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Characterization of Nutritional Components and Utilization of Pumpkin

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ABSTRACT

Pumpkin is a dicotyledonous seed vegetable with many nutritional components, including pumpkin polysaccharides, active proteins, essential amino acids, carotenoids, minerals and so on. It has been received considerable attentions in recent years because of the nutritional and health protective value of these components. Pumpkin is a traditional vegetable in many countries and is believed to have health benefits. In the past decades, many researches have focused on scientific evaluation of the characterization of pumpkin principal nutritional components and preparation as food or medicine functional components. Pumpkin extracts used for antidiabetic, antihypertensive, antitumor, immunomodulation, antibacterial, antihypercholesterolemia, intestinal antiparasitia, antiinflammation, and antalgic purposes have been reported. Pumpkin has tremendous potential as a raw material in the manufacture of natural health foods, but the contribution of pumpkin to minerals, vitamins and proteins in human nutrition is limited due to the presence of antinutrients. Although technologies such as germination and fermentation could be used to improve pumpkin's nutritional value and reduce these anti-nutritional substances, it is still important to identify and analyze the effect of functional components and antinutrients in pumpkin for further development of pumpkin-based health foods. This review will focus on the characterization of principal nutritional components and utilization areas of pumpkin, and point out the areas of future research to elucidate mechanisms whereby these compounds can reduce disease risk.

Keywords: *Cucurbita*, health food, nutritive composition, polysaccharides, technology

Abbreviations: body wt., body weight; **dm**, dry mass; **PP**, pumpkin polysaccharide; **PSO**, pumpkin seed oil

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INTRODUCTION

Pumpkin is an important horticultural crop that belongs to the family *Cucurbitaceae*. It is comprised of five domesticated species: *Cucurbita moschata* Duchesne ex Poiret, *C. pepo* L., *C. maxima* Duchesne, *C. mixta pangalo*, and *C. micifolia*. Three of these, *C. moschata C. pepo* and *C. maxima* represent economically important varieties cultivated worldwide and with high production (Whitaker *et al*. 1962; Robinson *et al*. 1997; Taylor *et al*. 2002; Zhao *et al*. 2004). Some researchers have provided a comprehensive introduction of fruit of both wild and domesticated forms of *Cucurbita*. A critical review on physiological aspects of productivity and quality in squash and pumpkins, seed production and germinability have been written (Jacks *et al*. 1972; Paris 1989; Brent Loy 2004; Nerson 2007).

Pumpkin is widely cultivated in temperate and subtropical zones of the world for edible and therapeutic purposes. It has been used traditionally as a vegetable as well as medicine in many countries, such as Yugoslavia, Mexico, India, Argentina, Brazil, America and China. In Mexico, *Cucurbita ficifolia* is consumed widely and several dishes and candies are prepared with the seeds or fruit (Andrade-Cetto and Heinrich 2005). *C. ficifolia* extracts are commonly used to treat diabetes mellitus. *C. moschata* is preferred by local consumers in Argentina and for its versatility, it is used for developing salty or sweet food products (de Escalada Pla *et al*. 2007). Pumpkin is considered by Traditional Chinese Medicine as being immensely valuable for human health. In the Compendium of the *Materia Medica*, a classical work in the history of Chinese science and technology development, "Buzhong Yiqi, Runfei Huatan", which means "it will do

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much good to our liver and lungs", is recorded for pumpkin (Popovic 1971; Jia *et al*. 2003). Over the past several decades, researchers have focused on the scientific evaluation of nutritional components in pumpkin, which has been frequently used as a functional food or exploited in medicine by several epidemiological studies (Ang-Lee *et al*. 2001; Marcus and Grollman 2002; de Smet 2002; Grover and Yadav 2004). Considerable evidence concerning pharmacological actions of pumpkin has accumulated through a number of animal models, cell culture studies and clinical trials.

Currently, therapeutic patterns of chronic disease have been converted from pharmaceutical therapy to treatment emphasizing prophylactics and reasonable nutritional supplements. Exploiting natural materials to extract active components, which can improve health condition of human bodies, has been highlighted by scientific evaluation.

The emphasis of this review is on the main nutritional components and medicinal properties of pumpkin, and to point out areas for future research to elucidate mechanisms whereby these compounds can reduce disease risk.

CHARACTERISTIC OF PRINCIPAL NUTRITIONAL COMPONENTS IN PUMPKIN

Pumpkin fruits consist mainly of pulp and seeds. Many nutritional components including pumpkin polysaccharides, carotenoids, minerals, amino acids and active proteins are found in pumpkin pulp (Buchbauer *et al*. 1998; Matsui *et al*. 1998; Appendino *et al*. 1999; Kuhlmann *et al*. 1999). Pumpkin seeds are a high-energy source as their embryo dry material is 40-50% lipids (Robinson 1975; Lazos 1986; Winkler 2000) and 30-37% proteins (Robinson 1975; Teppner 2000). In addition, pumpkin seeds are a good source of the elements K, P, Mg and Zn (Akwaowo *et al*. 2000; Seo *et al*. 2005). Besides pumpkin fruits, the remainder of pumpkin processing, or part of it, may be converted into nutrients for either food or feed, or into fertilizer.

It has been demonstrated that there are huge differences in nutritional components among pumpkins cultivated in various areas and of different species. Research aimed at determining the nutritional value of 17 pumpkin varieties in Lithuania showed a positive correlation between β-carotene and pumpkin diameter as well as between vitamin C and dry matter content (Danilcenko and Genyte 1999). Another study found that vitamin E content in pumpkin seeds of different species (*C. pepo* L.) were alterable (Murkovic *et al*. 1996).

Pumpkin polysaccharides

The main nutritional characteristic of *C. moschata* (**Fig. 1A**) pulp is that its dry material is abundant in polysaccharides, which include soluble polysaccharides and insoluble dietary fiber (Xiong 2000; Zhang and Yao 2002b, 2002c).

Many studies proved that *C. moschata* polysaccharides (PP) have an obvious effect on reduction of serum glucose. Hypoglycemic activity of water-extracted PP (6.88 g kg^{-1} body wt., twice daily for 3 weeks) were demonstrated and were superior to glibenclamide in alloxan®-induced diabetic rats (P<0.01) (Xiong 1998; Zhang *et al*. 2001; Zuo *et al*. 2001; Peng 2002; Zhang *et al*. 2004). Anti-hyperglycemic activity of water-extracted PP (3.8 g kg^{-1} body wt., twice daily for 3 weeks) was observed in normal rats (Xiong 2000). The antihyperglycemic effects of *C. ficifolia* fruit extract were investigated on streptozotocin®-induced experimental diabetes in rats. Oral administration of the extract (300 and 600 mg kg^{-1} body wt. per day) for 30 days resulted in a significant reduction in blood glucose, glycosylated haemoglobin, and an increase in plasma insulin and total haemoglobin. The effect was compared with 150 mg kg^{-1} body wt. tolbutamide® (Xia and Wang 2006). Pumpkin polysaccharides can increase the weight, decrease the blood glucose and lipids levels in diabetic rats, and have some good effects on diabetes and diabetic complications (Zhang and Yao 2002a; Liu *et al*. 2006). Crude polysaccharide from pumpkin fruit was reported to reduce the amount of branched chain amino acids and have better pharmacological effect on normal rats than on alloxan®-induced diabetic rats (Kong and Jiang 2002).

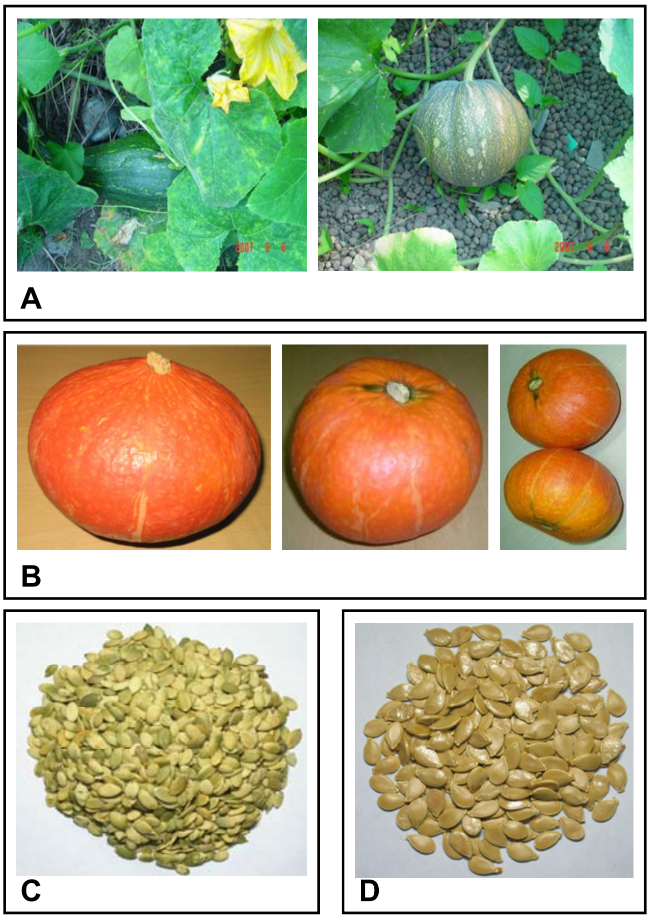

Fig. 1 Pumpkin. (**A**) *Cucurbita moschata* Duchesne in flowering (left) and fruiting (right) stages (Source: Institute of Botany, Chinese Academy of Sciences, Beijing); (**B**) Yellowflowergourd (*Cucurbita pepo* var. ovifera) (Source: a local plantation of Changping, Beijing); (**C**) Hull-less seeds of *C. moschata* (Source: a local plantation of Gansu, China). (**D**) Seeds of yellowflowergourd [*Cucurbita pepo* var. *ovifera*] (Source: a local plantation of Changping, Beijing).

In a clinical trial, pumpkin polysaccharide granules and pumpkin polysaccharide liquid administered orally all caused a significant reduction of post-prandial serum glucose and fasting glucose in NIDDM (non insulin dependent diabetes mellitus) subjects (Xiong 2001; Shi 2003; Lv *et al*. 2004). A daily supplement containing 30 g pumpkin powder significantly (P<0.01) reduced blood glucose concentrations in NIDDM patients (Chen *et al*. 1994).

The protein-bound polysaccharide from pumpkin fruits (PBPP) (**Fig. 1B**), which contains approximately 41.2% polysaccharides and approximately 10.13% protein can also distinctly increase the levels of serum insulin, reduce blood glucose levels and improve tolerance to glucose. A big PBPP dose group (1000 mg kg^{-1} body wt.) was superior to that of a small PBPP dose group (500 mg kg^{-1} body wt.) and glibenclamide® group. The average molecular weight was calculated to be 9.19×10^{6} Da and mannose and arabinose were present in a molar ratio of 1:2. Eighteen amino acids were identified to be components of the polymer. Alanine was the main amino acid (0.13%), followed by glutamic acid (0.113%) and serine (0.088%). From our previous studies, we presumed that pentose plays an important part in the hypoglycemic effect of pumpkin polysaccharides (Li *et al*. 2005; Fu *et al*. 2007).

The antitumour effects and immunocompetence of polysaccharide isolated from pumpkin have also been investigated. Mice were transplanted with S-180 or Ehrlich's ascetic (EAC). Drugs were given daily by oral administration. The dosage of pumpkin polysaccharide was 0.4 mL/day (10 mg/mL) for seven days. The inhibition rate of pumpkin polysaccharide for mice S-180 and EAC were 37.3% and 33.3%, respectively and the thymus rate was increased by 8.3% (Xu *et al*. 2000).

Pectin is a complex polysaccharide being a polymolecule, polydisperse and heterogenic due to its complicated chemical composition and structure (Gulfi *et al*. 2005). Pectin as an important structural constituent of the plant cell wall is widely used in the food industry as a gelling, thickening and stabilizing agent (King 1993). Pumpkin peels, which are discarded as agricultural byproducts after pumpkin processing, are rich in pectin. The process of fiber retrieval from *C. moschata* rendered, in a first step, for each 100 g of pumpkin, approximately, 38.0 g of juice, 38.2 g of pressed pulp, 16.0 g of peel and 4.4 g of seeds. In the second step of the process, 7 g of pectic substances are obtained from 100 g of peel (de Escalada Pla *et al*. 2007). The alcohol insoluble polysaccharide was extracted from *C. moschata* peels, and they were subsequently fractionated into three fractions: water-soluble, EDTA-soluble and alkali-soluble pectic substances. The three fractions possessed glucose- and bile acid-retardation effects and growth-promoting activities on good intestinal bacteria, which make pumpkin peel an attractive material for the development for functional foods (Jun *et al*. 2006). The pressed pulp remaining after the extraction of juice from pumpkins also seems a promising source of pectin (Shkodina *et al*. 1998).

It has been reported that pumpkin pectin and hydrolyzing pumpkin have remarkable effect on lowering cholesterin in blood plasma and improving metabolization of cholesterin of rats. Male Sprague-Dawley rats were fed a control diet (control group) and a diet containing 5% pectin from pumpkin for 45 days. Compared to the control group, serum cholesterol levels were significantly decreased by the pectin. Triacylglycerols were notablely reduced in liver, and fatty acids were obviously decreased in blood (Silveira and Alvarez Leite 1996; Kumar and Sudheesh 1997). Pumpkin pectin decreases the rate of fat assimilation, and at the same time causes quick dissimilation of fat. Pumpkin strongly controls 5-oxidationase of fat, so it can improve the condition of blood (Sekiya 1997). In addition, hypolipidemic activity was observed after feeding pumpkin polysaccharides to normal and diabetic mice (Koike *et al*. 2005).

In order to develop the use of pumpkin pulp residue after isolating soluble polysaccharides, xyloglucans were extracted by an alkaline method (Li *et al*. 2001, 2005; Fu *et al*. 2007).

Carotenoids

Carotenoids are a major source of vitamin A (V_A), which is necessary for normal eyesight, growth, and embryonic development. V_A deficiency is a common cause of blindness and infant mortality (Arima and Rodriguez-Amaya 1990; Rodriguez-Amaya 1999; Gonzalez *et al*. 2001; Murkovic *et al*. 2002). Pumpkin is an excellent source of pro-V_A carotenoids. The yellow to orange colour of the pumpkin flesh arises from carotenoids. The visual characterization of the colour correlates well with the carotenoid content. Varieties with a high content of carotenes have an orange appearance and varieties with a high lutein content and a low carotene content show a bright yellow colour. The major carotenoid in pumpkin is β-carotene, with small amounts of α-carotene, lutein, lycopene, and trace amounts of cryptoxanthin and *cis*-β-carotene. The varieties derived from three species, i.e. *C. pepo*, *C. maxima*, *C. moschata* and a cross breed of *C. maxima* and *C. moschata* have been analysed. The content of the carotenoids ranges from 0.06 to 7.4 mg/100 g for β-carotene, from 0 to 7.5 mg/100 g for α-carotene and from 0 to 17 mg/100 g for lutein. In detail, the content of α-carotene ranges from 0.03 to 0.17 mg/100 g for *C. pepo*, from 0 to 7.5 mg/100 g for *C. maxima*, from 0.98 to 5.9 mg/100 g for *C. moschata* and 2.4 mg/100 g for the cross breed of *C. maxima* and *C. moschata*. The content of β-carotene ranges from 0.06 to 2.3 mg/100 g for *C. pepo*, from 1.4 to 7.4 mg/100 g for *C. maxima*, from 3.1 to 7.0 mg/100 g for *C. moschata* and 3.5 mg/100 g for the cross breed. The content of lutein ranges from 0 to 1.8 mg/100 g for *C. pepo*, from 0.8 to 17 mg/100 g for *C. maxima*, from 0.08 to 1.1 mg/100 g for *C. moschata* and 12 mg/100 g for the cross breed (Murkovic *et al*. 2002).

A study incorporating 1902 men 40-60 years old in Japan showed that a pumpkin-rich diet could reverse the decrease in serum β-carotene levels caused by cigarette smoking and alcohol consumption (Fukao and Tsubono 1996). From 1985 to 1995, carotenoids from pumpkin were used for exploiting many kinds of medications, feedstuff and health foods in Austria (Franz and Jager 1997). Research on nutritional components in pumpkin indicated that pumpkin can be a primary vegetable to satisfy children's need for carotenoids (Suzuki *et al*. 2000). Infants lacking V_A and proteins benefited from milk powder by adding pumpkin powder to it (Fernandez and Guerra 1998). Along with aging and increase in tumour incidence, it is practical to develop pumpkin products rich in carotenoids (Suzuki *et al*. 2000; Xu *et al*. 2000; Dang 2004).

Minerals

Pumpkin is an eminent source of many minerals important for human health. *C. moschata* pulp contains high calcium (Ca) (205.45 μg/g) and potassium (K) (1840.30 μg/g) as well as low sodium (Na) (28.70 μg/g), which makes pumpkin a suitable food for middle-aged and aged people. It is also good for preventing osteoporosis and hypertension (Fan and Li 2005).

Pumpkin is superior to any other vegetable in chrome (Cr) content. Cr is a component of glucose tolerance factor (GTF), which is essential to advance the activity of insulin and accelerate the oxidation of blood glucose and thus improve blood glucose tolerance. Hypertensive diabetics are commonly deficient in Cr, which decreases insulin secretion and leads to a high level of blood glucose (Huang *et al*. 2005). The relationship between the relative Cr content in pumpkin and blood glucose level should also be further explored. The fruit and thin-husked seeds of *C. pepo* grown in Slovenia were analysed for selenium (Se) content. Se was determined based on hydride generation atomic fluorescence spectrometry. Results showed that pumpkin seeds (after lyophilizing) contained 108 ng g^{-1} of Se and Se content in lyophilized pumpkin fruit was 15 ng g^{-1} (Stibilj *et al*. 2004). *C. pepo* seeds were found to contain 0.005-0.013 mg kg^{-1} iodine (I), and the content in pumpkin seeds oil was 0.002-0.003 mg kg^{-1} of I. Thus, pumpkin seeds and oil from seeds may be a trace element supplement for areas deficient in I and Se (Kreft *et al*. 2002).

Cobalt (Co) is also an essential microelement in pumpkin which can improve the body's metabolic capacity, promote hematopoiesis and participate in the synthetic action of V_{B12}. Co is a necessary microelement for islet cells, a lack of which can induce diabetes. So it is necessary to balance insulin secretion of diabetic rats and hypoglycemic action. The relationship between hypoglycemic activity and Co content in pumpkin should be further investigated (Yang 2004).

Minerals in fluted pumpkin (*Telfairia occidentalis* Hook f.) were determined using an atomic absorption spectrophotometer (Akwaowo *et al*. 2000). Analyses were carried out at 12 and 50 weeks after planting (WAP) on stems, leaves and roots, while seeds were analysed at 8 and 32 weeks, respectively, after anthesis (WAA). Elemental composition generally increased with age, and antinutrients also increased with age in the stems, roots and seeds. In older stems (50 WAP), magnesium (50.5 mg/100 g) and calcium (40.5 mg/100 g) were higher than in younger ones (12 WAP). Phosphorus (10.6 mg/100 g) and zinc (6.80 mg/100 g) were higher in older leaves (50 WAP) than in younger ones (12 WAP), which contained higher magnesium (8.69 m/100 g) and iron (3.60 mg/100 g). Young leaves, which are often preferred for human consumption, were higher in cyanide (60.1 mg/100 g dm) and tannin content (40.6 mg/ 100 g dm) than older ones. Hence, young leaves should be properly cooked in order to remove antinutrient effects before consumption. At the same time, the oxalate (10.0 mg/100 g dm) and phytate (48.8 mg/100 g dm) contents were higher in the older leaves than in the younger ones. Older seeds (32 WAA) were richer in phosphorus (954 mg/ 100 g), potassium (632 mg/100 g) and iron (9.82 mg/100 g) than younger ones (8 WAA). Since younger pumpkin seeds contain less antinutrients than older ones, they may be nutritionally preferred for consumption. But due to high potassium (632 mg/ 100 g dm), iron (9.82 mg/ 100 g dm) and crude fat (56.24%) content, mature pumpkin seeds may be more appropriate to develop vegetable oil. Older roots (50 WAP) had higher potassium (883 mg/100 g), calcium (150 mg/100 g) and magnesium (103 mg/100 g) than their younger counterparts (12 WAP). Young roots, however, had higher levels of iron (24 mg/100 g) and copper 2.24 mg/100 g). Old fluted pumpkin roots had very high levels of antinutrients: oxalate (2600 mg/100 g dm), cyanides (84.2 mg/ 100 g dm), tannins (60.1 mg/100 g dm) and phytates (84.4 mg/100g dm) and may constitute potent human poisons. K, P, Magnesium (Mg), Zn and Ca distributions are relatively very high in the matured pumpkin seeds. P and Zn play crucial roles in actively metabolizing cells, particularly in relation to energy metabolism; photosynthesizing leaves play key roles in the energy cycle. Mg and iron are components of chlorophyll. It appears that minerals may be stored in mature fluted pumpkin seeds readying for the growth of new plants (Akwaowo *et al*. 2000).

Amino acids and active proteins

Although the protein content of pumpkin is less than 2.0% of dry matter weight, there are various essential amino acids in pumpkin pulp, e.g. the percentages of lysine (0.508%), valine (0.609%), leucine (0.700%), isoleucine (0.493%), phenylalanine (0.483%) and threonine (0.381%) are high in *C. moschata* (Zhang *et al*. 2003). Many immunocompetent proteins have also been found in pumpkin (Kato and Esaka 1996).

Antifungal PR-5 protein (28 KDa) isolated and purified from pumpkin leaves can rapidly lyse hyphal tips of *Neurospora crassa* at a concentration of >200 nM, and significantly inhibit the growth of *Fusarium oxysporum* and *Candida albicans* on agar-disc plates at >2 mM (Cheong *et al*. 1997). Another study showed that increasing the dose of sterilized pumpkin juice additives was associated with a reduction in the number of viable cells of lactic acid bacteria (LAB) in the cultured milk products (Arkhipova and Krasnikova 1995). More attention has been paid to antifungal proteins from pumpkin for developing safe and innoxious natural preservatives (Krishnamoorthi *et al*. 1990; Xia *et al*. 2003; Xie *et al*. 2004).

Several preliminary studies (*in vitro* as well as *in vivo*) with crude pumpkin extract and its various purified fractions, including proteins and polysaccharides, have shown anticancer activity against melanoma, ehrlich ascites and leukaemia (Ito *et al*. 1986).

Interestingly, enzyme preparations of pumpkin have been found to possess antitumor potential (Omura *et al*. 1974).

Pigments and others

Xanthophylls are apparently the predominant pigment present in pumpkin, as shown by absorption around 415 nm. This is in contrast to α- and β-carotenes which predominate in soy bean and red palm oil (Allen *et al*. 1982). Pigments from pumpkin pulp are widely used as additives in food, medicine, and cosmetics for their stable coloration, extensive available sources and simple extraction technology.

There is a special substance called gamma-aminobutyric acid (GABA) in pumpkin. It is a ubiquitous, non-protein amino acid which is produced primarily by the α-decarboxylation of Glu catalyzed by the enzyme glutamate decarboxylase (GAD) (Satya and Nair 1990). It is well known that GABA functions in animals as a major inhibitory neurotransmitter (Krogsgaard-Larsen 1989). GABA is involved in the regulation of cardiovascular functions, such as blood pressure and heart rate, and plays a role in the sensations of pain and anxiety (Mody *et al*. 1994). The consumption of GABA foods such as pumpkin could depress the elevation of systolic blood pressure in spontaneously hypertensive rats (SHRs) (Zhang 2003).

The distribution of antinutrients in pumpkin and their effects on other nutrients have been documented (Oke 1966; Broadhurst and Jones 1978; Desphande and Cheryan 1985). The most common anti-nutritional factors in pumpkin are tannins, phytates, oxalates and cyanides, but their contents are not constant. Antinutrients increased with age in the stems, roots and seeds in fluted pumpkin (*Telfairia occidentalis* Hook f.). Young leaves, which are often preferred for human consumption, were higher in cyanide (60.1 mg/ 100 g dm) and tannin content (40.6 mg/100 g dm) than older ones. Oxalate content (10.0 mg/100 g dm) and phytate content (48.8 mg/100 g dm) were higher in the older leaves than the younger ones. Some of the antinutrients in the leaves were above safety limits for human consumption. Old fluted pumpkin roots had very high levels of antinutrients, such as oxalate (2600 mg/100 g dm), cyanides (84.2 mg/100 g dm), tannins (60.1 mg/100 g dm) and phytates (84.4 mg/ 100 g dm), which may constitute potent human poisons (Akwaowo *et al*. 2000).

FUNCTIONAL COMPONENTS AND CHARACTERISTICS OF PUMPKIN SEEDS

Different varieties of pumpkins were developed considering the specific utilization of their pulp and seeds. Generally, more specialized varieties for developing pumpkin seeds are being applied. Lipids and proteins are the main nutritional components in pumpkin seeds. A cooked porridge blended with ground pumpkin seeds, which was provided to infants 4-6 months old in Kenya, resulted in a 82.5% increase in protein digestibility. All of the energy, protein, fat and carbohydrate supplied by the mix reached the recommended daily allowances. Sufficient unsaturated fatty acids from the mix can prevent eczema and enhance resistance to hypersusceptibility (Ward and Ainsworth 1998).

Pumpkin seeds contain useful amounts of linoleic and the following elements (on a μg per g dry weight basis): potassium (5,790), magnesium (5,690), manganese (49.3), zinc (113), selenium (1.29), copper (15.4), chromium (2.84), and molybdenum (0.81), but low amounts of calcium and iron. In conclusion, pumpkin seeds represent a useful source of many nutrients essential to humans (Glew *et al*. 2006).

Lipids

The lipid content is 40-50% of the dry weight of pumpkin seeds, and the percentage of the lipophilic extract that was composed of saturated fatty acids (palmitic and stearic acids combined) was 18.6-21.7% in *C. pepo*, 24.7-30.2% in *C. maxima*, 21.6-28.0% in *C. argyrosperma*, and 17.8-25.1% in *C. moschata* (**Fig. 1C**). Oleic acid constituted 21.0-36.7% of the total lipophilic extract weight in *C. pepo*, 18.3-29.4% in *C. maxima*, 20.1-33.7% in C. argyrosperma, and 13.5-25.8% in *C. moschata*; linoleic acid constituted 40.4-57.2% of the total lipophilic extract weight in *C. pepo*, 43.1-50.3% in *C. maxima*, 35.7-47.7% in *C. argyrosperma*, and 53.0-59.0% in *C. moschata* (Applequist *et al*. 2006). Pumpkin seed oil (PSO) provides a rich source of dietary polyunsaturated fatty acids (PUFAs), with mostly lipophilic antioxidants. PUFAs in pumpkin may enhance blood high-density lipoprotein, then regulate metabolization of serum lipids. Due to the abundance of PUFAs and other natural active substances in PSO, it is practical to use pumpkin seed oil to produce sap green salad oil.

PSO is a natural product commonly used in folk medicine for treatment of hypertension atherosclerosis and prostatic hypertrophy. It is rich in many antioxidants and beneficial nutritional supplements such as essential fatty acids, β-carotenes, lutein, γ and β-tocopherols, phytosterols and selenium (Zambo 1988).

PSO has a good effect on therapy and prevention of prostatic hypertrophy, whose etiology has not yet been completely explained, but different factors play a major role in its occurrence, among them, the sexual hormones (with a fundamental role of 5α-reductase). The 5α-reductase activity and inflammatory aspects in the prostate tissue can be effectively controlled with the use of highly standardized plant extracts. There is an active biologic activator – male sexual hormones – in PSO, which can eliminate earlier swelling of the prostate and prevent prostate cancer. The therapeutic use and safety of PSO were investigated in a clinical study with 2245 patients suffering from benign prostatic hyperplasia. Evaluation of the study was accomplished according to the International Prostate Symptom Score, which decreased by 41.4%, while live quality improved by 46.1% during the treatment (Bombardelli and Morazzoni 1997).

PSO as free radical scavenger helps to cure rats in which arthritis was induced using Freund's complete adjuvant. Upon chronic administration (100 mg kg^{-1} body wt. per day) for 29 days, PSO caused a remarkable elevation of serum sulphydryl groups, blood glutathione and plasma albumin levels which have been reduced during arthritis. It also reduced the levels of glucose-6-phosphate dehydrogenase activity in liver almost to 50% of the arthritic level, and produced a marked inhibition of paw oedema during the chronic phase by about 44% as compared with untreated arthritic group (Fahim *et al*. 1995).

Treatment of SHRs with felodipine (FEL) (one of the most vascular selective calcium antagonists which has antioxidant and antiplatelet activities in addition to a blood pressure lowering effect) or captopril (CPT) (as angiotensin converting enzyme inhibitor (ACE-inhibitor)) monotherapy or combined with PSO decreased the free radical scavengers in the heart and kidney. Co-administration of FEL (0.45 mg kg^{-1} body wt.) or CPT (9 mg kg^{-1} body wt.) with PSO (40 mg kg^{-1} body wt.) once daily for 4 weeks to SHRs led to beneficial effects on their free radical scavenging properties. It produced a significant effect of FEL and CPT actions on blood pressure as well as improving the biochemical parameters that were altered during hypertension (Zuhair *et al*. 2000). Another study suggested that PSO was effective against hypercholesterolemia. Administration of simvastatin® (one of 3-hydroxy-3-methyl glutaryl Coenzyme A reductase inhibitor, as antihypercholesterolemic drug) twice daily at oral dose of 1.86 mg kg^{-1} body wt. alone produced a significant increase in the activities of alanine aminotransferase (ALAT) (26%) and aspartate aminotransferase (ASAT) (32%) as well as creatine phosphokinase (CPK) (60%) while the combined therapy with PSO (daily at a dose of 40 mg kg^{-1} body wt.) ameliorated these effects (Zuhair *et al*. 1997).

In addition, the nutritive value of germination was studied for pumpkin seeds, and the results showed that there were slight decreases in the content of dry materials, protein and oil during germination. However, germinated pumpkin seeds contained a large quantity of linolenic acid, especially those germinated for 60 h and at 0.4 cm germ (possibly bud) length, and the biological value of lipids from germinated pumpkin, assessed with the ratio of PUFAs to saturated fatty acids was high. The content of linolenic acid obviously increased when pumpkin seeds were germinated in the cold or harvested late (Kostic and Lalic 1995).

In our research, we found that the oil from ungerminated pumpkin seeds (**Fig. 1D**) possessed hypoglycemic activity and improved blood glucose tolerance (Li *et al*. 2001).

Amino acids and active proteins

Analysis carried out to determine the seed protein components of fluted pumpkin (*T. occidentalis*) showed that there were enriched crude proteins (26.6%) and fat (47.5%). Albumin and globulin are the major proteins of fluted pumpkin seed and they constitute about 59% of the total protein (Giami and Barber 2004). Fluted pumpkin seed flour (the pumpkin seeds were dried and ground to a fine powder) is a good protein ingredient as it has a low gelation capacity of 14% (w/v), foaming capacity 12.8 ± 1.1% (Akintayo 1997).

Two proteins (designated α-moschin and β-moschin) with a molecular mass of 12 kDa and an N-terminal sequence rich in arginine and glutamate residues were found from fresh brown pumpkin (*C. moschata*) seeds. α- and β-moschins inhibited translation in the rabbit reticulocyte lysate system with an IC_{50} of 17 μM and 300 nM, respectively (Ng *et al*. 2002).

Three kinds of antifungal proteins (2249 Da, 4650 Da, 11696 Da) were obtained from pumpkin (*C. maxima*) seeds using chromatography and reverse-phase HPLC. The 11,696 Da component is a napin-like protein composed of a 3700 Da small chain and an 8004 Da large chain that are joined by two of the four disulphide linkages in the oxidised complex. Interestingly, the 11,696 Da napin-like protein is neither a substrate for Ca^{2+}-dependent protein kinase nor a calmodulin antagonist, which is different from many other basic antifungal proteins (Vassiliou and Neumann 1998).

A significant inhibitory effect of a purified protein (MW: 28000) from pumpkin seeds against the growth of the fungus, *Fusarium oxysporum* was exerted in an agar-disc plate at a concentration greater than 2 mM. It was shown that the MW 28000 possessed a synergistic effect with nikkomycin, a chitin synthase inhibitor, for the growth inhibition of *Candida albicans* (Cheong *et al*. 1997).

A peptide (MW: 8 kDa) purified from black pumpkin seeds, the N-terminal of which is abundant in arginine, glutamate and glycine residues, was proved to inhibit mycelial growth in fungi *Botrytis cinerea*, *Fusarium oxysporum* and *Mycosphaerella oxysporum* at a dose of 375 μg and inhibit translation in a cell-free rabbit reticulocyte lysate system with an IC_{50} of 1.2 μM (Wang and Ng 2003).

Additionally, were found to contain citrullin, a substance involved in the urea cycle. Thus pumpkin seed extracts can ward off insects and kill tapeworm in one hour. Study showed that ascarids can be prevented by feeding one limosis patient with ascariasis 50 to 100 g cooked pumpkin seeds in the morning (Youn 2004).

Studies revealed that the nutritional quality of fluted pumpkin seeds improved following a 5-day fermentation period. The unfermented fluted pumpkin seed contained crude protein (26.6%) and carbohydrate (13.6%). However, the crude protein content of fermented pumpkin seeds increased by 13.2% and the carbohydrate content decreased by 15.4% during a 7-day fermentation period. Albumin and globulin, which have been reported to have high levels of the amino acid lysine, are the primary seed proteins of fluted pumpkin seed. The albumin and globulin fractions increased during fermentation, reaching their maximum values (34.9% and 30.6% of total extractable protein, respectively) on the fifth day, but declined thereafter. Five days fermentation increased protein digestibility (from 58.2–78.2%) (in *vitro*) but decreased polyphenol (from 42.6-26.8 mg/100 g) and phytic acid (299-131 mg/100 g) contents of fluted pumpkin seeds. Fermentation for more than 5 days did not bring about visible effects. Germination can change the amino acid and carbohydrate constitution, then reduce blood glucose (Lee *et al*. 2001; Cai *et al*. 2003; Giami and Barber 2004). We also found that proteins from germinated pumpkin seeds have hypoglycemic activity. The protein components with molecular weight over 60 kDa and below 3 kDa after 4 days germination obviously increased the blood insulin level, and protein components of 3-60 kDa improved blood glucose tolerance. However, proteins from ungerminated pumpkin seeds did not possess hypoglycemic activity

(Li *et al*. 2001).

Vitamins

There is abundant vitamin K_1 (phylloquinone) in pumpkin seeds. Nutritional functions of sunflower oil, PSO, potatoes, eggs and milk were studied by feeding them to 298 healthy children and adolescents, 7 to 19 years old, 11 patients with cystic fibrosis 6 to 30 years old, 24 healthy adults 23 to 34 years old and 38 patients with diabetes 35 to 59 years old. Results showed that the effective component for cystic fibrosis and diabetes from the above five foods was V_{K1} (phylloquinone). PSO and sunflower oil had a high quantity of V_{K1} (Jakob and Elmadfa 1996). V_E is a primary lipid-dissolved vitamin from pumpkin (*C*. pepo) seeds, in which V_{E3} is 41-620 mg kg^{-1} dry material, 5-10 times that of V_{E1}. On the other hand, the contents of V_{E2} and V_{E4} are relatively lower (Hillebrand *et al*. 1996).

Pumpkin seeds contain relatively large amounts (0.02 ± 0.08%) of two triterpenes esterified with *para*-aminobenzoic acid (PABA), a folic acid constituent found for the first time in secondary plant metabolites. PAPB is a constituent of tetrahydrofolic acid and a versatile carrier of one-carbon fragments required for the synthesis of important primary metabolites like thymidylate, purines, and certain amino acids (Appendino *et al*. 1999).

CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

In pumpkin, there are varieties of health-protection components, and their function may be affected by some technologies (Omura *et al*. 1974; Ito *et al*. 1986; Krogsgaard-Larsen 1989; Satya and Nair 1990; Kong *et al*. 2000). The major process for extracting bioactive materials in pumpkin (mainly pumpkin pulp and seeds) have been summarized (**Fig. 2**). In order to gain higher yield and better function of bioactive components from pumpkin, further studies are needed to get a better understanding of the role of pumpkin extracts in protecting against disease.

Polysaccharides including protein-bound polysaccharides are primary bioactive materials in pumpkin. However, both of the structure property and relation between the structure and function are not clear. The correlation between amino acids contents and hypoglycemic activity of protein-bound polysaccharides still need further work to prove.

Pumpkin is rich in pectin. The pectic substance fractions from the alcohol insoluble polysaccharide of pumpkin peel possessed glucose and bile acid retardation effects as well as growth promoting activities on good intestinal bacteria. Pumpkin peel may be an attractive material for developing functional foods. Then compositional and structural properties of pectic polysaccharides from pumpkin peel could be of importance for the technological point of view. Many researchers have investigated compositions and physicochemical properties of pectins from various fruits and vegetables such as apple pomace, citrus fruits, grape peel, mango, soy hull, Japanese quince and plum. However, very little information is available concerning the physicochemical and physiological properties of pumpkin pectin. Moreover, the characteristic of pectin may be influenced by conditions of extraction as well as sources, location and many other environmental factors, so diverse extraction methods should be tried for the fractionation of pectin. In conclusion, more attentions should be paid to the composition, structure, physiological properties and extraction of pectin.

Pumpkin is a splendid source of carotenoids, especially β-carotene and other carotenoids that are good precursors of vitamin A. For better extraction efficiencies, methods of quantitatively extracting carotenoids from pumpkin should be developed. Because of harsh conditions, the official liquid–liquid extraction of carotenoids from dried vegetables might result in carotenoids degradation and not obtain com-

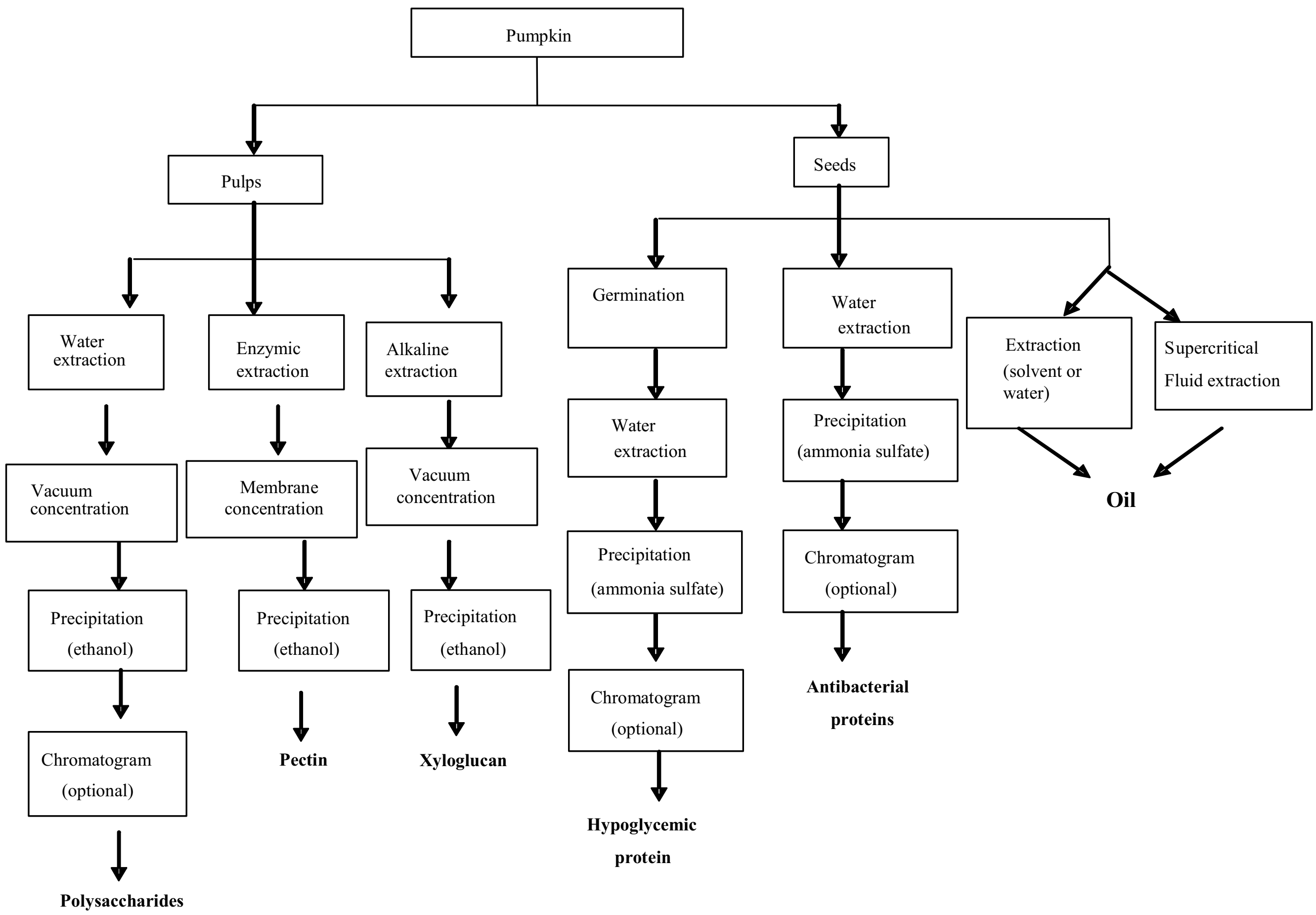

Fig. 2 Utilization of pumpkin bioactive materials.

plete recoveries. Supercritical fluid extraction is less toxic and less labor-intensive than liquid–liquid extractions, so it may be a better isolation technology of carotenoids in pumpkin.

Pumpkin seeds represent a useful source of many nutrients essential to humans. However, the usefulness of fluted pumpkin seeds as a protein source for human food is limited by the presence of antinutrients, which have been shown to have detrimental physiological effects on growing rats and chicks (Nwokolo and Sim 1987; Achinewhu and Isichei 1990). For reduction of antinutrients, technologies such as germination and fermentation are worth advocating. Although the functional, nutritional, microbiological and biochemical changes within pumpkin seed during fermentation have been examined, effect of fermentation on changes of the seed proteins, *in vitro* protein digestibilities, types and quantities of antinutrients in the seed is unknown. More attention should also be paid to new bio-activity of germinated pumpkin seeds.

Pumpkin seeds are fermented and used as a flavouring agent or protein supplement in a variety of local foods (Achinewhu 1987; Banigo and Akpapunam 1987). During fermentation, seeds are reported to undergo pronounced compositional changes as a result of proteolytic processes within the seeds, leading to degradation and alteration of storage proteins (Biehl *et al*. 1982). Compositional alterations, which occur during fermentation, including changes in protein fractions, such as albumin, globulin, prolamine and glutelin were found (El-Khalifa and El-Tinay 1994). Fermentation was observed to increase the crude protein content of fluted pumpkin seeds (Giami and Bekebain 1992). In a previous study, fermentation has been reported to improve the nutritive value of food legumes and cereals by decreasing the levels of antinutrients (Eka 1980; Dhankher and Chauhan 1987) and increasing protein digestibility (Chavan *et al*. 1988; Taylor and Taylor 2002). In China, people have used fermented pumpkin pulp to produce some traditional foods, such as sauce, vinegar, alcohol and preserved fruit (Kong and Li 2004).

Enzyme as special effective catalyst is helpful to extract the bioactive substances from plant. Enzyme-assisted extraction of pumpkin oil or protein is good method for research. Ultrasound treatment can significantly affect two types of physical phenomena: diffusion through the cell walls and washing out (rinsing) the cell contents once the walls are broken, involved in the extraction mechanism. For better industrialized utilization of pumpkin products, it is necessary to study ultrasound-assisted extraction of bioactive materials in pumpkin.

Synthesized utilization value of pumpkin is enormous. Its peel, pulp and seeds are all appropriate resources for multifarious foods. In China, pumpkin has been used for producing sauce, vinegar, alcohol, jam, beverage and preserved fruit. These categories of pumpkin foods have reached more than 20. However, most of them are produced by small-scale factories. Industrialization of functional foods of pumpkin is required to be developed. To benefit more people, we should do more deep research on pumpkin in the future.

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