Abstract

Introduction

Epidemiological studies have shown that fruits and vegetables consumption has many health benefits. [1, 2] The phytochemicals in fruits and vegetables helps in quenching free radicals and thus reduce the oxidative damage which can lead to several chronic degenerative diseases like heart diseases, neurodegenerative diseases, cancer and the ageing process. [2, 3] Population studies have revealed that the risk of cancer is reduced in individuals who eat 4-5 servings of fruits and vegetables daily.

Phytochemicals are grouped depending on their functions, structure, source of origin and cultivar. [4, 5] They include mainly polyphenolic compounds, vitamins, dietary fibre and carotenoids. The polyphenolic compounds are secondary metabolites of plants and act as inhibitor of lipid peroxidation, prevent DNA oxidative damage, scavenge free radicals, and prevent inhibition of cell communication, all of which are precursors to degenerative diseases. [6] Free radicals play an important role, both in health and disease. But, free radicals are useful only when they are produced in the right amount at the right place at the right time. Alterations to any of these parameters leading to free radical imbalance can lead to lipid peroxidation, cell death and genetic damage as a result of the extremely reactive nature of free radicals. [7] A variety of defense mechanisms do exist to quench potentially damaging free radicals, including enzymes like superoxide dismutase and glutathione peroxidase, micronutrients and excision and repair processes that remove free radical-induced damage. These defensive measures function as part of a complex system with significant interdependence and additive or synergistic effects. As antioxidant defenses are produced within the body and/or derived from the diet, efficient functioning of the antioxidant defense system is very much dependent on the optimal functioning of the body's metabolism and nutrition. Antioxidants terminate the free radical initiated chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants often act as reducing agents. Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from

lipid peroxidation. The antioxidants serve as physical barriers to prevent reactive oxygen species (ROS) generation or ROS access to important biological sites inside the body. They act as chemical traps that absorb energy and electrons and thus quench ROS (e.g. carotenoids, anthocyanidins). Some antioxidants act as metal chelators to prevent ROS generation (e.g. catechins) while, some are chain-breaking antioxidants which scavenge and destroy ROS (e.g. flavonoids, vitamin C, and vitamin E).

Fruits are commonly consumed raw; however, vegetables usually require to be processed before consuming. With the advent of industrialization and globalization, a rapid rise of processed food products market has occurred. Fruits and vegetables are increasingly being processed to make them available throughout the year. Processing methods can increase or decrease the health promoting properties of the fruits and vegetables.

Literature has cited that domestic cooking methods can result in significant changes in the composition and bioavailability of antioxidant compounds. [8] These changes could be both beneficial and detrimental depending on the extent and type of treatment conditions. Variety of effects like destruction, release and structural transformation of the phytochemicals take place during the cooking process. Cooking treatments like boiling, microwaving [8], baking, frying and griddling lead to changes in texture and nutritional properties of the vegetables. Studies have reported that cooking softens the cell walls which lead to increase in the extraction of carotenoids. [9] However, other studies have reported that cooking can also lead to loss in essential vitamins and antioxidants, mostly water soluble and heat labile compounds. The extent of loss is dependent on the type of cooking treatment [10] and the phytochemical composition of the cooked vegetables. The adverse effect of thermal processing in fruits, mainly during pasteurisation and spray drying has been reported for mulberry juice, durian juice and cashew apple juice on their bioactive components. [11-12] Overall, phytochemicals and their antioxidant properties in fruits and vegetables are dependent on a number of factors which includes variation among cultivars, environmental conditions, locations and agronomic factors, maturity stages and types of processing techniques. [13]

Residues from the processing of fruits and vegetables, considered to create an environmental problem, are being increasingly recognized as sources for recovery of valuable products. Efforts towards identifying alternative natural and safe sources of food antioxidants, especially of plant origin, have notably increased in recent years. [14-15] In this regard, the recovery of phenolic compounds from industrial wastes is gaining considerable attention, especially due to the antioxidant properties that these compounds exert. Studies have shown that the waste residues from fruits and vegetables after processing and the non-edible portions from various fruits (mainly seeds and peels) can be good sources of dietary fibres and antioxidants. [16]

Peels from various fruits have remarkably good polyphenol concentration. [17] Ajila et al. [18] studied the bioactive compounds and antioxidant potential in mango peel extract and reported that mango peel contains a number of valuable compounds such as polyphenols, carotenoids, enzymes and dietary fibre. Peel and pomace from lemon and apple contain good amount of polyphenolic compounds. [19] Studies have reported that citrus fruit peels and pomace exhibit good antioxidative, antihypertensive, antiobesity and antihyperglycemic properties.[20-21] Similarly, in avocado seeds, high phenolic content was reported. [22] Extracts of white and red grape pomace showed the presence of glycosylated flavonols such as quercetin and kaempferol. [23] Saura-Calixto et al. [24] described the association of polyphenolic compounds and carotenoids with the matrix of the dietary fibre. Pérez-Jiménez et al. [17] reported polyphenols with a concentration of 19740 mg/100 g dry weight in grape dietary fibre. The dietary fibre component has been proven to assist in regulation of blood glucose and lowering of serum cholesterol [25] and exert a number of protective effects on cardiovascular diseases, colorectal cancer and obesity. [26] In order to take advantage of the dietary and functional properties of fibre, some high dietary fibre formulated foods are currently being developed. [27-28]

A variety of fruits and vegetables are cultivated in Assam. The phytochemical content and antioxidant properties of the raw fruits and vegetables and the effects of processing on these properties have not been systematically studied. Further, the recovery of phenolics from processing wastes of fruits and vegetables of Assam and their composition have not been reported in literature. Pomace of *Averrhoa carambola* (also known as carambola/star fruit) is a byproduct obtained in large quantity after juice extraction. It is a good source of phytochemical compounds like phenolic acids and proanthocyanindins and has insoluble fibre-rich fraction (FRF) as the major fraction. [29] The fruit due to its good phytochemical content is used traditionally to cure many diseases. The carambola fruit is available in Assam and has immense potential for commercialization. Previous studies have reported that its fibre has desirable functional properties, *in vitro* hypoglycemic effects, and *in vivo* hypolipidemic and hypocholesterolemic effects. [30] This insoluble FRF could be a promising source of food fibre or low calorie bulk ingredient in functional food applications. However, the efficacy and functions of the fibre upon incorporation into a fruit beverage and subsequent *in vivo* study have not been reported.

The work reported in this thesis attempted to study the phytochemical and antioxidant content of fresh fruits and vegetables available in Assam, India and the effect of different processing methods on their phytochemical and antioxidant content. The pomace of carambola was used to extract polyphenol compounds and dietary fibre. The FRF from the pomace was incorporated in a mixture of fruit juices to develop a functional beverage powder and the quality and functional properties of the developed beverage powder were studied for health promoting properties *in vivo* using an animal model.

Objectives

1. To determine the phytochemical content and antioxidant capacities in selected fresh fruits and cooked vegetables of Assam.
2. To study the effect of pasteurisation and spray drying on the phytochemical content of selected fruits.
3. To optimize the phenolic extraction from *Averrhoa carambola* pomace and microencapsulate the phenolics by spray and freeze drying methods.
4. To study the *in vitro* physicochemical, phytochemical and functional properties of fibre rich fractions derived from by-products of selected fruits.
5. To develop a functional beverage powder fortified with carambola pomace fibre.
6. To study the effect of the functional beverage powder on the serum cholesterol and glucose levels and on the functions of liver enzymes.

Methodology of the present work

The methodology followed to accomplish the above mentioned objectives are given below.

Phytochemical content and antioxidant capacities in selected fresh fruits and cooked vegetables of Assam

- Thirteen fruit samples were collected in season from the local market, Tezpur. The fruits studied were *bogi jamun (Syzygium jambos* L.*), amla (Emblica officinalis*

Gaertn), Indian olive *(Elaeocarpus serratus* L.*), leteku_(Baccurea sapida* Muell. Arg)*,* carambola (*Averrhoa carambola* L.*), black jamun (Syzygium cumuni* L.Skeels.), watermelon (*Citrullus lanatus* var *lanatus*)*,* pineapple *(*Ananas comosus L. Merr*),* hog plum *(*Spondias pinnata L. Kurz*), pani jamun* or water apple (*Syzygium samarangense* (Blume) Merr. & Perry), Khasi mandarin orange (*Citrus reticulate* Blanco), *Poniol* or Coffee plum (*Flacourtia jangomas* (Lour.) Raeusch*)* and litchi (*Litchi chinensis* Sonn.).

- Fourteen vegetable samples were collected in season from local market, Tezpur. Vegetables included in the study were cauliflower (*Brassica oleracea* L. *var botrytis*), cabbage *(Brassica oleracea* L. *var captita)*, green pea (*Pisum sativa* L.), banana blossom (*Musa balbisiana* Colla, *ABB*), beetroot (*Beta vulgaris* L), teasel gourd *(Momordica dioica* Roxb.), black eyed pea (*Vigna unguiculata* subsp. *Unguiculata*), bottle gourd (*Lagenaria siceraria* (Molina) Standl.), tomato (*Solanum lycopersicum* L.*)*, carrot *(Daucus carota subsp. Sativus), kharua* brinjal (*Solanum melongena* L.*)*, radish (*Raphanus sativus* L.), knol-khol (*Brassica caulorapa L.) a*nd roselle leaves (*Hibiscus acetosella* Welw.).
- The acetone extract of the samples were analysed for total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant property (FRAP), DPPH radical scavenging activity (DPPH) and metal chelation capacity (MCC) by spectrophotometric methods.
- RP-HPLC studies of the extracts of selected fruit and vegetable samples were conducted.
- Processing treatments viz., steaming, boiling and microwaving at 600W power were given to the vegetables.
- Vegetable samples were extracted in acetone and analysed for total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant property (FRAP), DPPH radical scavenging activity (DPPH) and metal chelation capacity (MCC) by spectrophotometric methods.
- RP-HPLC studies of the extracts of selected vegetables having high phenolic content were conducted and changes in the phenolic acids after processing were determined.

Effect of pasteurisation on the phytochemical content of selected fruits

- Juice of pineapple, carambola, watermelon, black jamun and litchi were pasteurised. Pasteurisation methods included conventional method, microwaving (600W and 900W power) and ultrasound treatment.
- TPC, TFC, FRAP, DPPH were determined and RP-HPLC study of the treated fruit samples were conducted.
- Colour and microbial plate count of the pasteurised fruit juices were determined.

Effect of spray drying on the phytochemical content of selected fruits

- Selected fruit juice samples were spray dried with maltodextrin.
- Physicochemical and phytochemical properties of the spray dried fruit juice were studied.

Extraction from ***Averrhoa carambola*** **pomace and microencapsulation of phenolic compounds**

- Optimum conditions of phenolic extraction from carambola pomace were determined at different temperatures and solvent (ethanol) concentrations by applying central composite design (CCD) using response surface methodology (RSM). TPC, TFC, FRAP and DPPH were the response variables.
- The phenolic extract was studied for phenolic acid composition by RP-HPLC.
- The obtained encapsulates with maltodextrin were studied for physicochemical properties, and *in vitro* simulated gastric and intestinal release properties.

Extraction and characterization of the fibre from the fruit by-products

- Pomace of carambola (Averrhoa carambola) and pineapple (*Anona sativas*), peels of watermelon *(Citrullus lanatus),* Burmese grape (*Baccurea sapida*) *and* Khasi mandarin orange (*Citrus reticulate)*, and blossom of seeded banana (*Musa balbisiana, ABB*) were processed to obtain fibre.
- The fibre samples were subjected to hexane treatment for removal of crude lipid and pigments and further purified with ethanol.
- The obtained fibre samples were studied for physicochemical, phytochemical and functional properties.

Development of a functional mixed fruit juice powder fortified with carambola pomace fibre

- A mix of three fruit juices from pineapple: watermelon: carambola in 95:5:5 ratios, respectively, was made.
- The mix of fruit juices with added pomace fibre was homogenised and spray dried at different maltodextrin concentrations and temperature conditions to obtain optimised conditions by applying central composite design (CCD) using response surface methodology (RSM).
- The fibre fortified juice powder obtained with the optimised conditions was analysed for its proximate composition, color, pH, bulk density, moisture content, solubility, hygroscopicity and reconstitution property.
- The beverage was evaluated for sensory attributes on a 9 point Hedonic scale.

***In-vivo* studies on the effect of fibre-fortified mixed juice powder**

- Fibre-fortified mixed juice powder was fed to Sprague dawley rats. The rats were divided into three groups viz., control (given normal diet without test diet), groupI (test diet with refined wheat flour and vegetable fat) group II (test diet with refined flour), respectively. In the group I and group II rats, high serum cholesterol condition was induced before feeding them with the test diet. The rats were fed with their designated diets for 9 weeks. Blood samples were collected from the orbital sinus of the rat.
- The HDL, LDL and total triglycerides (TGL) levels, glucose lowering ability as well as the functions of two liver enzymes (SGOT and SGPT) were determined using specific enzyme kit and analyzed in an autoanalyser.
- At the end of the experimental period, the animals were sacrificed; heart and caecum were collected. The short chain fatty acid present in the caecal matter was analysed by GCMS method. The heart ventricle and aorta tissue were fixed, processed and studied for histopathological changes and compared.

The Thesis-

The thesis includes studies on the phytochemical and antioxidant properties of selected fresh fruits and vegetables of Assam, India. It also reported studies on the effect of cooking methods (steaming, boiling and microwave cooking) on the phytochemical and antioxidant properties of fourteen vegetables. The present work also determined the effect of different pasteurisation methods and spray drying on the total phenolic, flavonoid content and antioxidant properties of selected fruit juices. Optimisation of phenolic extraction from carambola pomace was carried out by response surface methodology (RSM) using a composite central design (CCD) and the extract was then microencapsulated and its physicochemical properties were studied. The dietary fibre obtained from by-products of six different fruits was studied for proximate content, phytochemical content, physicochemical and functional properties. From the studied fibre samples, one fibre source (carambola pomace) was added to a mix fruit juice system to develop a functional beverage powder. Optimisation by response surface methodology using central composite design was used to obtain the optimum conditions for the functional beverage powder development. The health promoting properties of the developed functional beverage powder were evaluated using a rat model.

The thesis is divided into ten chapters which are briefly discussed below

Chapter 1 includes the general introduction about the presence of phytochemicals in fruits and vegetables that act as natural antioxidants to quench the free radicals that are generated during the metabolic processes. Also, the effect of processing methods on the phytochemical content was mentioned. Further, the importance of the need to use fruits and vegetable by-products for extraction and production of polyphenol compounds, vitamins, and dietary fibre was discussed. The potential of the extracted bioactive compounds to be used in the development of functional food was also highlighted. It also gives the importance of conducting the present research in the light of reported gaps in available information.

Chapter 2 includes the prominent works reported on phytochemicals and antioxidant potential of some fruits, vegetables and their by products. The studies reported on the health promoting properties of dietary fibre. It also reviews works on the effect of different processing methods on the phytochemicals and antioxidants in processed fruits and vegetables. Some of the reviewed works were also taken as references for carrying out the experiments and analyzing the results obtained.

Chapter 3 includes the phytochemical content in thirteen fruit and fourteen vegetable samples commonly available in Assam. The TPC, TFC, FRAP, DPPH and MCC by spectrophotometric methods were determined. Different phenolic acids were identified in a few vegetables and all the fruit samples by RP-HPLC study. It also deals with the effect of steaming, boiling and microwave cooking on the phytochemical content and antioxidant properties of different vegetables of Assam. The fourteen vegetables selected for the study were first subjected to the processing steps and then analysed for TPC, TFC, FRAP, DPPH radical scavenging activity DPPH and MCC by spectrophotometric methods. Out of the above vegetable samples, banana blossom, roselle leaves, black eyed pea, teasel gourd and *kharua* brinjal which showed good antioxidant properties were then analysed for phenolic acids by RP-HPLC.

Chapter 4 includes the effect the spray dried fruit powders were studied for their physicochemical properties like moisture content, water activity, solubility, hygroscopicity, particle size distribution and colour. Samples were analysed for changes in TPC, TFC, FRAP and DPPH by spectrophotometric methods.

Chapter 5 includes the effect of different pasteurisation methods employed viz., conventional, microwave (600W and 900W power levels) and sonication treatments on the phytochemical and antioxidant activity of selected fruit samples. The variously pasteurised fruit juice samples were studied for total plate count and changes in color. The pasteurised fruit juice samples were analysed for changes in TPC, TFC, FRAP and DPPH by spectrophotometric methods. The phenolic acids present were determined by RP-HPLC.

Chapter 6 deals with optimisation of extraction of polyphenols from carambola pomace by response surface methodology and its microencapsulation by spray and freeze drying. Two independent variables viz., temperature and solvent (ethanol) concentration were taken at different combinations by applying central composite design using response surface methodology. The response variables were TPC, TFC and FRAP. The phenolic acids in the extract were determined by RP-HPLC. The obtained polyphenol extract was encapsulated with maltodextrin (≤20DE) by spray and freeze drying methods. A comparative study was carried out to determine TPC, surface phenolic content and encapsulating efficiency of encapsulates. Encapsulates were studied for moisture content, colour, solubility, bulk density and hygroscopicity. Also, the *in vitro* simulated gastric and intestinal digestion release properties of encapsulates were studied. Lastly, the surface morphology of the obtained encapsulates were determined by scanning electron microscopy.

Chapter 7 studied the physicochemical, phytochemical and functional properties of fibre rich fractions derived from by-products of six different fruits viz., pomace of carambola and pineapple, peels of watermelon, Burmese grape and Khasi mandarin, and blossom of seeded banana. The proximate composition of the samples was determined. The color and dietary fibre were estimated. Physicochemical properties viz., moisture content, bulk density, water holding capacity, oil holding capacity and swelling capacity of the obtained fibre were determined. The obtained fibre rich fraction samples were also studied for TPC, TFC, FRAP, DPPH and MCC by spectrophotometric methods. The phenolic acid composition was also determined using RP-HPLC. Lastly, the functional properties of the fibre samples viz., glucose adsorption capacity, alpha amylase activity inhibition rate, glucose diffusion rate and glucose diffusion rate index were studied.

Chapter 8 deals with the development of a beverage powder consisting of three fruit juices viz., carambola, pineapple and watermelon mixed together and fortified with fibre rich fractions derived from carambola pomace powder. The concentrations of maltodextrin and inlet temperature of spray drier were optimised using response surface methodology for spray drying of the juice fibre mixture. The response variables were moisture content, yield, hygroscopicity, and solubility and bulk density. Apart from these, the colour and proximate content were also determined. Also, the particle size distribution, flowability and surface morphology was studied. The sensory analysis was done by 9 point Hedonic rating test.

Chapter 9 studied the positive effect of the fibre fortified mix juice powder on the lowering of cholesterol and glucose levels as well as on the functions of two liver enzymes viz., serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) when given in diet to hypercholesterolemic Sprague dawley rat. Blood samples were studied for serum high density lipoprotein, low density lipoprotein, total cholesterol, total triglycerides, SGOT and SGPT. The short fatty acid content of the caecal matter of the two groups was determined by GCMS. Histopathological study of the heart ventricle and aorta tissue was carried out.

Chapter 10 presents the salient findings of the present investigation. It concludes that the studied fruits and vegetables significant amount of phytochemicals and showed antioxidant properties. Processing altered the phenolic content and antioxidant activities in vegetables due to variety of effects like destruction, release and transformation of the phytochemicals. The antioxidant activity of the selected vegetables was enhanced in most of the cases. In most cases, thermal treatment like pasteurisation had a positive effect on the phytochemical content of the fruit juices. Depending on the type of fruit sample and treatment, increase or decrease in phytochemical values was observed. HPLC study showed different phenolic acids depending on the sample type and also a few new phenolic acids were detected in some treated samples. Optimisation of conditions of extraction of phenolics from carambola pomace revealed that variation in ethanol concentration and temperature had both positive and adverse effects on TPC, FRAP and DPPH activity. However, their combined interaction had a positive effect on the antioxidant activities. The proposed extraction model of CCRD through RSM could be used for the polyphenols extraction from pomace of carambola and the extracted polyphenols can be used in different food models as such or in encapsulated form depending on the requirements of the end products. The carambola pomace was found to be a good source for phenolic compounds and dietary fibre. The extracted phenolic compounds from carambola pomace can be added to other complex food systems to develop health promoting food products. Out of the six fibre sources studied, the fibre obtained from the carambola pomace was selected for fortification in the mix fruit juice system. The carambola pomace fibre was comparatively better in retaining their polyphenol content and antioxidant properties as well as it exhibited good water- and oil-holding capacities. Apart from that, it also showed good functional properties of glucose adsorption and amylase inhibition properties. An optimised model was obtained for spray drying the fibre fortified mixed juice powder with maltodextrin. The developed functional beverage powder showed cholesterol and glucose lowering properties and had positive effects on SGOT and SGPT. The analysis of the caecal matter showed presence of short chain fatty acids which are desirable for maintaining colon health. The functional beverage powder obtained may have future scope in the functional foods segment.

Bibliography

1. McCann, S.E., Ambrosone, C.B., Moysich, K.B., Brasure, J., Marshall, J.R., Freudenheim, J.L., Wilkinson, G.S. and Graham, S. Intakes of selected nutrients, foods, and phytochemicals and prostate cancer risk in western New York. *Nutr. Cancer.* **53**(1), 33–41 (2005).
2. Hung, H.C., Joshipura, K.J., Jiang, R., Hu, F.B., Hunter, D., Smith-Warner, S.A., Colditz, G.A., Rosner, B., Spiegelman, D. and Willett, W.C. Fruit and Vegetable Intake and Risk of Major Chronic Disease. *J. Nat. Cancer Inst.* **96**(21), 1577-1584 (2004).
3. Prior, R.L. Fruits and vegetables in the prevention of cellular oxidative damage. *American J. Clinical Nutr.* **78**(3), 570S-578S (2003).
4. Sariburun, E., Sahin, S., Demir, C., Türkben, C. and Uylaser, V. Phenolic content and antioxidant activity of raspberry and blackberry cultivars. *J. Food Sci.* **75**(4), C328-C335 (2010).
5. Singh, J., Upadhyay, A.K., Prasad, K., Bahadur, A. and Rai, M. Variability of carotenes, vitamin C, E and phenolics in Brassica vegetables. *J. Food Comp. Anal.* **20**, 106–112 (2007).
6. Cao, Y.H. and Cao, R.H. Angiogenesis inhibited by drinking tea. *Nature.* **398**, 381(1999).
7. Kaur C. and Kapoor H.C. Antioxidants in fruits and vegetables – the millennium's health. *Int. J. Food Sci. & Tech.* **36**, 703–725 (2001).
8. Zhang, D., and Hamauzu, Y. Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. *Food Chem.* **88,** 503–509 (2004).
9. Rodriguez-Amaya, D.B. Changes in carotenoids during processing and storage of foods. *Arch Latinoamericanos de Nutri.* **49**, 38S–47S (1999).
10. Lin, Ch-H. and Chang, Ch-Y. Textural change and antioxidant properties of broccoli under different cooking treatments. *Food Chem.* **90**, 9–15 (2005).
11. Aramwit, P., Bang, N. and Srichana, T. The properties and stability of anthocyanins in mulberry fruits. *Food Res. Int.* **43**, 1093-1097 (2010).

12. Chin, S., Nazimal, S. A. H., Quek, S., Man, Y. B. C., Rahman, R. A. and Hashim, D. M. Effect of thermal processing and storage condition on the flavour stability of spray-dried durian powder. *LWT-Food Sci. Technol.* **43**, 856–861(2010).
13. Naczk, M. and Shahidi, F. Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *J. Pharm. Biomed. Anal.* **41**, 1523–1542 (2006).
14. Singh, R. P., Chidambara Murthy, K. N. and Jayaprakasha, G. K. Studies on the antioxidant activity of pomegranate (Punica granatum) peel and seed extracts using in vitro models. *J. Agri. Food Chem.* **50,** 81–86 (2002).
15. Negi, P. S., Jayaprakasha, G. K. and Jena, B. S. Antioxidant and antimutagenic activities of pomegranate peel extracts. *Food Chem.* **80,** 293–297 (2002).
16. Moure, A., Cruz, M. J., Franco, D., Dominguez, J. M., Sineiro, J., Nunez, M. J., and Parajo, J. C. Natural antioxidants from residual sources. *Food Chem.* **72**, 145–171 (2001).
17. Pérez-Jiménez, J., Arranz, S., Tabernero, M., Díaz-Rubio, M. E., Serrano, J., Goñi, I. Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. *Food Res. Int.* **41**, 272–285 (2008).
18. Ajila, C.M., Naidu, K.A., Bhat, S.G. and Rao, U.J.S.P. Bioactive compounds and antioxidant potential of mango peel extract. *Food Chem.* **105,** 982–988 (2007).
19. Schieber, A., Stintzing, F. C. and Carle, R. By-products of plant food processing as a source of functional compounds- recent developments. *Trends in Food Sci. Tech.* **12**(11), 401-413 (2001).
20. Kang, S. H., Shin, H. S., Kim, H. M., Hong, Y. S. and Yoon, S. A. Immature *Citrus sunki* peel extract exhibits anti-obesity effects by β-oxidation and lipolysis in high-fat diet-induced mice. *Biol. Pharm. Bull.* **35**, 223–230 (2012).
21. Ono, E., Inoue, J., Hashidume, T., Shimizu, M. and Sato, R. Anti-obesity and anti-hyperglycemic effects of the dietary citrus limonoid nomilin in mice fed a high-fat diet. *Biochem. Biophy. Res. Comm.* **410,** 677–681(2011).
22. Pahua-Ramos, M.E., Ortiz-Moreno, A., Chamorro-Cervallos, G., Hernàndez-Navarro, M.D., Garduño-Siciliano, L. and Necoechea-Mondragòn, H. Hypolipidemic effect of

avocado (*Persea americana* Mill) seed in a hypercholesterolemic mouse model. *Plant Foods Hum. Nutr.* **67** (1), 10−16 (2012).

23. Rubilar, M., Pinelo, M., Shene, C., Sineiro, J. and Nunez, M.J. Separation and HPLC-MS identification of phenolic antioxidants from agricultural residues: almond hulls and grape pomace. *J. Agric. Food Chem.* **55** (25), 10101–10109 (2007).
24. Saura-Calixto, F., Serrano, J. and Goni, I. Intake and bioaccessibility of total polyphenols in a whole diet. *Food Chem.* **101**(2), 492-501(2007).
25. Anderson, J. W., Smith, B. M. and Guftanson, N. S. Health benefit and practical aspects of high-fibre diets. *American J. Clinical Nutr.* **595**, 1242–1247 (1994).
26. Salmeron, J., Manson, J.A.E., Stampfer, M.J., Colditz , G.A., Wing, A.L. and Willet, W.C. Dietary fibre, glycaemic load and risk of non-insulin dependent diabetes mellitus in women. *J. American Med. Assoc.* **277**, 472-477 (1997).
27. Griguelmo-Miguel, N. and Martin-Belloso, O. Influence of fruit dietary fibre addition on physical and sensorial properties of strawberry jams. *J. Food Eng.* **41**, 13–21(1999).
28. Tudorica, C. M., Kuri, V. and Brennan, C. S. Nutritional and physicochemical characteristics of dietary fibre enriched pasta. *J. Agri. Food Chem.* **50**, 347–356 (2002).
29. Shui, G., Leong, L.P. Residue from star fruit as valuable source for functional food ingredients and antioxidant nutraceuticals. *Food Chem.* **97**, 277–284 (2006).
30. Chau, C.F., Chen, C.H. and Lee, M.H. Insoluble fibre-rich fractions derived from *Averrhoa carambola*: hypoglycemic effects determined by in vitro methods. *LWT-Food Sci. Technol.* **37**, 331–335 (2004).