acids have the same basic structure containing a central carbon atom surrounded by four groups: a hydrogen atom, an amine group (NH_2), an acid group (COOH), and a distinctive side group. The side groups, which vary from a single hydrogen atom to a complex ringed structure, distinguish the 21 different amino acids. Generally, dietary protein sources are divided into two categories: (i) animal protein, such as meat, poultry, fish, eggs, milk and milk product; (ii) plants protein, such as legumes (peanuts, peas, beans and lentils), cereal, vegetables and fruits. Animal-product food are generally higher in protein than plant products and the proportion of amino acid in the protein of animal origin comes closer to meet the human needs than in most plant sources. Plant protein is potentially important in undeveloped and developing countries. Several countries in South Asia such as Bangladesh, Bhutan, India, Maldives, Nepal, Pakistan and Sri Lanka, the most regular foods come from plant sources where rice and wheat are the main staples; wheat, legumes and edible oil are main import foods. Although a variety of animal foods are used by economically privileged people, for a large section of the population animal foods do not constitute part of their diet due to economic reasons (Khalil 2000). Similarly, human populations of the western Sahel region of Africa normally depend upon a number of edible wild plants to satisfy a substantial part of their nutritional requirements. The dependency on wild plant foods is heightened during times of drought that lead to poor cereal harvests. Thus, the wild plant foods of the western Sahel play an essential role in the survival and health of human populations who utilize them (Salih *et al.* 1991). Bean proteins are found to be relatively high in essential amino acids, in particular lysine, threonine, isoleucine, leucine, phenylalanine and valine. But, they are deficient in sulphur-containing amino acids, in particular methionine and cystine (Uebersax and Occena 2003). Methionine is the first limiting essential amino acid in legumes because the major storage proteins, globulin, is low in this amino acid (Jeong *et al*. 2003).

Amino acids can be divided into essential and nonessentials. The nonessentials are those that can be synthesized by the body. In adults, 12 of the amino acids can be synthesized, and nine are essential. Early in infancy, several amino acids that are non-essential later in life are conditionally essential, namely cystein and arginine (Kretchmer and Zimmermann 1997). Protein is an essential component of the diet needed for the survival of living beings. The basic function of protein in nutrition is to supply adequate amounts of needed amino acids.

In developing countries, legumes are a cheap and valuable potential source of protein and they are consumed in large quantities. Additionally, legumes are consumed as eco-nomical supplementary protein with cereals (Philips and Baker 1987; Lam and Lumen 2003). For instance, in India, legumes are the only high-protein sources of the average diet and are eaten mainly with rice, wheat and other minor cereals (Jeong *et al*. 2003). According to Guillon and Champ (1996), legumes are currently of interest to the consuming public because they provide high nutrient levels without supplying excessive calories, and the perception of their food values has dramatically shifted from the 'poor man's steak' label. In developing countries, legume proteins can now be regarded as versatile ingredients or as biologically active components more than as essential nutrients. The partial replacement of animal foods with legumes is claimed to improve overall nutritional status.

Today, despite many advances in agriculture, food and nutrition, there are still large segments of the population in developing and underdeveloped countries that suffer from protein malnutrition. Protein energy malnutrition (PEM) remains the common nutritional disorder facing by the majority of the world's poor population. In Malaysia, PEM still affects children in rural and native communities (Ismail 1992; Tee 1999). Major treatment and prevention of PEM is through sufficient consumption of dietary protein. As population growth continues to increase, and as the main sources of food may be approaching maximum per capita output, demand seems likely to outpace food production. Furthermore, current trends have indicated a widening gap between human population and protein supply. Therefore, the most logical way to increase the world protein supply would be to make more plant proteins available for human consumption. Hence, research efforts have been directed toward identification and evaluation of under exploited sources, such as alternative protein crops (Siddhuraju *et al.* 1996).

Among plant-based protein sources, cereals are the most important sources of dietary protein as well as energy for people in most of the developing countries of the world (Massey 2003). However, cereal protein is found to be low in concentration and poor in quality. In this regard, the potential of legumes that are still not widely used as dietary sources of protein are increasingly being assessed for the improvement of traditional legume crops (Siddhuraju *et al*. 1996). Although several researchers showed that soya beans (*Glycine max* L.) can supply more potential protein than any other crop (Messina 1999) and be excellent sources of low-cost protein, the use of soya beans is currently limited to undeveloped countries especially in African countries. Therefore, there is a need to explore other sources of concentrated plant proteins (Vose 1980) which ideally should be crops that are widely grown in tropical countries.

Many studies have been carried out to assess the potential of roselle seeds as a new source of protein and oil (El-Adawy and Khalil 1994; Rao 1996; Abu-Tarboush *et al.* 1997). Cook *et al.* (2000) analyzed the amino acid and total protein compositions of the 13 Nigerian plants in order to contribute to the growing body of knowledge on the wild plants' chemical and nutrient composition. They reported that the seeds of *Parkia biglobosa* contained the highest amount of protein (294 mg/g dry wt). In addition to *P. biglobosa* seeds, three other plants contained at least 20% protein: *Eptadenia hastata* (22.8%), *Bombax costatum* (22.5%), and ataruhu (scientific name unknown) (21.2%). Although no single plant would provide humans with adequate levels of all nutrients, since plant foods are commonly prepared with other foods such as oils, peanut residue, sesame or millet (Humphry *et al.* 1993), they could contribute useful amounts of a number of essential nutrients to the diet.

El-Adawy and Khalil (1994), Abu-Tarboush *et al.* (1997) and Hainida *et al*. (2008) found high protein content in roselle seeds while Samy (1980) and Rao (1996) noted a lower protein content. These differences may be related to the variations of cultivated regions. The protein content of the seeds was higher than that of other common seeds and grains legumes such as black seeds (Salleh 1992), sunflower (*Helianthus annuus* L.) seeds, melon (*Cucumis melo* L.) seeds, chickpeas (*Cicer arietinum* L.), cowpeas (*Vigna sinensis*), pigeon peas (*Cajanus cajan* L.), soybeans, and groundnuts (*Arachis hypogaea* L.) (FAO 2001). Flour made from whole roselle seeds flour contained a high amount of protein (27%). Roselle protein isolates showed higher protein content (88%) than seeds. El-Adawy and Khalil (1994) published the amino acid profiles of Egyptian roselle seeds from three different cultivars (**Table 3**). They found that roselle seed protein content was as high as 31.02%, which was a potential high protein source. Its lysine content was similar to that of the FAO reference protein. The limiting amino acids were found to be valine, isoleusine and trytophan, while total sulfur-containing amino acids were not limiting. Roselle seeds exhibited high digestibility by an *in-vitro* digestibility assay. They concluded that roselle seeds could be used as a protein source and could be used as a supplement food mixture for poor lysine sources. Rao (1996) analysed two varieties of roselle seeds for their nutrient composition and biological evaluation and found their protein content (18.8–22.3%) to be high. Lysine and tryptophan contents were high while sulphur-containing amino acids were limiting. This finding was contradictory with a previous finding by El-Adawy and Khalil (1994) where total sulfur-containing amino acids were not found to be limiting. Biological protein quality analysis of raw and cooked roselle seeds showed that the protein digestibility of roselle seed diets was lower than that of casein control diet.

Previous research indicated that roselle seeds were rich in lysine, arginine, leucine, phenylalanine and glutamic acid (Al Wandawi *et al*. 1984; Rao 1996; Hainida *et al*. 2008). Methionine and cysteine were the main limiting amino acids present (Rao 1996; Abu-Tarboush *et al.* 1997; Hainida *et al*. 2008). However, Al-Wandawi *et al*. (1984) reported that tryptophan was the main limiting amino acid in their study. El-Adawy and Khalil (1994) indicated that globulin was the major protein fraction of the seeds rather than albumins. According to Murray and Roxburgh (1984), high levels of albumin will elevate sulphur-containing amino acids (cysteine and methionine). This might have resulted in lower values of cysteine and methionine in roselle seeds (Hainida *et al*. 2008). Nevertheless, Hainida *et al*. (2008) revealed adequate cysteine and methionine contents present in roselle seeds for human requirement. In addition, amino

Table 3 Amino acid profiles of different roselle seed varieties.

Amino acid	El-Adawy and Khalil 1994			Rao 1996	
	Light red	Early dark red	Late dark red	AMV-2	Bhimili-1
Aspartic acid	10.91	10.50	10.52	9.1	8.3
Threonine [a]	4.86	4.74	4.39	4.2	3.8
Serine	4.40	4.65	4.70	5.2	5.2
Glutamic acid	21.30	21.78	21.38	21.6	21.8
Proline	4.14	4.13	4.21	5.2	4.6
Glycine	4.27	4.27	4.32	6.4	5.1
Alanine	4.69	4.55	4.72	6.1	4.2
Cystine [a]	2.64	2.91	2.88	1.0	4.2
Valine [a]	3.26	3.32	3.32	5.0	4.8
Methionine [a]	1.13	1.20	1.15	1.2	1.9
Isoleucine [a]	3.24	3.03	3.64	5.0	3.7
Leucine [a]	7.32	7.29	7.28	6.5	5.9
Tyrosine [a]	3.46	3.39	3.54	2.7	3.2
Phenylalanine [a]	5.09	5.25	5.15	4.8	4.7
Lysine [a]	5.37	5.46	5.56	4.3	3.3
Histidine	2.97	2.74	2.77	NA	NA
Tryptophan [a]	0.37	0.29	0.34	1.2	1.4
Arginine	10.58	10.50	10.13	9.9	10.2

Note: [a] Essential amino acid according to FAO/WHO (1991)

Table 4 Chemical composition of roselle seeds varieties.

Component	El-Adawy and Khalil 1994 % (g/100 g) (Mean ± standard deviation)			Rao 1996 % (g/100 g)	
	Light red	Early dark red	Late dark red	AMV-2	Bhimili-1
Protein	31.02 ± 1.32	30.11 ± 0.78	30.94 ± 0.94	18.8	22.3
Ash	6.89 ± 0.23	5.80 ± 0.12	6.52 ± 0.32	5.4	5.4
Fat	21.60 ± 0.57	22.53 ± 0.64	23.26 ± 0.78	19.1	22.8
Total carbohydrate	36.37 ± 1.86	38.12 ± 1.12	38.05 ± 1.05	NA	NA
Crude fiber [a]	4.12 ± 0.14	3.44 ± 0.08	1.23 ± 0.06	NA	NA
Total dietary fiber	NA	NA	NA	42.6	39.5
Insoluble fiber	NA	NA	NA	30.5	28.3
Soluble fiber	NA	NA	NA	12.1	11.2
Moisture	9.25 ± 0.98	11.66 ± 1.08	11.45 ± 0.86	8.6	6.7

Notes: [a] Crude fiber is determined as 100 – (total protein + total ash + total lipids + total carbohydrates)
NA: not available

acid profiles of the roselle protein concentrate and its protein isolate were similar to that of the roselle defatted flour. They also showed that the extraction method to obtain roselle proteins has no adverse effect on the amino acid profile. Arginine (34 g/100 g), lysine (14 g/100 g), leucine (15 g/100 g) and glutamic acid (24 g/100 g) were found to be high in roselle seed products. The lysine content of seeds was similar to that of the FAO reference protein (FAO/ WHO 1991). Moreover, relative to the FAO reference pattern, the limiting amino acids were found to be valine, isoleucine, and tryptophan (El-Adawy and Khalil 1994).

Some nutritional and functional properties of roselle seed products have been studied by Abu-Tarboush *et al.* (1997). Their studies showed that roselle seed flour contained 26% of protein. In Malaysia, Wong (2003) reported that the protein content of dried roselle seed flour was 20% on a dry weight basis. The protein content was significantly lower than the protein content of roselle seeds reported by El-Adawy and Khalil (1994). This probably due to variations of the roselle seeds used as the plants were grown under different soil and climate conditions, and agricultural practices that may exhibit different chemical and physical attributes. Comparison of the chemical composition of roselle seed varieties from two published studies are shown in **Table 4**. Roselle seeds have an excellent foaming capacity (El-Adawy and Khalil 1994). Most probably this capacity is associated with the high protein content in the seeds. Giami (1993) had suggested that foam capacity and stability are related to the amount of native protein. Amino acid profiles of roselle seed varieties, as reported by El-Adawy and Khalil (1994) and Rao (1996) are shown in **Table 3**. The results from El-Adawy and Khalil (1994) reveal that all amino acids were either the same or slightly different for all cultivars. There were no significant differences among the roselle seed cultivars in amino acid content. The chemical score of roselle seed protein was 40 and 57 for var. AMV-2 and Bhimili-1 varieties respectively.

Lipids

The value of roselle seed fat was considerable even when compared with other seeds (**Table 2**). Palmitic, oleic, linoleic acid (Rao 1996) and stearic acids (El-Aldawy and Khalil 1994) were the major fatty acid constituents in roselle seeds. A high content of unsaturated fatty acids constituted more than 70% of the polyunsaturated fatty acid. The ratio of saturated to unsaturated fatty acids was 1:2 (El-Adawy and Khalil 1994). In contrast, Mohiuddin and Zaidi (1975) found a ratio of saturated to unsaturated fatty acids to be 1:3. Roselle seeds have been reported to have similar properties to cotton seed oil as a substitute for crude caster oil. There are different fatty acids present in roselle seeds, namely myristic (2.1%), palmitic (35.2%), palmitoleic (2.0%), stearic (3.4%), oleic (34.0%), linoleic (14.4%), 13-expoxy-*cis*-9-octadeceonic (12, 13-epoxoliec) (4.5%), sterulic (2.9%) and malvalic (1.3%) (Ahmad *et al.* 1979). However, there is limited published data on roselle seeds oil or lipids.

Other components

Carbohydrates supply the greatest percentage of calories and bulk in an average diet, yet they make up less than 1% of total body weight. The main function of dietary carbohydrate is to supply energy (Kretchmer and Zimmermann 1997). The carbohydrate content of roselle seeds has been understudied. Nevertheless, El-Adawy and Khalil (1994)

Table 5 Comparison of TDF, SDF and IDF values of roselle seeds with common source of fiber.

	Dietary Fibers (g/100 g)		Ratio SDF:IDF	Total
	Soluble (SDF)	**Insoluble (IDF)**		
Roselle seeds [a]	4.84	13.42	3.0	18.26
Roselle seeds [b]	11.2-12.1	28.3-30.5	2.5	39.5-42.6
Wheat bran [c]	4.6	49.6	10.7	54.2
Oat [c]	1.5	73.6	49.1	75.1
Rice bran [c]	4.7	46.7	9.9	51.4
Apple fiber [c]	13.9	48.7	3.5	62.6
Tomato fiber [c]	8.3	57.6	6.9	65.9

[a] Hainida *et al.* 2008
[b] Rao 1996
[c] Claye *et al.* 1996
TDF: Total dietary fiber; SDF: Soluble dietary fiber and IDF: Insoluble dietary fiber.

found that the roselle seeds of different cultivars have a high content total carbohydrate content ranging from 36-38%. A study by Hainida *et al.* (2008) however, found a low (~ 13%) carbohydrate content in the seeds.

Dietary fiber is a collective term for a variety of plant substances that are resistant to digestion processes of human gastrointestinal enzymes. Different types of fiber are distinguished by their differing physiological properties and systemic effects. Dietary fiber is grouped into two different categories namely water-soluble and -insoluble depending on their solubility in water. Water-soluble fiber will dissolve in hot water whereas -insoluble fibers will not (Anderson *et al.* 1990). The structural or matrix fibers (lignins, cellulose, and some hemicellulose) are insoluble. The natural gel-forming fibers which are pectins, gums, mucilages, and the remainder of the hemicelluloses are soluble (Brown *et al.* 1999).

According to Rao (1996), roselle seeds are rich in dietary fiber (39-42%). This contributes to the strength of the seed if compared to other varieties of common sources of dietary fiber such as wheat and rice bran, oat and fiber from fruits (**Table 5**). However, El-Adawy and Khalil (1994) indicated that the seeds from different cultivars contained a low amount of crude fiber (1.23-4.12%).

ANTIOXIDATIVE EFFECT OF ROSELLE SEEDS

Antioxidants can be broadly defined as any substance that, when present at low concentrations compared to those of an oxidizable subtrate, significantly prevents or delays any oxidation of that substrate (Halliwell and Gutteridge 1990). In other words, antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reaction. Studies have shown that free radicals present in the human organism cause oxidative damage to different molecules, such as lipids, proteins and nucleic acids and thus are involved in the initiation phase of some degenerative illnesses. As a consequence, antioxidants are able to neutralize oxygen-free radicals for example by inhibiting low density lipoprotein oxidation (Hoffman and Garewal 1997; Prior *et al.* 1998). Antioxidants can be divided into two basic categories, namely synthetic and natural (Velioglu *et al.* 1998).

Anthocyanin is one type of flavonoid component that can be found in high amounts in roselle calyces (Wang *et al.* 2000; Tsai *et al.* 2002). Anthocyanins are universal plant colorants as they are largely responsible for the brilliant orange, pink, scarlet, red, mauve, violet and blue colors of flower petals and fruits of higher plants. Tsai *et al.* (2002) suggested that anthocyanin is the major source of antioxidant capacity in roselle petal extract, based on the observation that the antioxidant capacity of roselle extract increased when the weight of petals and extraction time increased. There was also a relationship between anthocyanin colour and antioxidant capacity. Tee *et al.* (2002) observed stronger antioxidant properties in roselle calyces extract than BHA or tocopherol in a linoleic acid model system. Meanwhile, Duh and Yen (1997) reported that the calyces of roselle possessed high contents of phenolic compounds. The water extracts of calyces also showed good hydrogen donating abilities, indicating that they had effective activities as radical scavengers. Many agricultural industries have an adverse environmental impact because of the wastes from the plant raw materials they use. Many agricultural wastes contain a considerable amount of natural antioxidant components. Several studies have reported the presence of antioxidative components in industry by-products such as in peanut hull (Duh and Yen 1995), wild rice hull (Asamarai *et al.* 1996), canola hull (Amarowicz *et al.* 2000), and many others. Some studies also investigated their application in the meat model system (Asamarai *et al.* 1996). The outer layers of plant materials usually contain a great amount of polyphenolic compounds, as expected from their protective function in the plants (Moure *et al.* 2001). There are also many studies that reported antioxidative compounds present in a wide variety of seeds. For example, in grape (*Vitis vinifera* L.) seed (Ricardo da Silva *et al.* 1991; Fuleki and Ricardo da Silva 1997; Jayaprakasha *et al.* 2001, 2003; Negro *et al.* 2003), apple (*Malus domestica*) seed (Lu and Foo 1998), citrus fruits seeds (Bacco *et al.* 1998), black-currant (*Ribes nigrum* L.) seed (Lu and Foo 2003), borage (*Borago officinalis* L.) seed (Wettasinghe and Shahidi 1999), fenugreek (*Trigonella foenumgraecum*) seed (Mansour and Khalil 2000) and many others which are usually the by-products from industries. Their application in the meat and linoleic acid model systems were also investigated (Wettasinghe and Shahidi 1999; Mansour and Khalil 2000; Jayaprakasha *et al.* 2001). Other agricultural industry by-products such as citrus fruits peels (Bacco *et al.* 1998), red and white grape pomace peels (Larrauri *et al.* 1998), red grape peels (Negro *et al.* 2003), potato peels (Mansour and Khalil 2000) and apple pomace (Lu and Foo 2000) are reported to possess antioxidative bioactive components.

Tee *et al.* (2002) determined the antioxidative properties of roselle extract prepared from calyces by monitoring the formation of diene-conjugated compounds and thiobarbituric acid reactive substances in a linoleic acid model system. The properties were compared with tocopherol and BHA. Results indicated that the roselle extract showed stronger antioxidant properties than tocopherol and BHA. The inhibitory effect on lipid oxidation observed was probably through the action of anthocyanins, a group of phenolic compounds found in roselle.

The mean values of the antioxidant activity of roselle seed extract at different concentrations using β-carotene bleaching assay are shown in **Fig. 7**. According to Chew (2003), as the concentration of roselle seed extract increased from 1500 to 3500 ppm, so too did the antioxidant activity of the extract increase. When the concentration of extract increased beyond 3500 ppm, the antioxidant activity of the extract decreased, from 3500 to 9500 ppm. Roselle seed extract exhibited the antioxidant activity of 40, 89, 79, 60 and 55% at 1500, 3500, 5500, 7500 and 9500 ppm, respectively (**Fig. 7**). Statistical analysis (ANOVA test) indicated that differences in the means of antioxidant activity at different concentrations of roselle seed extract were all significant ($p < 0.05$). However, there was no significant difference in the means of antioxidant activity between roselle

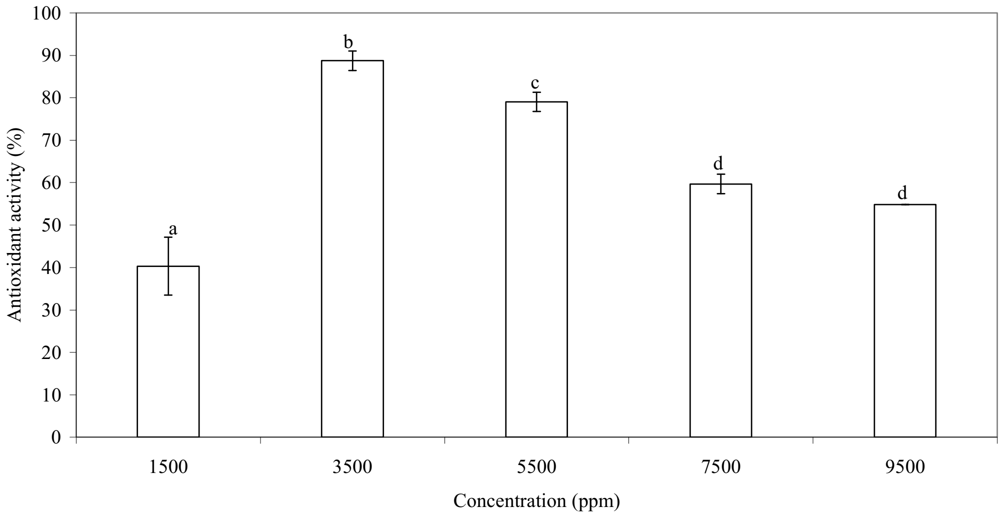

Fig. 7 Antioxidant activity of roselle seed extract at different concentrations. Antioxidant activity was measured using a β-carotene bleaching assay. Values with different letters are significant different at the level of $p < 0.05$. Source: Based on and modified from Chew (2003).

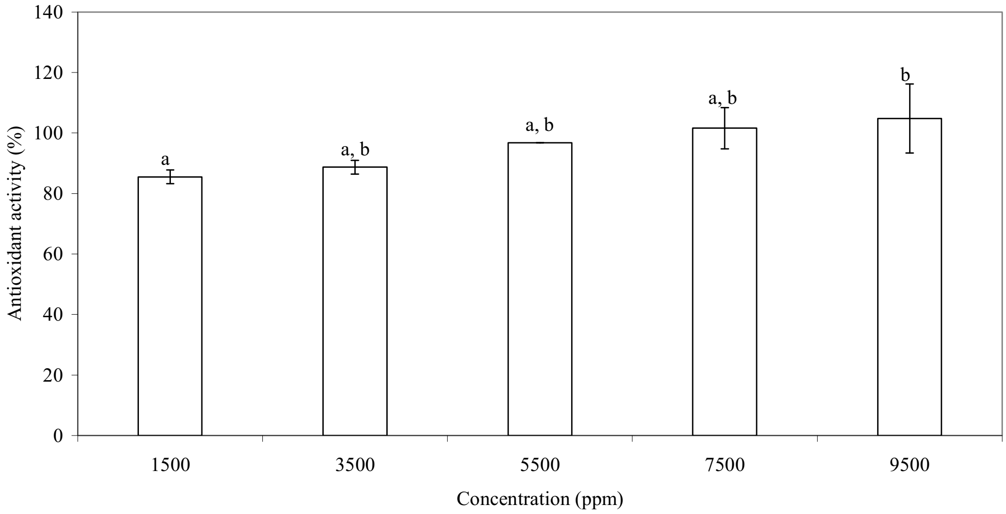

Fig. 8 Antioxidant activity of a combination of roselle seed and cocoa shell extracts at different concentrations. Antioxidant activity was measured using a β-carotene bleaching assay. Values with different letters are significant different at the level of $p < 0.05$. Source: Based on and modified from Chew (2003).

seed extract at 7500 and 9500 ppm (**Fig. 7**). Amin and Mukhrizah (2006) reported that roselle seed extract exhibited the highest antioxidant activity when extracted with water compared to methanol. In their study, the antioxidant activity of roselle seed extract was compared to other extracts, namely those of cocoa shells, okara (by-product of soy milk processing), mango peel and pink guava by-product extracts. The means of antioxidant activity of water extracts decreased in the order of roselle seeds > mango peel > okara > cocoa shell. In the methanolic extract, pink guava by-product and cocoa shell exhibited higher antioxidant activity than roselle seed extract.

Chew (2003) showed that the antioxidant activity of a combination of roselle seeds and cocoa shell increased as the concentration of extract increased from 1500 to 9500 ppm. The means antioxidant activity of the extract was 85, 89 and 97% at 1500, 3500 and 5500 ppm, respectively. The corresponding values were 102 and 105% at 7500 and 9500 ppm, respectively (**Fig. 8**). Amin and Chew (2006) evaluated the antioxidant activity of roselle seeds and its combination with cocoa shell at 7.5 g/L and found that the antioxidant activity of the extracts was in the order of cocoa shell ≈ cocoa shell + roselle seeds > roselle seeds.

Besides antioxidant activity evaluated by the β-carotene bleaching assay, the 2,2-diphenyl-2-picrylhydrazyl radicals (DPPH) assay was also used to determine the scavenging activity of roselle extract of stable radical species of DPPH. The proton radical scavenging action is known to be one of the various mechanisms for antioxidation. DPPH is one of the compounds that possesses a proton-free radical and shows a maximum absorption at 517 nm. When DPPH encounters proton radical scavengers, its purple colour fades rapidly. Chew (2003) showed that the scavenging activity of roselle seeds increased when the concentration of extract increased from 1500 to 9500 ppm (**Fig. 9**). The means free radical scavenging activity for roselle seeds at the concentrations of 1500, 3500, 5500, 7500 and 9500 ppm were 54, 70, 74, 88 and 94%, respectively. It was suggested that the increase in the free radical scavenging activity was due to the increase in the amount of antioxidants present in the extract. Amin and Chew (2006) showed that roselle seed extract at 7.5 g/L exhibited a significant lower free radical scavenging activity than cocoa shell. Amin and Mukhrizah (2006) reported that roselle seeds exhibited the highest free radical scavenging activity among other by-products. The mean free radical scavenging of several water extracts was in the descending order of roselle seeds > pink guava by-products > okara > cocoa shell > mango peels. Roselle seed extract in 50% methanol was an efficient DPPH scavenger with scavenging activity of 80% (Amin and Mukhrizah 2006).

The free radical scavenging activity at different concentrations of combination of cocoa shell and roselle seed extracts is shown in **Fig. 10** (Chew 2003). As the concentration of combination of cocoa shell and roselle seed extract increased from 1500 to 9500 ppm, the free radical scavenging activity of the extract increased. It was similar to the findings for cocoa shell and roselle seed extracts alone. Unlike cocoa shell extract, the increment in the free radical scavenging activity for a combination of cocoa shell and roselle seed extract was greater. The mean free radical scavenging activities were 65, 76, 81, 94 and 96% for the extract at 1500, 3500, 5500, 7500 and 9500 ppm, respectively. Amin and Chew (2006) estimated the phenolic content of roselle seeds, and found that cocoa shell had higher phenolic content than roselle seeds.

To evaluate the reducing power of roselle seeds, an assay based on the reduction of ferric (Fe^{3+}) to the ferrous

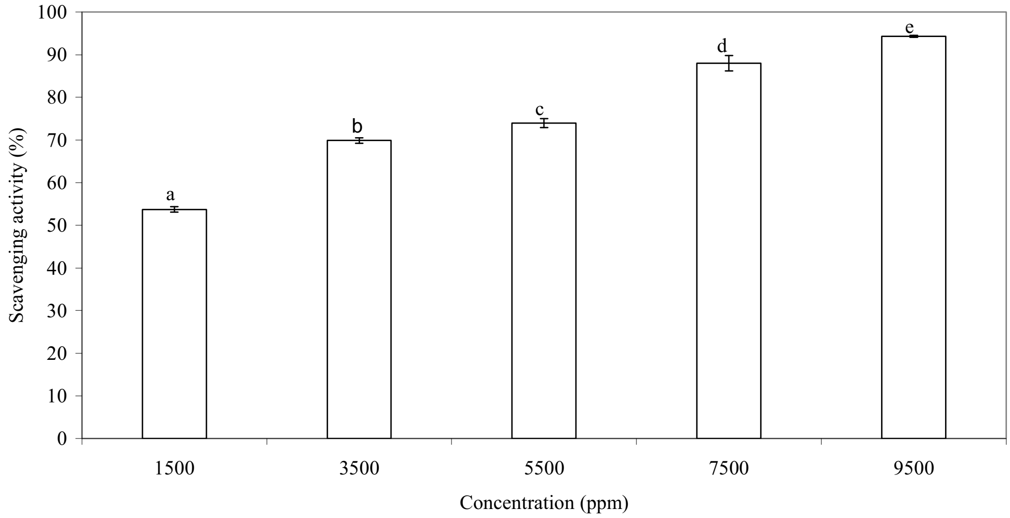

Fig. 9 Scavenging activity of roselle seed extract at different concentrations. Scavenging activity was estimated based on the capability of the extract to scavenge the DPPH radical. Values with different letters are significant different at the level of $p < 0.05$. Source: Based on and modified from Chew (2003).

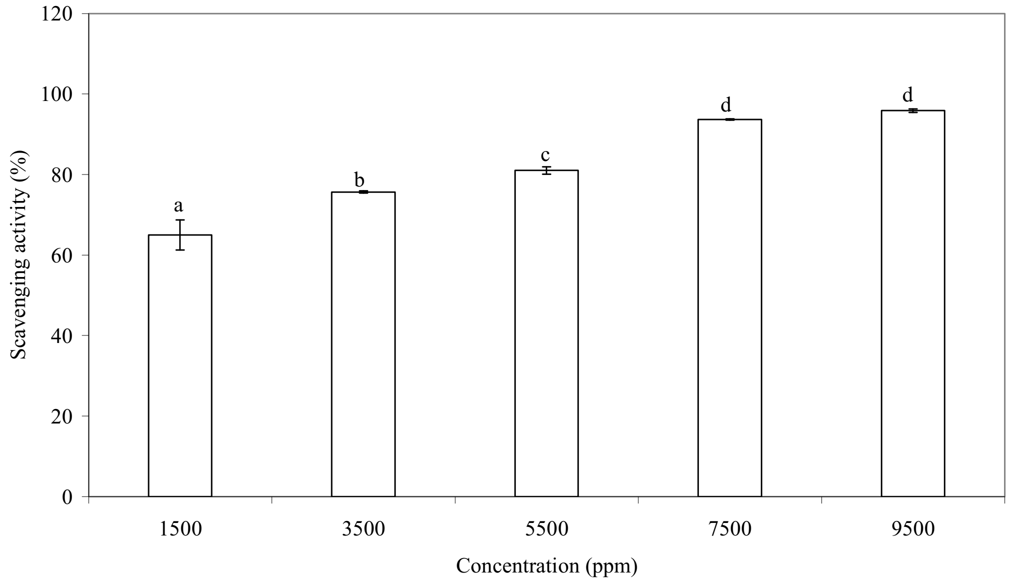

Fig. 10 Scavenging activity of a combination of roselle seed and cocoa shell extracts at different concentrations. Scavenging activity was estimated based on the capability of the extract to scavenge the DPPH radical. Values with different letters are significant different at the level of $p < 0.05$. Source: Based on and modified from Chew (2003).

Table 6 Antioxidative effects of roselle seed extract and synthetic antioxidants on the oxidative stability of cooked beef patties stored at 4°C for 2 weeks.

Time (day)	Increasing order of antioxidative effect
1	α-tocopherol < BHT < a combination of roselle seeds and cocoa shells < roselle seed extract
3	α-tocopherol < BHT < a combination of roselle seeds and cocoa shells < roselle seed extract
7	BHT < α-tocopherol < roselle seed extract < a combination of roselle seeds and cocoa shells
14	BHT < α-tocopherol < roselle seed extract < a combination of roselle seeds and cocoa shells

Source: Chew (2003)

form or when more electrons are donated by antioxidant components was used. In this assay, the colour of Perl's Prussian Blue will be darker resulting in an increased absorbance reading. The highest reducing power was found in the methanolic extract of pink guava, whereas in the water extract the highest reducing power was mango peel. Roselle seeds exhibited the lowest reducing power compared to other by-products. The reducing power in methanolic extracts at 0.16, 0.32 and 0.64 mg/ml decreased in the order of pink guava by-products > cocoa shells > okara > mango peels > roselle seeds, whereas the reducing power of the water extract decreased in the order of mango peels > cocoa shells > roselle seeds ≈ okara ≈ pink guava by-products (Amin and Mukhrizah 2006).

To evaluate the antioxidative efficacy of roselle seeds on the susceptibility of cooked beef to lipid oxidation, Amin and Chew (2006) monitored the formation of thiobarbituric acid reactive substances (TBAR) in cooked beef by the addition of roselle seed extract stored at 4C for 2 weeks. The study found that the roselle seed extract have potential to reduce lipid oxidation in cooked-refrigerated beef (**Table 6**) (Chew 2003).

PROTEIN QUALITY

The topics of protein and amino acid requirements have generated substantial controversy the past 10 years. Some consensus began to emerge that a portion of the controversy resulted from confusion over the meaning of the generic term requirement. However, the use of three separate terms: (i) biological need, which defines the quantities of nutrient in question that are consumed in its various metabolic pathways (ii) dietary requirement or estimated average requirement (EAR), which define the quantity of the nutrient that must be supplied in the diet to satisfy biological needs (iii) recommended dietary allowance (RDA) or recommended dietary intake (RDI), which are practical expressions of the dietary requirement, would eliminate this confusion (Reeds and Beckett 1997).

Reeds and Garlick (2003) discussed factorial models of the dietary requirements for protein and nitrogen and developed individual indispensable amino acids from published information. A relationship was found between age and protein deposition and between protein (amino acid intake) and nitrogen balance. The results were then used to develop recommendations on the protein-energy ratio and the amino

Table 7 Protein efficiency ratio (PER) and net protein ratio (NPR) of two different varieties of roselle seeds.

Growth study	Halimatul *et al.* (2007)			Rao (1996)			
	Dried roselle seed	**Boiled roselle seed**	**Casein**	**AMV-2 Raw**	**AMV-2 Cooked**	**Bhimili-1 Raw**	**Bhimili-1 Cooked**
PER	1.15 ± 0.60 b	1.71 ± 0.34 ab	2.11 ± 0.20 a**	1.09 ± 0.12	2.35 ± 0.05	1.73 ± 0.17	2.35 ± 0.05
NPR	0.44 ± 0.60 b	1.12 ± 0.33 ab	1.49 ± 0.18 a**	ND	ND	ND	ND
NPU	ND	ND	ND	37 ± 3.7	45 ± 1.2	39 ± 2.1	46 ± 0.6

ND: not determined.
In the same row, values with different superscript letters are significantly different at $p < 0.05$ or $p < 0.01$ indicated by (**).

acid pattern of the diet. Over the age range of 6-24 months the models predict a fall in the weight-specific protein and amino acid requirement that result almost entirely from changes in the growth rate of children. It also concluded that this contrasts markedly with the relationship between age and energy requirements. The amino acid modeling implies that the optimum pattern of individual essential amino acids also changes only marginally across the age range considered in the report. The protein quality, also known as the nutritional or nutritive value of a food, depends on its amino acid content and on the physiological utilization of specific amino acids after digestion, absorption and minimal obligatory rates of oxidation (Friedman 1996). In order to assess the protein quality of each plant, by comparing amino acid compositions with that of the World Health Organization (WHO) standard protein (FAO/WHO/UNU 1985).

The amino acid composition of proteins is often used to define their nutritional quality where protein quality is affected by the concentration and pattern (ratio of constituent amino acids making up a specific protein) of indispensable amino acids. The ratio of amino acids is useful in differentiating closely related proteins or highlighting decreases in specific amino acids following chemical modifications of protein during food processing (Eggum and Sorensen 1989). According to Sarwar and Sepehr (2003), amounts of indispensable amino acids, digestibility of protein and bioavailability of amino acids are basic parameters in determining the quality of a protein source. Generally, proteins that are deficient in one or more amino acids are “poor quality” whilst “good quality” proteins are those that are readily digestible and contain the indispensable amino acids in quantities that correspond to the human requirement.

However, before a protein can serve as a nutritional source of amino acids, it must be digested. Digestibility means the susceptibility of peptide bonds to hydrolysis. McDonough *et al*. (1990), Sarwar and McDonough (1990) and Sarwar and Paquet (1989) found that (a) the apparent digestibility of crude protein varied with the concentration of protein in the diet (b) true digestibility was independent of the concentration of protein (c) the low digestibility of proteins in pinto (*Phaseolus vulgaris* L.) beans, kidney (*P. vulgaris*) beans, and lentils were comparable to those reported earlier for beans (Fabaceae), peas (*Pisum sativum* L.) and lentils (*Lens culinaris*) (d) true digestibility for methionine, cystine and tryptophan were up to 27% lower than values for crude protein and (e) true digestibility of lysine in wheat, oat, rye and sorghum were 14% lower than those in crude protein. Friedman (1996) cited that digestibility and amino acid availability are not synonymous. Nevertheless, both significantly influence protein quality of foods. Rather, digestibility means the susceptibility of peptide bonds to hydrolysis, while availability refers to the chemical integrity of the amino acid; for instance, its resistance to processing by heat, high pH, oxidation and other factors. These treatments can limit both availability and digestibility, especially of lysine, methionine, threonine, and tryptophan. Furthermore, high digestibility does not always mean high protein quality; instead, digestibility of protein is a good predictor of bioavalability of amino acids (Sarwar and Sepehr 2003). Therefore, both amino acid availability and digestibility must be considered to define the nutritional value of proteins.

Any factor that alters digestibility or amino acid availability of foods would in turn affect the protein quality (Friedman 1996). The following are studies of factors which were found to influence the nutritive value of protein foods or protein ingredients. Basically, proteins are rarely consumed as the only element of a meal; rather they are eaten with other foods, the susceptibility of food proteins to digestion are influenced by other nutrient such as fiber and phytic acid (Begbie and Pusztai 1989; Lathia and Koch 1989). Besides, digestibility of proteins is influenced by the nature and structure of protein and the effects of processing (Friedman 1996). Abdel-Aal and Hucl (2002) determined and compared the total protein, amino acid composition and *in vitro* protein digestibility of selected ancient wheats and their pasta, breakfast cereal and bread products with those produced from durum and common wheat. In general, this research showed that the amino acid composition is similarly influenced by milling and processing.

A study done by Halimatul *et al*. (2007) reported the protein quality of two differently processed (dried and boiled) roselle seed powder. This study showed that the protein quality of dried roselle seeds was similar to that of roselle seeds boiled at 100C for 30 min. To conduct growth and nitrogen balance studies, rats were fed with 10% (w/w) protein from dried (DS) and boiled (BS) roselle seed powder for 4 weeks where casein was used as the standard reference protein. There was a significantly higher ($p < 0.05$) food intake and weight gain of rats fed with BS compared with DS. In the growth study, there was no significant difference ($p < 0.05$) in the protein efficiency ratio (PER) and in the net protein ratio (NPR) of BS compared to DS, but it was significantly different to casein (CD) (**Table 7**). In the nitrogen balance study, true nitrogen absorption (TNA) and nitrogen balance (NB) of the BS group were significantly higher ($p < 0.05$) than the DS group. However, apparent digestibility (AD), true digestibility (TD) and biological value (BV) for both diets was not significantly different (**Table 8**). Rao (1996) reported that food intake, gain in body weight, PER and NPU values of rats administered cooked roselle seed diets were significantly higher than a raw seed diet. Heat treatment improved the nutritive value of legumes and other edible seeds by reducing the levels of anti-nutritional factors and increasing protein digestibility (Giami *et al*. 2001; Siddhuraju and Becker 2001; El-Adawy 2002; Fagbemi *et al*. 2005; Giami 2005). PER and NPR values of both BS and DS were lower than cowpea (Giami 2005). AD, TNA, TD, NB and BV values for both DS and BS were lower than casein diet. AD and TD values for roselle seeds were lower than 12 sunflower seed cultivars (Canibe *et al*. 1999), cooked jack (*Canavalia ensiformis*) bean and roasted devil (*Mucuna pruriens*) bean (Agbede and Aletor 2005), 11 cultivars of lupin (*Lupinus* sp.) seed (Eggum *et al*. 1993) and *japonica* and *indica* cooked milled rice (Boisen *et al*. 2001), but it exhibited higher BV values (**Table 8**). Enzymatic hydrolysis towards digestibility of protein is directly related to its structure (Rasco 1998). In addition, chemical changes in roselle seeds during boiling could reduce the protein susceptibility to digestive enzymes.

In addition, roselle seeds had high *in-vitro* protein digestibility (IVPD) as reported by several groups (El-Adawy and Khalil 1994; Abu-Tarboush and Ahmed 1996; Abu-Tarboush *et al*. 1997; Yagoub *et al*. 2004). A study done by El-Adawy and Khalil (1994) revealed that three different roselle seed cultivars from Egypt exhibited around 79.00–81.3% IVPD, whereas Abu-Tarboush and Ahmed (1996) reported defatted roselle seed flour from Sudan exhibited 82% IVPD where it was significantly higher than defatted soybean flour. Domestic processing was reported to de-

Table 8 Apparent digestibility (AD), true nitrogen absorption (TNA), true digestibility (TD), nitrogen balance (NB) and biological value (BV) of varieties of seeds.

Varieties of seeds	Nitrogen balance study					References
	AD (%)	TNA (%)	TD (%)	NB	BV (%)	
Casein	83.41 ± 1.66	1.07 ± 0.13 b	85.83 ± 1.41 b	1.04 ± 0.13 b	97.18 ± 0.33	Halimatul *et al.* 2007
Dried *Hibiscus sabdariffa* L. seed	66.42 ± 3.28	0.78 ± 0.11 a	69.10 ± 3.01 a	0.75 ± 0.11 a	96.10 ± 0.56	
Boiled *Hibiscus sabdariffa* L. seed	64.26 ± 3.76	1.05 ± 0.17 b	66.18 ± 3.56 a	1.02 ± 0.17 b	97.08 ± 0.46	
12 cultivars of sunflower seed	81.6–84.3	ND	92.9–95.8	ND	65.3–73.2	Canibe *et al.* 1999
Cooked jack bean	52.7	ND	66.4	ND	80.5	Agbede and Aletor 2005
Roasted devil bean	45.2	ND	60.0	ND	42.5	
11 cultivars of lupin seed	ND	ND	84.1–89.8	ND	63.7–86.6	Eggum *et al.* 1993
Japonica cooked milled rice	ND	ND	96.9–98.0	ND	76.5–77.2	Boisen *et al.* 2001
Indica cooked milled rice	ND	ND	93.1–97.2	ND	71.4–73.7	

ND: not determined.
In the same row, values with different superscript letters are significantly different at $p < 0.05$ or $p < 0.01$.

crease the percent of IVPD of roselle seeds as investigated by Yagoub *et al.* (2008). IVPD of roselle seeds was significantly reduced by all processing methods (soaking, sprouting and cooking). Soaking resulted in the highest reduction of IVPD while cooking had the lowest effect.

Protein quality is also known as the nutritional or nutritive value of a food, and depends on its amino acid content, ratios of essential amino acids and on the physiological utilization of specific amino acids after digestion, absorption, and minimal obligatory rates of oxidation (Friedman 1996). Sarwar and McDonough (1990) reported that lower digestibility of a protein source or protein ingredient may be due to the occurrence of amino acids in less digestible parts and the present of protease or amylase inhibitors, lectins, phytates and tannins. Friedman (1981) reported that alkali-induced racemization of amino acids and concurrent formation of lysinoalanine in food proteins can impair the nutritional quality and safety of foods by decreasing the amount of indispensable L-amino acids, thus decreasing digestibility and bioavailability of proteins and forming toxic products. Ruiz-Lopez *et al.* (2000) reported that antinutritional factors such as lectins, antitrypsin activity, cyanogenic glycosides, alkaloids, phytates and α-galactosides were found in the seeds of several wild lupin cultivars. However, these could be considered a good source of protein after a suitable reduction in the content of alkaloids.

A study done by El-Adawy and Khalil (1994) revealed that there are no distinct differences in three cultivars of roselle seeds from Egypt and indicated a low tannin and phytic acid content and the absence of hemagglutinin activity. Yagoub *et al.* (2004) reported that the percentage of total polyphenol and phytic acid contents was significantly decreased by cooking and fermentation treatments. However, results reported by Yagoub *et al.* (2008) were contradictory. This study revealed that cooking only significant decreased ($p < 0.05$) the polyphenol content inherent in roselle seeds but soaking and sprouting treatments did not. Heat degradation, leaching out of the compounds in water during soaking, change in chemical reactivity and formation of insoluble complexes might be factors that caused a significant reduction of these anti-nutrients by cooking (Saikia *et al*. 1999; Alonso *et al*. 2000; Yagoub *et al*. 2004). Moreover, the phytic acid content of roselle seeds was unaffected by soaking, sprouting and cooking (Yagoub *et al*. 2008).

Al-Wandawi *et al.* (1984) and Abu-Tarboush and Ahmed (1996) reported that whole roselle seeds had a lower percentage or trace of free and bound gossypol compounds. However trypsin and α-chymotrypsin inhibitor activities in defatted roselle seed flour were reported to be higher than its protein isolate, however, these were lower than soybean defatted flour (Abu-Tarboush and Ahmed 1996). Roselle seeds also reportedly had a high phytic acid content than soybean (Abu-Tarboush and Ahmed 1996).

According to Cook *et al.* (2000), in assessing the nutritional value of the plant foods, it is important to consider how well amino acids and other nutrients are absorbed through the gut. If any of the plant foods contain significant amounts of protease inhibitors, the utilization of amino acids will be impaired. The effects of protein quality to human health have been studied. Previous studies have shown that the intake of a low quality cereal protein, during either a short or a long period causes thymus atrophy of growing rats with a significant decrease in cellular proliferation, altered differentiation and maturation of T-cell population and altered the distribution of plasma zinc (Slobodianik *et al.* 1989; Pallaro *et al.* 1991; Pallaro *et al.* 1997; Pallaro and Slobodianik 1999).

Although anti-nutritional factor are found to largely affect the protein quality of foods, many of the anti-nutritional factors in legumes can be eliminated or inactivated, to a large extent, by appropriate heating and processing during food preparation (Mwanjala *et al.* 1999). Phillips and Baker (1987) examined the effects of various processing techniques on cowpea protein quality, and found that the greatest improvement over raw seeds (expressed by an increase in protein effciency ratio from 2.04 to 2.81) occurred when the meal was extruded. Giami (2004) studied the effect of fermentation, for 7 days, on levels of nitrogenous constituents, protein fractions, antinutrients and protein quality of fluted pumpkin (*Telfairia occidentalis* Hook) seeds. Fermentation significantly increased crude protein and *in vitro* protein digestibility but decreased polyphenol and phytic acid contents of the seeds. The values obtained for the protein efficiency ratio, the net protein ratio and true digestibility of diets formulated with pumpkin seeds fermented for 5 days were similar to those of casein.

Longvah and Deosthale (1998) studied the effect of processing on the protein quality of *Perilla frutescens*. The effects of dehulling, cooking and roasting on the protein quality of *P. frutescens* seeds were evaluated. This study found that cooking increased the net protein ratio, net protein utilization, and true digestibility of protein significantly, whereas roasting exerted a negative effect; dehulling significantly increased the net protein ratio, net protein utilization and true digestibility of protein. In term of biological evaluation of protein, total biological utilization of legume protein is relatively low, with digestibility less than 76%. The true digestibility of legumes varies from 73% (canned pinto beans) to 98% (soya protein isolate) (Lam and Lumen 2003). Protein digestibility may be impaired by the presence of numerous anti-nutritional compounds that must be removed or destroyed during processing (Uebersax and Occena 2003). However, some of the unwanted components can be removed or reduced by manipulation of processing techniques such as soaking, boiling, autoclaving, heating, cooking and fermentation (Bishnoi and Khetarpaul 1994; Fernandez *et al*. 1997; Alonso *et al*. 1998, 2000).

TOXICOLOGY EFFECTS

An experiment was conducted to study the effect of roselle seeds on broiler performance, function and histopathology in the liver. Histopathological studies demonstrated no damage in the liver due to roselle seeds in the diet. An *in-vivo* study indicated that roselle seeds can be included in levels up to 30% without adverse effect on weight gain or liver function (Perry 1980).

Roselle seeds, like other seeds and legume may have anti-nutritional factors that have been associated with a reduction in food digestibility, a decrease in nutrient bioavailability and flatulence production. Abu-Tarboush and Ahmed (1996) reported defatted flour and protein isolate of roselle seeds contained protease inhibitors, phytic acid and gossypol, but they would not pose a problem if the seeds are properly processed. Moreover, a study done by Fagbenro (2005) found that the replacement of soybean meal protein by 60% of roselle seed meal into a catfish diet did not affect weight gain, growth response, feed conversion, protein utilization or carcass composition and histological abnormalities in the liver. Hainida *et al*. (2008) found that diets with or without the inclusion of roselle seeds did not interfere with the food intake (19-20 g/day) among the groups of normal and hypercholesterol rats studied. On the basis of daily observations, all rats remained healthy and active throughout the experiments. There were no significant differences in final body weights among the groups, however, the final weight of rats fed with 50 g/kg and 150 g/kg increased slightly compared with normal and hypercholesterol rats groups.

It was observed, by earlier workers, that different cooking methods improve the nutritional quality of food legumes to various extents (Chau *et al*. 1997). Halimatul *et al*. (2007) found that the protein quality of studied dried roselle seeds was similar to that of boiled seeds. Therefore, the anti-nutrient of dried roselle seeds might not affect food digestibility and biological value. Nevertheless, further research is needed to investigate the anti-nutritional factors of seeds.

POTENTIAL HEALTH BENEFITS

Madani *et al*. (2004) compared the effects of diets based on soya bean protein (plant protein) and casein (animal protein) supplemented or not with 0.1% cholesterol on plasma lipoprotein lipid amounts and their fatty acid composition, lecithin:cholesterol acyl-transferase activity, and lipid peroxidation. Their results suggested that the origin of dietary protein affects lipid peroxidation and polyunsaturated fatty acid biosynthesis and distribution among liver and different lipoprotein lipid classes, but plays only a minor role in the regulation of plasma and lipoprotein cholesterol concentrations. Baldwin (2002) compared of soya protein with animal proteins or even the casein of cow's milk and established that soya protein helps prevent atherosclerosis, whereas animal protein from beef and milk contribute to atherosclerosis.

Prospective epidemiological evidence is conflicting regarding the role of animal protein vs. plant protein in bone loss. Frassetto *et al*. (2002) found cross-cultural relationship between hip fracture rates and dietary protein was positively related to animal protein intake and inversely related to vegetable protein intake. They explained that increases in dietary animal protein are associated with increases in urinary Ca excretion; an increase in osteoporotic fractures has frequently been attributed to an increase in dietary animal protein. On the other hand, a report by Massey (2003) summarized that urinary Ca excretion is strongly related to net renal acid excretion. The catabolism of dietary protein generates ammonium ion and sulfates from sulfur-containing amino acids. Bone citrate and carbonate are mobilized to neutralize these acids, so urinary Ca increases when dietary protein increases. However, vegetable proteins are known to have poorer nutritional quality than animal proteins for humans because they are imbalanced in the ratio of cysteine to methionine needed to meet requirements, not because they are all lower in sulfur per g of protein. Furthermore, common plant proteins such as soy, corn, wheat and rice have similar total sulfur per g of protein as eggs, milk and muscle from meat, poultry and fish. Therefore increasing intake of purified proteins from either animal or plant sources similarly increases urinary Ca. Plus, the effects of a protein on urinary Ca and bone metabolism are modified by other nutrients found in that protein food source. This concluded that animal and plant foods may have different effects on bone health, although these effects are mainly attributable to other constituents of the food and diet, not protein.

Studies revealed that fiber-rich foods such as wheat bran, oat bran, beans, grains and legumes have important effects on serum cholesterol (Sharma and Kawatra 1995; Smit *et al*. 1999; Lairon 2001; Marlett 2001). Hainida *et al*. (2008) indicated that the inclusion of 50 g/kg and 150 g/kg of dried roselle seeds exhibited positive hypocholesterolemic effects but did not hold true when added at less than 50 g/kg in hypercholesterolemic diets. Hainida and co-workers observed significant substantial negative correlations between high-density lipoprotein (HDL-C) with total cholesterol (TC) and low-density lipoprotein (LDL-C) respectively. A positive correlation was observed between LDL-C and TC. Since HDL-C is negatively correlated to coronary heart disease, consumption of roselle seeds might be beneficial for cardio protective effect.

Numerous studies have been conducted to evaluate the possible effects of ingesting various sources of dietary fiber. Cara *et al*. (1992) found that serum triglyceride responses were lowered in the presence of oat bran, wheat fiber, or wheat germ and that chylomicron triglycerides were reduced by wheat fiber. Moreover, cholesterolemia decreased postprandially for 6 h, and was further lowered in the presence of oat bran. This was in agreement with Hundemer *et al*. (1991) and Anderson *et al*. (1994) who found that diets rich in soluble fibers had significantly lowered cholesterol concentration in the plasma and liver. Subsequently, sufficient consumption of dietary fiber could reduce the risk of civilization diseases, such as cardiovascular disease, colon cancer and obesity (Marlett 2001; Slavin 2001). Some dietary fibers have also been reported to decrease the digestion and absorption of carbohydrate and postprandial serum glucose levels (Flourie 1992; Ou *et al*. 2001).

Roselle seeds had a considerable good ratio of soluble to insoluble fractions (Hainida *et al*. 2008) compared to other common source of dietary fiber, such as wheat bran, oat fiber and rice bran (Claye *et al*. 1996) (**Table 5**). Grigelmo *et al*. (1999) reported that the ratio of soluble to insoluble fractions in dietary fibers must be within the range of 1.0 to 2.3 in order to be able to exert a physiological effect. Dietary fibers may lower serum cholesterol by modifying the bile acid absorption and metabolism, interfering with lipid absorption and metabolism, producing short-chain fatty acids from fiber fermentation in colon, and altering the concentrations of insulin and hormones (Anderson *et al*. 1990). Previous research demonstrated that bile acids bind strongly to vegetable fiber (Sugano 1983; Jenkins *et al*. 1999; Alonso *et al*. 2001). The hypocholesterolemic effects may be due to binding of the dietary fiber from roselle seeds with bile acids which in turn increases its fecal excretion (Lairon 2001; Marlett 2001; Chau *et al*. 2004). A subsequent increase in demand for cholesterol by the bile acid synthesis pathway, may divert cholesterol away from the lipoprotein synthetic pathway (Story and Lords 1987; Morita *et al*. 1997), thereby reducing the serum cholesterol. Nevertheless, since other dietary components in the seeds could also influence the total cholesterol and distribution of cholesterol in different lipoprotein classes, it is not possible to definitively assume that dietary fibers alone were linked to cholesterol-lowering effects.

Despite dietary fiber, a high content of protein in the seeds might also be the reason for the hypocholesterolemic effect. Alonso *et al*. (2001) reported the consumption of vegetable proteins may lead to changes in the level of hormones that modulate plasma cholesterol. However, Alonso *et al*. (2001) did not indicate any clear relationship between hormone levels and serum lipids. Morita *et al*. (1997) reported that methionine in the diets play a central role in cholesterol-lowering effects, through the regulation of Apo A-I secretion from liver into the blood circulations. Roselle seed powder contained a higher methionine content (Al-Wandawi *et al*. 1984; Rao 1996; Hainida *et al*. 2008) than other established cholesterol-lowering foods, such as soybean and grains (Morita *et al*. 1997; Abdel-Aal and Hucl 2002). Hence, it is reasonable to assume that amount of methionine in seeds might play an important role in cholesterol-lowering effects.

Kritchevsky *et al.* (1982) and Morita *et al*. (1997) showed that the ratio of lysine to arginine might be an important determinant of atherogenicity. A higher ratio of lysine to arginine is associated with enhanced atherosclerosis (Kritchevsky *et al.* 1982). Rao (1996), Abu-Tarboush *et al.* (1997) and Hainida *et al*. (2008) found that the lysine to arginine ratio was low in roselle seeds (0.4-0.5). Thus, despite dietary fibre, protein and methionine content, this amino acid ratio could also be one of the indicators of cholesterol lowering effects of roselle seeds.

Antioxidants are also considered to be important in the prevention of disease-like cardiovascular disease. However, Hainida *et al*. (2008) indicated that the antioxidant capacity of roselle seeds was low and might not be an important determinant in lowering the total cholesterol level. Truswell (2002) reported that some grains such as oat had significantly reduced LDL-C, but that HDL-C and triglyceride levels remain unaltered. According to Pizzorno *et al.* (2002) for every drop of 1% in LDL, myorcardial infarction (MI) risk drops by 2%, and for every 1% increase in HDL, MI risk drops 3-4%. This suggests that even a slight increase in HDL and decreased in LDL helps to reduce the risks of cardiovascular disease (CVD). When the risk of CVD is reduced, this eventually could reduce the number of deaths due to heart diseases and cardiovascular disorders. Therefore, the inclusion of roselle seeds in the diet might potentially prevent the risk of atherosclerosis and coronary heart disease. However, to utilize these significant contributions, further investigations of seed composition is warranted.

FUTURE RESEARCH

Previous studies showed that roselle seeds have their own potential nutritional benefits that should be further analysed and exploited especially their protein, dietary fibers, oils and hypocholesterolemic agents. Future advanced studies are needed to facilitate a clearer understanding of their functional ingredients. Below are some recommendations.

Dietary fiber and protein fraction are potential nutrients, which should be extracted and used as functional ingredients for the development of nutraceuticals or functional food product. It would be beneficial if we could utilized and develop diversified products, such as high dietary fiber powder and protein concentrate as food additives.

Antioxidants activity of roselle seeds is considerable compared with other seed legumes. Solubility is an important physical factor in sample preparation for antioxidant capacity. High concentration of the seed extract is needed to exert maximum antioxidant activity. More research should be done to examine the antioxidative effects of roselle seeds that may be added as a natural antioxidant or food additive in the replacement of artificial antioxidants.

More animal models should also be considered to further confirm the cholesterol-lowering effect of roselle seeds. Animal models with physiological conditions that are more similar to human for hypocholesterolemic effects should be used such as the rabbit model. Toxicology with specific biomarkers should be investigated to evaluate the toxic constituents present in the seeds. This is needed in order to transfer this present finding to humans and to further standardize the dose for human intake.

Although roselle seeds exhibit good protein quality through *in-vivo* and *in-vitro* techniques, further studies should be conducted more to evaluate its digestibility, bioavailability, and measurements of reactive lysine, dye binding and the rate of hydrolysis by proteolytic enzymes.

In addition, utilization of roselle seeds may surely minimize the production of waste from roselle processing and contribute to the beneficial outcome of the food industry. *Hibiscus sabdariffa* is very easy to grow, and the public can grow roselle as a home garden-crop due to its ease to maintain as the plant does not need much attention. Results of the nutritional composition, protein quality and potential health benefits of roselle seeds should be referred for future research, development and commercialization. It would be useful, if we could fully utilize roselle seeds, hence minimizing the waste production from roselle processing. The complete utilization of the seeds may be beneficial in developing a diversity of health-care products, functional foods, nutraceuticals and food supplement products.

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