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Effect of Irradiation and High Pressure Processing Technologies on the Bioactive Compounds and Antioxidant Capacities of Vegetables

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Abstract: Thermal treatment of food is done in the food industry for preservation purposes. Food preservation prevents deteriorative reactions, extends a food's shelf life and assures its safety. Thermal processing has most of the characteristics of an ideal food preservation method. However, in some foods the high thermotolerance of certain enzymes and microorganisms, mainly bacterial spores, entails the application of extreme heat treatments, which changes the nutritional, phytochemical and organoleptic food properties. Therefore, alternatives to thermal processing as the main means of inactivating pathogenic and spoilage microorganisms are being developed by researchers. The present review focuses on the effect of some non thermal processing technologies on the bioactive compounds and antioxidant activities of some vegetables.

Key words: Antioxidant activity, bioactive compounds, non-thermal processing, vegetables

INTRODUCTION

Over the last few years consumption of processed fruits and vegetables has increased and there is convincing epidemiological evidence that consumption of fruits and vegetables is in general beneficial to health (Duthie *et al*., 2000; Netzel *et al*., 2007). Some of our studies showed that fruits and vegetables are rich sources of phytochemical substances (Muchuweti *et al*., 2005, 2006, 2007; Chitindingu *et al*., 2007; Nhukarume *et al*., 2010). The potential health benefits of phytochemical substances have been related to the action of the compounds as antioxidants and inhibitors of lipid peroxidation (Li-Chen *et al*., 2005). Phytochemical compounds such as phenolics are strong antioxidants against free radicals and reactive oxygen species. Free radicals and reactive oxygen species have been implicated as the major cause of chronic human diseases such as cardiovascular diseases, diabetes and degenerative diseases (Kyung-Hee *et al*., 2005; Chen and Yen, 2007).

Conventional thermal processing of fruits and vegetables remains the most widely adopted technology for shelf-life extension and preservation of fruits and vegetables. However, consumer demand for nutritious foods, which are minimally and naturally processed, has led to interest in non thermal technologies. The effect of thermal treatment on the total phenolics contents and antioxidant activities of vegetables has been reviewed recently (Chipurura *et al*., 2010). Convectional cooking of vegetables was in general found

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to decrease the total phenolics contents and antioxidant activities of vegetables. Interestingly Tiwari *et al.* (2009) concluded that apart from ozone; high pressure processing, pulsed electric field, ultrasound, irradiation, dense phase carbon dioxide have minimal influence on anthocyanins content of fruit juices. Most studies on the effects of non thermal technologies were done on fruit juices. Hence, the present study focuses on the effects of some of these novel processing technologies on the phenolics contents and antioxidant activities of some vegetables.

ANTIOXIDANTS IN CELLULAR SYSTEMS AND FOODS

Antioxidants are defined as the substance that when present in low concentrations compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substance. For the *in vivo* situation the concept of antioxidants includes antioxidant enzymes, iron binding and transport proteins and other compounds affecting signal transduction and gene expression (Percival, 1996). In case of foods and beverages, antioxidants are related to the protection of specific oxidation substrates. Antioxidants are divided into two major classes, namely endogenous antioxidants and exogenous antioxidants.

Endogenous antioxidants are mainly enzymes and three groups of enzymes play important roles in protecting cells from oxidant stress (Becker *et al.*, 2004). Firstly, Superoxide Dismutases (SOD) are enzymes that catalyze the conversion of two superoxides to hydrogen peroxide and oxygen. Secondly, catalase, found in peroxisomes in eukaryotic cells, degrades hydrogen peroxide to water and oxygen and hence completes the detoxification reaction started by SOD. Finally, glutathione peroxidase, a group of enzymes which are the most abundant contain selenium and like catalase, degrade hydrogen peroxide. Glutathione peroxidase reduces organic peroxides to alcohols. In addition to the three enzymes, glutathione transferase, ceruloplasmin, hemoxygenase may participate in enzymatic control of oxygen radicals and their products.

Exogenous antioxidants are mainly obtained from the diet. The common exogenous antioxidants are vitamin E, vitamin C/ascorbic acid, carotenoids, glutathione and phenolics compounds. Vitamin E is the major lipid-soluble antioxidant and plays an important role in protecting membranes from oxidative damage. The primary activity vitamin E is to trap peroxy radicals in cellular membranes. Vitamin C is a water-soluble antioxidant that can reduce radicals from a variety of sources. Vitamin C participates in recycling vitamin E radicals. Vitamin C functions as a pro-oxidant under certain circumstances and sometimes produces oxygen by-products of metabolism that can cause damage to cells. To date carotenoids are known to act as antioxidants *in vitro*. In addition; to quenching of singlet oxygen, carotenoids may react with radical species either by additional reactions or through electron transfer reactions, which results in the formation of the carotenoid radical cation. Glutathione is the most important intracellular defense against damage by reactive oxygen species. The cysteine on the glutathione molecule provides an exposed free sulphydryl group that is very reactive, providing an abundant target for radical attack. Reaction with radicals oxidizes glutathione but the reduced form is regenerated in a redox cycle that involves glutathione reductase and the electron acceptor NADPH membranes (Percival, 1996).

In addition to vitamin E and vitamin C, phenolic compounds can function as antioxidants. The antioxidant properties of some plant extracts have been attributed partially to their phenolic compound contents (Coinu *et al.*, 2007). There are many classes of phenolic compounds, with phenolic acids, flavonoids and tannins as the main dietary phenolics.

Phenolic acids include hydroxybenzoic and hydroxycinnamic acids. Flavonoids are a diverse group of secondary plant metabolites that include flavonols, flavanols, flavanones and flavones. The major classes of tannins in the plant kingdom are hydrolysable and condensed tannins.

ANALYTICAL METHODS FOR TOTAL PHENOLICS CONTENT AND ANTIOXIDANT ACTIVITY

One of the common methods that have been developed for quantification of phenolic compounds is the Folin Ciocalteu (Folin C.) method, which is based on the reduction of phosphomolybdic acid in acid by phenols in aqueous alkali. The method is used to determine the total free phenolic groups (HTs and PAs). The method does not differentiate between tannins and many other phenolics that are not tannins. Interfering substances such as ascorbic acid, tyrosine and glucose are also measured (Singleton and Rossi, 1965; Makkar, 1999). Other methods used for the quantification of phenolic compounds are the butanol-HCl, vanillin-HCl and rhodanine assays.

The radical scavenging assays involve direct measurement of hydrogen atom donation or electron transfer from the potential antioxidant to free radical molecules in lipid free systems. Such assays are available as commercial kits and or a laboratory can prepare their own reagents (Becker *et al*., 2004). The assays for detection of antioxidant activity, scavenging of stable radicals and scavenging of short lived radicals include; the Ferric Reducing Antioxidant Power (FRAP) assay, Trolox Equivalent Antioxidant Capacity (TEAC) assay, 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\bullet}$) assay, the Oxygen Radical Absorbance Capacity assay (ORAC) and model systems. The evaluation of antioxidants in model systems is based on measuring changes in the concentration of compounds being oxidized, on depletion of oxygen or on the formation of oxidation products (Becker *et al*., 2004; Kumazawa *et al*., 2004). The variety of different methods/assays and experimental techniques also causes problems when comparing the antioxidant status of foods and biosystems. In view of this, an effort to adopt a standardized widely-applicable testing method is a logical consequence and has been the subject of numerous scientific discussions. From the general point of view, these radical scavenging assays should be recommended for standardization.

THERMAL PROCESSING TECHNOLOGIES

In addition to HPP and irradiation, high intensity Pulsed Electric Field (PEF) and ultrasound processing technologies are also being used in the food industry for preservation purposes. Other non thermal technologies with promising applications in the food industry include the use of Dense Phase Carbon Dioxide (DPCD) and ozone.

High intensity Pulsed Electric Field (PEF) processing involves the application of short high power electrical pulses (typically 20-80 kV cm^{-1}) for short time periods (<1 sec) to fluid foods placed between 2 electrodes (Senorans *et al*., 2003). Typical systems for the treatment of pumpable fluids consist of a PEF generation unit, which is in turn composed of a high voltage generator and a pulse generator, a treatment chamber, a suitable product handling system and a set of monitoring and controlling devices as shown in Fig. 1.

PEF has been demonstrated to be effective against various pathogenic and spoilage microorganisms and enzymes without appreciable loss of flavour, colour and bioactive compounds (Cserhalmi *et al*., 2006; Elez-Martinez *et al*., 2006; Hodgins *et al*., 2002;

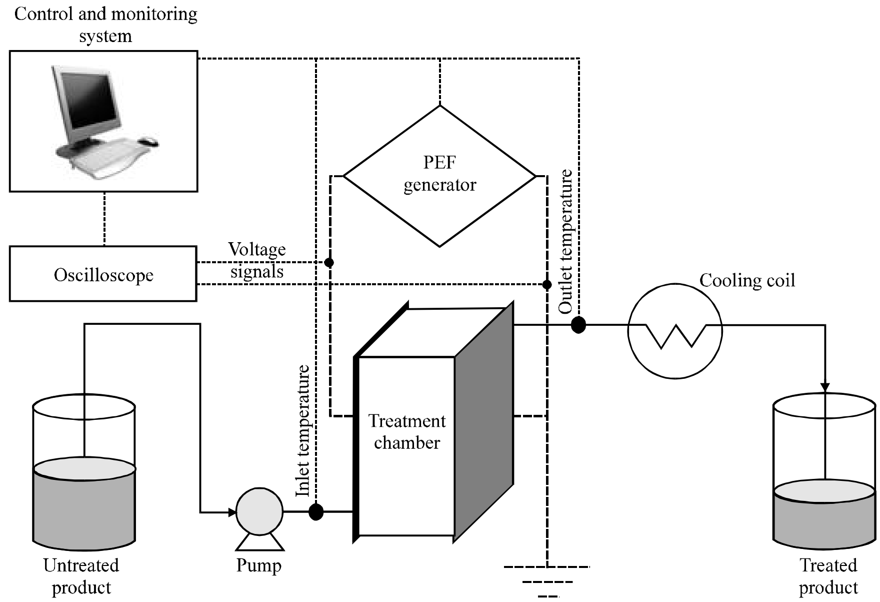

Fig. 1: Schematics of a PEF processing system for pumpable products (Soliva-Fortuny *et al*., 2009)

Yeom *et al*., 2000). PEF treatment involves short treatment times to inactivate microorganisms at temperatures below those adversely affecting food qualities (Raso and Barbosa-Canovas, 2003). In comparison to the extensive research devoted to the destruction of microorganisms by PEF and the significant information existing about the effects of PEF on enzymes, there are few studies about the impact of PEF on the nutritional quality and health-related compounds of foods (Martin-Belloso *et al*., 2005).

Soliva-Fortuny *et al*. (2009) reviewed some studies done on impart of PEF on some bioactive compounds in foods. The bioactive compounds include proteins, peptides, amino acids, phenolic compounds, isoprenoid compounds, chlorophylls, fatty acids, vitamins and flavour compounds. The author noted that the application of these treatments in order to obtain safe and stable products while maintaining their fresh-like bioactive potential looks promising and requires further investigation.

Ultrasound is defined as sound waves with frequencies higher than 16 kHz. These waves can be propagated in a liquid media as alternating compression. If ultrasound has sufficient energy, a phenomenon known as cavitation occurs. Cavitation involves the formation, growth and rapid collapse of microscopic bubbles. Based on theoretical considerations, extremely high temperatures and pressures are momentarily delivered to the liquid media during the collapse of bubbles (Suslick, 1988). Electrical discharge and production of free radicals have also been associated with extreme conditions occurring inside the collapsing bubble (Leighton, 1998). However, the ultimate reason for microbial inactivation via ultrasound is believed to be the mechanical damage caused by cavitation (Raso *et al*., 1998).

Ultrasound processing was reported to have minimal effects on the degradation of key quality parameters such as colour and ascorbic (Tiwari *et al*., 2008), enhances the extraction of phenolic and other bioactive compounds from grape must or wine (Cocito *et al*., 1995).

There are few studies on the effect of ultrasound processing on antioxidant capacities of foods. Hence, there is need to harness this technology which has shown potential in the preservation of foods.

IRRADIATION

Irradiation of food products is achieved by exposing the product to a source of ionizing or non ionizing energy for the purpose of extending the products shelf life. The ionizing radiation source could be high-energy electrons, X-rays (machine generated), or gamma rays (from Cobalt-60 or cesium-137), while the non-ionizing radiation is electromagnetic radiation mainly from ultraviolet rays (UV-A, UV-B and UV-C), visible light, microwaves and infrared (Mahapatra *et al*., 2005).

Irradiation is not being widely used because of some misconceptions by consumers about its role in causing cancer. Scientific studies have established that 10 kGy and above does not produce any toxicological hazards or nutritional or microbiological problems in food (WHO, 1999). Radiation processing has also been shown to decrease the antinutritional components in some proteinaceous leguminous seeds (Bhat *et al*., 2007). The use of irradiation combined with other methods especially low heat has been shown to be effective against a number of pathogenic microbes (Pan *et al*., 2004).

The results on the effects of irradiation on the phytochemical compounds and antioxidant activities are contradicting. Some researchers reported an improvement while others a decrease in antioxidants of irradiated samples. The observed phenomenon might be attributed by the dose applied (usually low and medium doses have insignificant effects on antioxidants), exposure time, raw material used and the solvent systems used in extracting the phenolics compounds. Alothman *et al*. (2009) reviewed the possible mechanisms for the radiation-induced changes on antioxidants.

Some authors (Fan, 2005; Costa *et al*., 2006; Song *et al*., 2007; Lemoine *et al*., 2007) reported an increase in the antioxidants of the irradiated vegetables. However, Schindler *et al*. (2005) reported significant reductions of *p*-hydroxybenzaldehyde, *p*-coumaric acid, ferulic acid, rutin, naringenin in tomatoes. A study by Costa *et al*. (2006) gave interesting results, were the phenolics compounds which contributes to the antioxidant activity were reduced significantly while the antioxidant activity of the broccoli florets increased.

To fully appreciate the benefits of irradiation, studies on the effects of the process on the nutritional, microbiological and organoleptic properties of the vegetables should be conducted. In one such study by Song *et al*. (2007), carrot and kale juices were irradiated and their microbiological, nutritional and sensory properties evaluated. The results showed that irradiation was effective in reducing the microbial load and preserving the nutritional and organolepric properties of the vegetable juices. Applications of the technology on a variety of vegetables juices should also be explored as literature reveals that most of the reported applications of irradiation are limited to fruit juices.

HIGH HYDROSTATIC PRESSURE PROCESSING (HHP)

High Hydrostatic Pressure Processing (HHP) is a non thermal food preservation method for microbial and enzyme inactivation. The method has nominal effects on nutritional and quality parameters when compared to thermal treatments. HHP is derived from material science in which products are treated above 100 MPa. HPP has been extensively reviewed

Table 1: Effect of HPP on bioactive compounds and antioxidant activities of some vegetables

Vegetable	Treatment conditions	Major findings	References
Carrot, tomatoes and broccoli (crushed or liquid extracts)	500-800 MPa, 25 or 75°C	Chlorophyll a and b in broccoli, lycopene and beta-carotene in tomatoes and antioxidative activities of water soluble carrot and tomato homogenates were not affected	Butz *et al.* (2002)
Tomato puree	100-600 MPa for 12 min at 20°C	The highest stability of lycopene was found when tomato puree was pressurized at 500 MPa and stored at 4±1°C	Qiu *et al.* (2006)
Green peas	900 MPa/20°C/5-10 min	Vitamin C retention of 82%	Quaglia *et al.* (1996)
Green beans	500 MPa/25°C/1 min	Vitamin C retention of 92% soon after HPP processing	Krebbers *et al.* (2002)
Cowpeas sprout seeds	500 MPa/25°C/15 min	Vitamin C retention of 59% was reported soon after HPP processing	Doblado *et al.* (2007)
Vegetable soup gazpacho	250 MPa/35°C/15 min, 30 days storage at 4°C	Antioxidant capacity retention of 80% after 30 days storage under refrigeration	Dede *et al.* (2007)
Tomato juice	250 MPa/35°C/15 min, 30 days storage at 25°C	Antioxidant capacity retention of 80% after 30 days storage under ambient conditions	Dede *et al.* (2007)
Broccoli, lettuce and cabbage	400 MPa and 600 MPa	Antioxidant capacity and total carotenoid content differed between vegetables but were unaffected by HPP treatment	Butz *et al.* (2002)
Tomato puree	700 MPa, 20°C	Lycopene content was unaffected by HPP	Krebbers *et al.* (2003)
Tomato juice	200 MPa at 4 and 25°C,	Pressure treatments at and below 200 MPa at 4 and 25°C maintained the extractable total carotenoids and lycopene and radical-scavenging capacity	Hsu (2008)

(Rastogi *et al*., 2007). Literature reveals that HPP preserves the nutritional value of HPP processed food and food products. Although commercial interest in the use of high-pressure technology in food processing has occurred only since the early 1990s, the effects of high pressure in inactivating microorganisms have been known for more than a century. Earliest investigations using HHP involved the application of pressures in milk and meats (Beattie and Lewis, 1924). The findings were promising as the process was found to reduce the microbial loads of the products and subsequently increased the shelf life.

The use of HPP in the food industry can lead to the production of many products with superior nutritional, functional and organoleptic properties. The technology has found some applications in blanching (Eshtiaghi and Knorr, 1993), dehydration and osmotic dehydration (Farr, 1990; Dornenburg and Knorr, 1993; Rastogi *et al*., 1994, 2005; Rastogi and Niranjan, 1998), rehydration (Zhang *et al*., 2005), frying (Knorr, 1999). Other application of the technology include solid liquid extraction, pressure shift freezing and pressure assisted thawing, gelation and rheology (Benet *et al*., 2004; Fernandez-Garcia *et al*., 2001; Knorr, 1999).

There are few reports concerning the retention of phenolics compounds and antioxidant activities in vegetables, one such report is that by Patras *et al*. (2008) who reported an insignificant reduction in antioxidant activity of tomato and carrot purée. The authors also reported retention of more than 90% of ascorbic acid after treatments at 600 MPa. Some reports on the effect of HPP on bioactive compounds and antioxidant activities are shown in Table 1.

CONCLUSION

It would appear from a nutritional point of view that high pressure processing and irradiation are exceptional food processing technologies which have the potential to preserve compounds with pharmacological properties in foods. Therefore, high pressure processed and irradiated foods could be sold at a premium over their thermally processed counterparts as they will have retained their fresh-like properties. However, these methods should be combined with other preservation techniques to ensure that thermotolerant microbes are inactivated.

REFERENCES

Alothman, M., R. Bhat and A.A. Karim, 2009. Effects of radiation processing on phytochemicals and antioxidants in plant produce. Trends Food Sci. Technol., 20: 201-212.

Beattie, J.M. and F.C. Lewis, 1924. Application of pressures in milk and meats. Int. J. Hygiene, 24: 123-137.

Becker, E.M., L.R. Nissen and L.H. Skibsted, 2004. Antioxidant evaluation protocols: Food quality or health effects. Eur. Food Res. Technol., 219: 561-571.

Benet, G.U., O. Schlueter and D. Knorr, 2004. High pressure-low temperature processing. Suggested definitions and terminology. Innovative Food Sci. Emerging Technol., 5: 413-427.

Bhat, R., K.R. Sridhar and B. Bhushan, 2007. Free radicals in velvet bean seeds (*Mucuna pruriens* L. DC.) and their status after gamma-irradiation and conventional processing. LWT-Food Sci. Technol., 40: 1570-1577.

Butz, P., R. Edenharder, F.A. Garcia, H. Fister, C. Merkel and B. Tauscher, 2002. Changes in functional properties of vegetables induced by high pressure treatment. Food Res. Int., 35: 295-300.

Chen, H.Y. and G.C. Yen, 2007. Antioxidant activity and free radical-scavenging capacity of extracts from guava (*Psidium guajava* L.) leaves. Food Chem., 101: 686-694.

Chipurura, B., M. Muchuweti and F. Manditsera, 2010. Effects of thermal treatment on the phenolics content and antioxidant activity of some vegetables. Asian J. Clin. Nutr.,

Chitindingu, K., A.R. Ndhlala, C. Chapano, M.A. Benhura and M. Muchuweti, 2007. Phenolic compound content, profiles and antioxidant activities of *Amaranthus hybridus* (Pigweed), *Brachiaria brizantha* (*Upright brachiaria*) and *Panicum maximum* (Guinee grass). J. Food Biochem., 31: 206-216.

Cocito, C., G. Gaetano and C. Delfini, 1995. Rapid extraction of aroma compounds in must and wine by means of ultrasound. Food Chem., 52: 311-320.

Coinu, R., S. Carta, P.P. Urgeghe, N. Mulinacci, P. Pinelli, F. Franconi and A. Romani, 2007. Dose-effect study on the antioxidant properties of leaves and outer bracts of extracts obtained from *Violetto di Toscana* artichoke. Food Chem., 101: 524-531.

Costa, L., A.R. Vicente, P.M. Civello, A.R. Chaves and G.A. Martinez, 2006. UV-C treatment delays postharvest senescence in broccoli florets. Postharvest Biol. Technol., 39: 204-210.

Cserhalmi, Z., A. Sass-Kiss, M. Toth-Markus and N. Lechner, 2006. Study of pulsed electric field treated citrus juices. Innovative Food Sci. Emerging Technol., 7: 49-54.

Dede, S., H. Alpas and A. Bayindirli, 2007. High hydrostatic pressure treatment and storage of carrot and tomato juices: Antioxidant activity and microbial safety. J. Sci. Food Agric., 87: 773-782.

Doblado, R., F. Juana and C. Vidal-Valverde, 2007. Changes in vitamin C content and antioxidant capacity of raw and germinated cowpea (*Vigna sinensis* var. carilla) seeds induced by high pressure treatment. Food Chem., 101: 918-923.

Dornenburg, H. and D. Knorr, 1993. Cellular permeabilisation of cultured plant tissues by high electric field pulses or ultra high pressure for the recovery of secondary metabolites. Food Biotechnol., 7: 35-48.

Duthie, G.G., S.J. Duthie and J.A.M. Kyle, 2000. Plant polyphenols in cancer and heart disease: Implications as nutritional antioxidants. Nutr. Res. Rev., 13: 79-106.

Elez-Martinez, P., R. Soliva-Fortuny and O. Martin-Belloso, 2006. Comparative study on shelf life of orange juice processed by high-intensity pulsed electric fields or heat treatment. Eur. Food Res. Technol., 222: 321-329.

Eshtiaghi, M.N. and D. Knorr, 1993. Potato cubes response to hot water blanching and high hydrostatic pressure treatment. J. Food Sci., 58: 1371-1374.

Fan, X., 2005. Antioxidant capacity of fresh-cut vegetables exposed to ionizing radiation. J. Sci. Food Agric., 85: 995-1000.

Farr, D., 1990. High-pressure technology in food industry. Trends Food Sci. Technol., 1: 14-16.

Fernandez-Garcia, A., P. Butz and B. Tauscher, 2001. Effects of high pressure processing on carotenoid extractability, antioxidant activity, glucose diffusion and water binding of tomato puree, (*Lycopersicon esculentum* Mill). J. Food Sci., 66: 1033-1038.

Hodgins, A.M., G.S. Mittal and M.W. Griffiths, 2002. Pasteurization of fresh orange juice using low-energy pulsed electrical field. J. Food Sci., 67: 2294-2299.

Hsu, K.C., 2008. Evaluation of processing qualities of tomato juice induced by thermal and pressure processing. LWT - Food Sci. Technol., 41: 450-459.

Knorr, D., 1999. Process Assessment of High Pressure Processing of Foods: An Overview. In: Processing Foods: Quality Optimization and Process Assessment, F.A.R. Oliveira and J.C. Oliveira (Eds.). CRC Press, Boca Raton, FL., pp: 249-267.

Krebbers, B., A.M. Matser, M. Koets and R.W. Van den Berg, 2002. Quality and storage-stability of high pressure preserved green beans. J. Food Eng., 54: 27-33.

Krebbers, B., A.M. Matser, S.W. Hoogerwerf, R. Moezelaar, M.M.M. Tomassen and R.W. Van den Berg, 2003. Combined high pressure and thermal treatments for processing of tomato puree: evaluation of microbial inactivation and quality parameters. Innovative Food Sci. Emerging Technol., 4: 377-385.

Kumazawa, S., T. Hamasaka and T. Nakayama, 2004. Antioxidant activity of propolis of various geographic origins. Food Chem., 84: 329-339.

Kyung-Hee, K., T. Rong, R. Yang and W.C. Steve, 2005. Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. Food Chem., 95: 466-473.

Leighton, T.G., 1998. The Principles of Cavitation. In: Ultrasound in Food Processing, Povey, M.J.W. and T.J. Mason (Eds.). Blackie Academic and Professional, London, pp: 151-178.

Lemoine, M.L., P.M. Civello, G.A. Martnez and A.R. Chaves, 2007. Influence of postharvest UV-C treatment on refrigerated storage of minimally processed broccoli (*Brassica oleracea* var. Italica). J. Sci. Food Agric., 87: 1132-1139.

Li-Chen, W., H. Hsiu-Wen, C. Yun-Chen, C. Chih-Chung, L. Yu-In and A.H. Ja-an, 2005. Antioxidant and antiproliferative activities of red Pitaya. Food Chem., 17: 341-346.

Mahapatra, A.K., K. Muthukumarappan and J.L. Julson, 2005. Applications of ozone, bacteriocins and irradiation in food processing: A review. Crit. Rev. Food Sci. Nutr., 45: 447-461.

Makkar, H.P.S., 1999. Quantification of tannins in tree foliage: A laboratory manual for FAO/IAEA coordinated research project on the Use of nuclear and related techniques to develop simple tannin assay for predicting and improving the safety and efficiency of feeding ruminants on the tanniniferous tree foliage. Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, pp: 1-29.

Martin-Belloso, O., S. Bendicho, P. Elez-Martinez, G.V. Barbosa-Canovas, M.S. Tapia and M.P. Cano, 2005. Does High Intensity Pulsed Electric Fields Induce Changes in Enzymatic Activity, Protein Conformation and Vitamin and Flavor Stability? Marcel Dekker/CRC Press, Boca Raton, FL, USA., pp: 87-104.

Muchuweti, M., G. Zenda, A.R. Ndhlala and A. Kasiyamhuru, 2005. Sugars, organic acid and phenolic compounds of *Ziziphus mauritiana* Fruit. Eur. Food Res. Technol., 221: 570-574.

Muchuweti, M., A.R. Ndhlala and A. Kasiyamhuru, 2006. Analysis of phenolic acids including tannins, gallotanins and flavonols in *Uapaca kirkiana* Fruits. Food Chem., 94: 415-419.

Muchuweti, M., C. Mupure, A. Ndhlala, T. Murenje and M.A.N. Benhura, 2007. Screening of antioxidant and radical scavenging activity of *Vigna ungiculata*, *Bidens pilosa* and *Cleome gynandra*. Am. J. Food Technol., 2: 161-168.

Netzel, M., G. Netzel, D.R. Kammerer, A. Schieber, R. Carle and L. Simons *et al*., 2007. Cancer cell antiproliferation activity and metabolism of black carrot anthocyanins. Innovative Food Sci. Emerging Technol., 8: 365-372.

Nhukarume, L., B. Chipurura, Z. Chikwambi and M. Muchuweti, 2010. Phenolic content and antioxidant capacities of *Parinari curatelifolia*, *Strychnos spinosa* and *Adansonia digitata*. J. Food Biochem., 34: 207-221.

Pan, J., A.R. Vicente, G.A. Martínez, A.R. Chaves and P.M. Civello, 2004. Combined use of UV-C irradiation and heat treatment to improve postharvest life of strawberry fruit. J. Sci. Food Agric., 84: 831-1838.

Patras, A., N. Brunton, S. Da Pieve, F. Butler and G. Downey, 2008. Effect of high pressure processing on antioxidant activity and instrumental colour of tomato and carrot purees. Innovative Food Sci. Emerging Technol., 10: 16-22.

Percival, M., 1996. Antioxidants. Clin. Nutr. Insights, 1: 1-6.

Qiu, W., H. Jiang, H. Wang and Y. Gao, 2006. Effect of high hydrostatic pressure on lycopene stability. Food Chem., 97: 516-523.

Quaglia, G.B., R. Gravina, R. Paperi and F. Paoletti, 1996. Effect of high pressure treatments on peroxidase activity, ascorbic acid content and texture in green peas. Lebensmittel-Wissenschaft und Technologie, 29: 552-555.

Raso, J., R. Pagan, S. Condon and F.J. Sala, 1998. Influence of temperature and pressure on the lethality of ultrasound. Applied Environ. Microbiol., 64: 465-471.

Raso, J. and G.V. Barbosa-Canovas, 2003. Nonthermal preservation of foods using combined processing techniques. Crit. Rev. Food Sci. Nutr., 43: 265-285.

Rastogi, N.K., R. Subramanian and K.S.M.S. Raghavarao, 1994. Application of high-pressure technology in food processing. Indian Food Ind., 13: 30-34.

Rastogi, N.K. and K. Niranjan, 1998. Enhanced mass transfer during osmotic dehydration of high-pressure treated pineapple. J. Food Sci., 63: 508-511.

Rastogi, N.K., K.S.M.S. Raghavarao and K. Niranjan and D.W. Sun, 2005. Developments in Osmotic Dehydration. In: Emerging Technologies for Food Processing, Sun, D.W. (Eds.). Elsevier Academic Press, London, UK., pp: 221-250.

Rastogi, N.K., K.S. Raghavarao, V.M. Balasubramaniam, K. Niranjan and D. Knorr, 2007. Opportunities and challenges in high pressure processing of foods. Crit. Rev. Food Sci. Nutr., 47: 69-112.

Schindler, M., S. Solar and G. Santog, 2005. Phenolic compounds in tomatoes: Natural variation and effects of gamma-radiation. Eur. Food Res. Technol., 221: 439-444.

Senorans, F.J., E. Ibanez and A. Cifuentes, 2003. New trends in food processing. Crit. Rev. Food Sci. Nutr., 43: 507-526.

Singleton, V.L. and J.A. Jr. Rossi, 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic., 16: 144-158.

Soliva-Fortuny, R., A. Balasa, D. Knorr and O. Martin-Belloso, 2009. Effects of pulsed electric fields on bioactive compounds in foods: A review. Trends Food Sci. Technol., 20: 544-556.

Song, H.P., M.W. Byun, J. Cheorun, H. L. Cheol, S.K. Kyong and H.K. Dong, 2007. Effects of gamma irradiation on the microbiological, nutritional and sensory properties of fresh vegetable juice. Food Control, 18: 5-10.

Suslick, K.S., 1988. Ultrasound: Its Chemical, Physical and Biological Effects. Vch Publisher, New York, SBN-13: 9780895733283.

Tiwari, B.K., C.P. O'Donnell, K. Muthukumarappan and P.J. Cullen, 2008. Effect of ultrasound processing on quality and nutrational properties of fruit juices. Stewart-Post Harvest Rev., 4: 1-6.

Tiwari, B.K., C.P. O'Donnell and P.J. Cullen, 2009. Effect of non thermal processing technologies on the anthocyanin content of fruit juices. Trends Food Sci. Technol., 20: 137-145.

WHO, 1999. High-Dose Irradiation: Wholesomeness of Food Irradiated with Doses Above 10 kGy. World Health Organization, Geneva.

Yeom, H.W., C.B. Streaker, Q.H. Zhang and D.B. Min, 2000. Effects of pulsed electric fields on the quality of orange juice and comparison with heat pasteurization. J. Agric. Food Chem., 48: 4597-4605.

Zhang, H., L. Li, E. Tatsumi and S. Isobe, 2005. High-pressure treatment effects on proteins in soymilk. Lebensmittel-Wissenschaft und Technologie, 38: 7-14.