

Review paper

Antimicrobial, antioxidant and phyto-chemicals from fruit and vegetable wastes: A review

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Paper no: 47 Received: 04 Sept, 2012 Received in revised form: 01 Nov, 2012 Accepted: 03 Nov, 2012

Abstract

Food processing industry including fruit and vegetable processing is the second largest generator of wastes into the environment only after the household sewage. The generation of biodegradable waste increased linearly with the growth and development of food processing industry. A huge amount of waste in the form of liquid and solid is produced in the fruit and vegetable processing industries is valuable but biodegradable natural resources with large economic potential. It causes pollution problem if not utilized or disposed-off properly. The waste obtained from fruit processing industry is extremely diverse due to the use of wide variety of fruits and vegetables, the broad range of processes and the multiplicity of the product. Different fruits and vegetable possess various quantities of waste. Waste product which is thrown into the environment has a very good antimicrobial and antioxidant potentiality. These are novel, natural and economic sources of antimicrobics and antioxidants, which can be used in the prevention of diseases caused by pathogenic microbes. These all benefits will open up as a scope for future utilization of the waste for therapeutic purpose. However, lack of pilot testing of the developed technologies, negative attitude of the industrialists and perhaps, less helping hand from the government sector are the major constraints in utilization of the waste.

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Keywords: Vegetable, utilization, environment, antioxidant, antimicrobial, waste, food processing.

Introduction

The growing, processing and preparation of food result in the production of varying degree of waste material. The waste material may be in the form of leaf/straw, waste during harvesting, processing industry waste and after processing waste (Joshi and Devraj, 2008). The waste obtained from fruit processing industry is extremely diverse due to the use of wide variety of fruits and vegetables, the broad range of processes and the multiplicity of the product (William, 2005). Vegetables and some fruits yield between 25% and 30% of non-edible products (Ajila *et al.*, 2010). The full utilization of horticultural produce is a requirement and a demand that needs to be met by countries wishing to implement low-waste technology in their agribusiness (Kroyer, 1995). Depending

on plant species, variety and tissue, high levels of health-protecting antioxidants, such as vitamin C and E, phenolic compounds including phenyl-propanoids and flavonoids, and or carotenoids such as lycopene can be found. The waste materials such as peels, seeds and stones produced by the fruit and vegetable processing can be successfully used as a source of phytochemicals and antioxidants. The entire tissue of fruits and vegetables is rich in bioactive compounds, such as phenolic compounds, carotenoids, vitamins and in most cases, the wasted by products can present similar or even higher contents of antioxidant and antimicrobial compounds than the final produce can (Ayala-Zavala *et al.*, 2010). The new aspects concerning the use of these wastes as by-products for further exploitation on the production of food

additives or supplements with high nutritional value have gained increasing interest because these are highvalue products and their recovery may be economically attractive. The by-products represent an important source of sugars, minerals, organic acid, dietary fibre and phenolics which have a wide range of action which includes antitumoral, antiviral, antibacterial, cardioprotective and antimutagenic activities (Jasna *et al.*, 2009). Because of increasing threat of infectious diseases, the need of the hour is to find natural agents with novel mechanism of action. Natural products provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Fruit and vegetable peels are thrown into the environment as agro waste which can be utilized as a source of antimicrobics. Utilisation of by-products is, however, limited due to the poor understanding of their nutritional and economic value (Schroeder, 1999).

According to Indian Agricultural Research Data Book (2012), the estimated area (In 000'HA) of fruits and vegetables is 6383 and 8495 and the production (In 000'MT) is 74878 and 146554 (Table 1).

The extent of total losses in these commodities is approximately estimated as 20-30% of the total production, amounting to a loss of Rs. 30,000 crores per annum. Sliced apples produced 10.91% of pulp and seed (core) by-products and 89.09% of the final products. Peeled mandarins produced 16.05% of peels and 83.95% of final products. Diced papayas produced 6.51% of seeds, 8.47% of peels, 32.06% of unusable pulp (due to the lack of shape uniformity in a cube), and 52.96% of final products. Pineapples produced 9.12% of core, 13.48% of peels, 14.49% of pulp, 14.87% of top, and 48.04% of finished products. Mangoes produced 13.5% of seeds, 11% of peels, 17.94%

Table 1: Area and Production estimates for Horticulture crops (Area in 000' HA, Production in 000' MT and Productivity = MT/HA)

	2008-09			2009-10			2010-11		
	Area	Production	Pdy.	Area	Production	Pdy.	Area	Production	Pdy.
Fruits									
Banana	709	26217	37.0	770	26470	34.4	830	29780	35.9
Mango	2309	12750	5.5	2312	15027	6.5	2297	15188	6.6
Citrus	924	8623	9.3	987	9638	9.8	846	7464	8.8
Papaya	98	3629	37.1	96	3913	40.9	106	4196	39.6
Guava	204	2270	11.1	220	2572	11.7	205	2462	12.0
Apple	274	1985	7.2	283	1777	6.3	289	2891	10.0
Pineapple	84	1341	16.0	92	1387	15.1	89	1415	15.9
Sapota	156	1308	8.4	159	1347	8.5	160	1424	8.9
Grapes	80	1878	23.6	106	881	8.3	111	1235	11.1
Pomegranate	109	807	7.4	125	820	6.6	107	743	6.9
Litichi	72	423	5.9	74	483	6.5	78	497	6.4
Others	1083	7234	6.7	1105	7201	6.5	1265	7583	6.0
Fruits-Total	6101	68466	112	6329	71516	11.3	6383	74878	11.7
Vegetables									
Potato	1828	34391	18.8	1835	36577	19.9	1863	42339	22.7
Tomato	599	11149	18.6	634	12433	19.6	865	16526	19.1
Onion	834	13565	16.3	756	12159	16.1	1064	15118	14.2
Brinjal	600	10378	17.3	590	10165	17.2	680	11896	17.5
Tapioca	280	9623	34.3	232	8060	34.8	221	8076	36.5
Cabbage	310	6870	22.1	331	7281	22.0	369	7949	21.5
Cauliflower	349	6532	18.7	338	6410	19.0	369	6745	18.3
Okra	432	4528	10.5	452	4803	10.6	498	5784	11.6
Peas	348	2916	8.4	365	3029	8.3	370	3517	9.5
Sweet Potato	124	1120	9.0	119	1095	9.2	113	1047	9.3
Others	2275	28006	12.3	2332	31724	13.6	2083	27557	13.2
Veg.-Total	7981	129077	162	7985	133738	16.7	8495	146554	17.3

Source: Anonymous, 2011-2012

unusable pulp, and 57.56% of final products. It is emphasized that considerable amounts of fruit material are the by-products of the minimal processing, and the possibility of creating alternative processes to give added value to this wasted material must be considered (Ayala-Zavala *et al.*, 2010). While according to FAO (2003), the total waste generated from fruits was estimated as 3.36 million tonnes (MT) out of the total production of 16.8 MT and particularly for banana it was 6.4 MT. India is producing 3 million tonnes of citrus fruits like mandarins, lime, lemon, and sweet orange. Citrus wastes are rich source of oil, pectin and variety of by-products. The failure or inability to salvage and reuse such materials economically results in the unnecessary waste and depletion of natural resources (Bhalerao *et al.*, 1989). The extent of the waste produced and available from processing industries of some of the important fruits and vegetables, is given in Table 2 and 3.

Table 2: Quantities of various fruit and vegetable processing wastes

Commodity	Percent weight basis
Apple	12-47
Apricot	8-25
Grape fruit	3-58
Orange	3
Peach	11-40
Pear	12-46
Asparagus	3.2-30
Bean, green	5-20
Beet	7-4
Broccoli	20
Cabbage	5-25
Carrot	18-52
Cauliflower	8
Peas	6-79
Potatoes	5
Spinach	10-40
Sweet potato	15
Tomato	5-25

Source: Gera IB and Kramer, 1969

Table 3: Fruits and vegetable processing wastes available in India

Vegetable	Nature of waste	Production(content)(tones)	Approx.waste(%)	Potentialquantities ofwaste (tones)
Mango	Peel, stones	6987.7	45	3144.4
Banana	Peel	2378.0	35	832.3
Citrus	Peel, rag and seed	1211.9	50	6-06.0
Pineapple	Skin, core	75.7	33	24.7
Grapes	Stem, skin and seeds	565	20	-
Guavas	Peel and core and seeds	565	10	-
Peas	Shell	107.7	40	68.3
Tomato	Skin, core and seeds	464.5	20	90.3
Potato	Peel	2769.0	15	415.3
Onion	Outer leaves	1102.0	-	-
Apple	Peel, pomace and seeds	1376.0	-	412.0

Source: Gupta and Joshi, 2000.

Chemical composition

The amount of pollution load and characteristics of the waste depend on the food being processed. Chemical composition of the wastes from fruits and vegetables show that it is a rich source of various nutrients. Some of these fruit and vegetable wastes are a rich source of vital constituents like carbohydrates, proteins, fats, minerals, fibres etc. Nutrient composition of some of the solid wastes from fruits and vegetables is given in the Table 4.

The association between the diet rich in fruits and vegetables and a decreased risk of cardiovascular diseases and certain forms of cancer is supported by considerable epidemiological evidence (Ness and Powles, 1997; Riboli and Norat, 2003). Different studies have shown that free radicals present in the human organs cause oxidative damage to various molecules, such as lipids, proteins and nucleic acids, and are thus involved in the initiation phase of the degenerative diseases. Phenolic and other phytochemical antioxidants found in fruits and vegetables are capable of neutralising free radicals and may play a major role in the prevention of certain diseases (Kaur and Kapoor, 2001). Numerous studies have provided evidence for decreased risk of some chronic dis-eases e.g., some types of cancer, cardiovascular and neurodegenerative disorders with increased dietary intake of vegetables, fruits, teas, spices and other plant-based foods and supplements. The most abundant by-products of minimal processing of fresh-cut fruit and vegetable are peel and seed and those are reported to contain high amounts of phenolic compounds with antioxidant and antimicrobial properties (Shrikhande 2000; Muthuswamy *et al.*, 2008; Tuchila *et al.*, 2008).

Phytochemicals

Plants synthesize a diverse array of secondary metabolites (phytochemicals) known to be involved in plant defence against microbial and fungal pathogens and insect pests, and in the last few decades several classes of phytochemicals have

Table 4: Composition of different fruit wastes (per 100g)

Waste	Moisture(g)	Protein(g)	Fat (g)	Minerals(g)	Fibre(g)	Carbohy-drate (g)
Apple pomace	–	2.99	1.71	1.65	16.16	17.35
Mango seed kernel	8.2	8.50	8.85	3.66	–	74.49
Jack fruit (inner and outer portion)	8.5	7.50	11.82	6.50	30.77	14.16
Jack fruit seeds	64.5	6.60	0.40	1.20	1.50	25.80
Jack seed flour	77.0	2.64	0.28	0.71	1.02	18.12
Passion fruit peel	81.9	2.56	0.12	1.47	5.01	–
Banana peel	79.2	0.83	0.78	2.11	1.72	5.00
Sweet orange seeds	4.00	15.80	36.90	4.00	14.00	–
Watermelon seeds	4.3	34.10	52.60	3.70	0.80	4.50
Muskmelon seeds	6.8	21.00	33.00	4.00	30.00	–
Pumpkin seeds	6.0	29.50	35.40	4.55	12.00	12.53
Banana stem						
Central core	93.1	0.30	0.03	1.04	0.68	1.20
Outer hard fibrous sheath	91.9	0.12	0.06	0.98	1.81	2.44
Press juice from stem	98.6	0.05	–	0.63	–	0.41

Source: Maini and Sethi, 2000.

been shown to help reduce the risk of various diseases e.g. cancer and coronary heart disease. Nowadays, there is a growing interest in finding phytochemicals as an alternative to synthetic substances, which are commonly used in the food, pharmaceutical and cosmetic industry. Epidemiological studies have pointed out that the consumption of fruits and vegetables imparts health benefits, e.g. reduced risk of coronary heart disease and stroke, as well as certain types of cancer. Apart from dietary fibre, these health benefits are mainly attributed to organic micronutrients such as carotenoids, poly-phenols, tocopherols, vitamin C and others. Flavonoids from fruits and vegetables probably reduce risks of diseases associated with oxidative stress, including cancer. Apples contain significant amounts of flavonoids with antioxidative potential.

The products and byproducts obtained during the minimal processing of the fruits were analyzed for the phytochemical content and antioxidant status. It was found that the total phenolics and flavonoid contents were higher in the byproducts as compared to the final products, being more pronounced in mango seeds and peels. These compounds could be responsible for free radical inhibition activity. Several studies have shown that the content of phytochemical compounds is higher in peel and seeds with respect to the edible tissue. Gorinstein *et al.*, (2001) found that the total phenolic compounds in the peels of lemons, oranges, and grapefruits were 15% higher than that of the pulp of these fruits. Peels from apples, peaches, pears as well as yellow and white flesh nectarines were found to contain twice the amount of total phenolic compounds as that contained in fruit pulp (Gorinstein *et al.*, 2001). While the edible pulp of bananas (*Musa paradisiaca*) contains 232 mg/100 g of dry weight

phenolic compounds, this amount is about 25% of that present in the peel (Someya *et al.*, 2002). Similarly, other studies have reported that pomegranate peels contain 249.4 mg/g of phenolic compounds as compared to only 24.4 mg/g phenolic compounds found in the pulp of pomegranates. Apple peels were found to contain up to 3300 mg/100 g of dry weight of phenolic compounds (Wolfe and Liu 2003). Grape seeds and skins, the byproducts of grape juice and white wine production, are also sources of several phenolic compounds, particularly mono, oligo, and polymeric proanthocyanidins (Shrikhande, 2000). It has been reported that the total phenolic compounds of seeds of several fruits, such as mangos, longans, avocados, and jackfruits, were higher than that of the edible product, and that the byproducts could be a valuable source of phytochemicals (Soong and Barlow, 2004). The peels and seeds of tomatoes are richer sources of phenolic compounds than the pulp of the tomatoes are. The phenolic compounds of 12 genotypes of tomatoes have been studied, and, in general, lower levels were found in the flesh, ranging from 9.2 to 27.0 mg/100 g, as compared to 10.4 to 40.0 mg/100 g in the peels (George *et al.*, 2004). A similar observation was reported, and the total phenolic compounds (expressed as milligram of gallic acid equivalents per 100 g) of the skin, seeds, and pulp of tomatoes were found to be 29.1, 22.0, and 12.7 mg/100 g, respectively (Toor and Savage, 2005). It was also found that the peel byproduct of tomato cultivars (Excell, Tradiro, and Flavorine) had significantly higher levels of total phenolic compounds, total flavonoids, lycopene, ascorbic acid, and antioxidant activity as compared with the pulp and seeds (Toor and Savage, 2005). In general, there are up to 10-fold higher occur between the phenolic contents of byproducts than the

Table 5: Examples of functional food components

Classy' components	Source	Potential benefit
Beta-carotene	Various fruits	Neutralizes free radicals which may damage cells; bolsters cellular antioxidant defences
Lutein, Zeaxanthin	Citrus	May contribute to the maintenance of healthy vision
Flavonoids		
Anthocyanidins	Berries, cherries, red grapes	Bolster cellular antioxidant defences; may contribute to the maintenance of brain function
Flavanols catechins, epicatechins, procyanidins	Apples, grapes	May contribute to the maintenance of heart Health
Flavanones	Citrus foods	Neutralize free radicals which may damage cells; bolster cellular antioxidant defences
Flavonols	Apples	Neutralize free radicals which may damage cells; bolster cellular antioxidant defences
Proanthocyanidins	Cranberries, apples, strawberries, grapes, wine, peanuts, cinnamon	May contribute to the maintenance of urinary tract health and heart health

Source: Jasna *et al.*, 2009.

pulp. Some of the examples of functional food components are shown in Table 5.

Antimicrobials

The antimicrobial constituents are present in all parts of the plant viz. bark, stalks, leaves, fruits, roots, flowers, pods, seeds, stems, latex, hull and fruit rind. The antimicrobial activities of a variety of naturally occurring phenolic compounds from different plant sources have been studied in detail (Burt 2004). Recent research has revealed that the fruit peels and seeds, such as grape seeds and peels, pomegranate peel and mango seed kernel (Kabuki *et al.*, 2007) may potentially possess antimicrobial property. Various fruits (peel, flesh or seed) have been used in traditional medicine for stomach ache, sore eyes, fever, etc. Papaya has been shown to contain sulphhydroxyl protease which can inhibit viral or microbial infection (Rajashekhara *et al.*, 1990).

These compounds play an important role in fruits' protection against pathogenic agents, penetrating the cell membrane of microorganisms, causing lysis. Phenolic compounds from spices such as gingeron, zingerone, and capsaicin have been found to inhibit the germination of bacterial spores (Burt 2004). Polyphenols contained in green tea (*Camellia sinensis*) combat against *Vibrio cholerae* O1, *Streptococcus mutans* and *Shigella* (Si *et al.*, 2006). The fruit and vegetable peel extracts showed better antifungal activity than antibacterial activity; Gram-negative bacteria were more susceptible than Gram-positive bacteria. The most susceptible organism was fungi and Gram-negative *K. pneumoniae*. *M. indica* showed maximum and best antimicrobial activity. The antimicrobial

activity of an ethanol extract from mango seed kernels against food-borne pathogenic bacteria has also been reported. The mango extract was more effective against Gram-positive than Gram-negative bacteria, with a few exceptions (Kabuki *et al.*, 2007). In addition, flavonoids have been reported to enhance the antibacterial, antiviral, or anticancer activities of compounds such as naringenin, acycloguanosine, and tamoxifen (Bracke *et al.*, 1999). The mixture of phytochemical constituents in plant extracts can be an advantage due to the synergistic effect that the constituents may have (Bakkali *et al.*, 2008).

Citric, succinic, malic, acetic, and tartaric acids are commonly found in fruits and fresh-cut byproducts. They have been traditionally used in the food industry as preservative agents, attributing their antimicrobial efficacy to the pH changes of the treated media. In general, bacteria grow at a pH close to 6.5 to 7.5, but tolerate a pH range from 4 to 9. Yeasts are more tolerant to low pH values than bacteria are, whereas molds can grow in the widest pH range. One effective way of limiting microbial growth is increasing the acidity of a particular food by adding an acidic substance (Massilia *et al.*, 2009). Acids attack cell walls, cell membranes, metabolic enzymes, protein synthesis systems, and the genetic material of microorganisms (Tripathi and Dubey, 2004).

The usage of bioactive extracts as applied to fruit preservation is an alternative to chemical preservatives and helps to achieve consumer demand for fresh, nutritious and safe fruits, and vegetables that are free of synthetic additives. Some bioactive extracts have been proven to be effective antimicrobials and antioxidants; however, their addition to fruit may cause changes in sensorial attributes. For example, green tea extract (GT) has

been evaluated as being able to act in the preservative treatment of fresh-cut lettuce. Different quality markers, such as respiration, browning, ascorbic acid, and carotenoid content were evaluated. Several GT concentrations (0.25, 0.5, and 1 g/100 ml) at different temperatures (20 °C and 50 °C) were tested. Optimal GT treatments (0.25 g/100 ml at 20 °C) were compared with chlorine (120 ppm at 20 °C). High GT concentrations (0.5 g/100 ml and 1.0 g/100 ml) to a large extent prevented ascorbic acid and carotenoid losses of 0.25 g/100 ml GT as did chlorine. However, GT enhanced the browning of the samples, probably as a result of the high polyphenol content of the treatment, though heat-shock reduced this negative effect. No significant differences were observed between chlorine and the optimal GT (0.25 g/100 mL at 20 °C) in the browning appearance and sensory properties. GT kept the antioxidant activity of the samples better than chlorine did.

Ethanol extract of cinnamon bark (1% w/v) reduced the aerobic growth of bacteria inoculated fresh-cut apples significantly during storage at 6 °C up to 12 d. Catechin, chlorogenic acid, and phloridzin, 3 phenolic compounds that are abundant in apple processing byproducts, exhibited varying degree of inhibitory action toward the growth of tested food pathogenic and spoilage bacteria, fungi, and yeasts (Muthuswamy and Rupasinghe, 2007). However, it is important to note that these phenolics (except 25 mm phloridzin) did not inhibit the probiotic bacterium *Lac. rhamnosus* suggesting no or minimal threat to the beneficial colon microflora, if the phenolics are used as food additives at the desirable concentrations. Also these authors suggest that the major phenolic compounds of apple byproducts could find use as food additives, however, the regulatory aspects of the use of plant extracts as fresh-cut fruit additives must be contemplated.

Bacterial infections remain an important problem for human health. The control of bacterial infections has been traditionally treated by inhibiting microbial growth using different types of

antibiotics. Therefore, the search of non toxic compounds which inhibit QS and so, the virulence of pathogenic bacteria can bring new alternatives for the treatment of bacterial infections in humans also notable are the antibacterial properties of berries. The cloudberry (*Rubus chamaemorus*), raspberry (*Rubus idaeus*), and bilberry (*Vaccinium myrtillus*) and crowberry (*Empetrum nigrum*) were effective against all of the bacterial strains tested. Bog bilberry (*V. uliginosum*) inhibited all the Gram-positive bacteria, but not Gram-negative *E. coli*, *S. aureus*, *B. subtilis* and *M. luteus* (Rauha et al., 2000).

Essential oils from citrus offer the potential for all natural antimicrobials for use in improving the safety of organic or all natural foods (Joshi et al., 2011). Subba et al., (1967) determined that orange and lemon oil had *in vitro* antibacterial effects on *Salmonella* and other food-borne microorganisms. However, Fisher and Phillips (2006), on the other hand, found that Gram-positive bacteria were more sensitive than Gram-negative *in vitro*. Seven citrus essential oils were screened by disc diffusion assay for their antibacterial activity against 11 serotypes/strains of *Salmonella* (Bryan et al., (2008)). The 3 most active oils were selected to determine the minimal inhibitory concentration (MIC) against the some *Salmonella*. Orange terpenes (C4), singles-folded d-limonene (C5), and orange essence terpenes (C6) all exhibited inhibitory activity against the *Salmonella* spp. On the disc diffusion assay orange terpenes and d-limonene both had MICs of 1%. The most active compound, terpenes from orange essence, produced MIC that ranged from 0.125% to 0.5% against the 11 salmonella tested. (Table 6)

The antimicrobial activity of some plant peels against microorganisms causing infection is summarised in Table 7.

Mohamed et al., 1994 evaluated antimicrobial activity of extracts of ripe, unripe and leaves of guava (*Psidium guajava*); ripe, unripe and leaves of starfruit (*Averrhoa carambola*); ripe

Table 6: Mic (in percent, v/v) of orange oils against 11 *Salmonella* spp.

	C4	C5	C6
<i>S. enteritidis</i> 1773-92	1	1	0.25
<i>S. senftenberg</i> 43845	1	1	0.5
<i>S. senftenberg</i> 1402-94	1	1	05.
<i>S.tennessee</i> 825-94	1	1	0.5
<i>S. kentucky</i> 1271-94	1	1	0.25
<i>S. eidelberg</i> 8326	1	1	0.25
<i>S. enteritidis</i> 13076	1	1	0.13
<i>S. montevideo</i> G4639	1	1	0.25
<i>S. Michigan</i>	1	1	0.25
<i>S. typhimurium</i> (Copenhagen)	1	1	0.5
<i>S. stanfey</i> H1256	1	1	0.5

Source: Bryan et al., 2008.

and unripe banana (*Musa sapientum* variety *Montel*); ripe and unripe papaya (*Carica papaya*); passionfruit (*Passiflora edulis* *F. Flavicarpa*) peel; two varieties of *Lansium domesticum* peel (langsat and duku); rambutan (*Nephelium lappaceum*) peel and rambai (*Baccaurea motleyana*) against Gram positive bacteria, Gram negative bacteria, yeast and fungi (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Lactobacillus bulgaricus*; *E. coli*, *Proteus vulgaricus*, *Pseudomonas aeruginosa*, *Salmonella typhi*; *Saccharomyces cerevisiae*, *Candida lypolytica*; *Rhizopus spp.*, *Aspergillus niger*, and *Chlamydomucor spp*) by using both the filter paper disc diffusion and tube dilution assays. Extracts from ripe starfruit, guava leaves and rambai peel showed strong activity against all the bacteria tested, in most cases with activity stronger than 50µg streptomycin. Passionfruit peel, ripe and unripe guava showed activity against all the bacteria tested except *E. coli*. Rambutan peel too showed activity against all the bacteria tested except towards *Pseudomonas aeruginosa*. Most of the fruit wastes showed some activity towards bacteria but poor activity against yeast or fungi. Extracts from bananas, papayas, passionfruit peel, *Lansium domesticum* peels and rambutan peels showed activity against *Candida lypolytica*

while extracts from guava showed strong activity against *Saccharomyces cerevisiae*. Other than guava, ripe starfruit, rambai peel and rambutan peel showed potential for use against bacteria.

Antioxidants

There is evidence that chronic diseases, such as cancer and cardiovascular disease, may occur as a result of oxidative stress. Free radicals are endogenous initiators of degenerative processes, as they damage lipids, proteins and DNA, thus favouring development of a number of degenerative diseases. The consumption of food rich in natural antioxidants, as well as food enriched with them, ensure the desirable antioxidant status and helps in prevention of the development of diseases caused by oxidative stress. The most publicized phytochemicals with antioxidant properties have been vitamin C, vitamin E, and beta-carotene (which the body converts into vitamin A). Evidence exists that vitamin E can help prevent atherosclerosis by interfering with the oxidation of low-density lipoproteins (LDL), a factor associated with increased risk of heart disease.

Table 7: Antimicrobial activity of some plant peels against some microorganisms causing infectious diseases

Plant name	Extract	Microorganisms
<i>Citrus grandis</i> (Rutaceae)	Hexane, ethyl acetate, butanol, methanol, benzene: acetone	<i>Bacillus subtilis</i> , <i>Bacillus cereus</i> , <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Salmonella enteritidis</i>
<i>Citrus reticulata</i> Blanco (Rutaceae)	Oil	<i>Alternaria alternata</i> , <i>Rhizoctonia solani</i> , <i>Curvularia lunata</i> , <i>Fusarium oxysporum</i> , <i>Helminthosporium oryzae</i>
<i>Vitis vinifera</i> (Vitaceae)	80% ethanol	<i>Staphylococcus aureus</i> , <i>Bacillus cereus</i> , <i>Escherichia coli</i> , <i>Salmonella infantis</i> , <i>Campylobacter coli</i>
<i>Citrus reticulata</i> Blanco (Rutaceae)	Flavonoid extract	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Staphylococcus epidermidis</i> , <i>Enterococcus faecalis</i> , <i>Salmonella typhimurium</i> , <i>Enterobacter cloacae</i>
<i>Citrus acida</i> Roxb. (Rutaceae)	Oil	<i>Bacillus subtilis</i> , <i>Bacillus cereus</i> , <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Enterobacter aerogenes</i> , <i>Salmonella typhimurium</i> , <i>Aspergillus ficuum</i> , <i>Aspergillus niger</i> , <i>Aspergillus fumigatus</i> , <i>Aspergillus flavus</i> , <i>Fusarium saloni</i> , <i>Fusarium oxysporum</i> , <i>Pencillium digitatum</i> , <i>Candida utilis</i>
<i>Ficus carica</i> L. (Moraceae)	Aqueous	<i>Bacillus cereus</i> , <i>Staphylococcus epidermidis</i> , <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Pseudomonas fluorescens</i>
<i>Citrus bergamia</i> Risso (Rutaceae)	Ethanol fraction	<i>Escherichia coli</i> , <i>Pseudomonas putida</i> , <i>Salmonella enterica</i> , <i>Listeria innocua</i> , <i>Bacillus subtilis</i> , <i>Staphylococcus aureus</i> , <i>Lactobacillus lactis</i> , <i>Sacharomyces cerevisiae</i>
<i>Nephelium lappaceum</i> L. (Sapindaceae)	Ether, methanol, aqueous	<i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , <i>Pseudomonas aeruginosa</i> , <i>Salmonella typhi</i> , <i>Vibrio cholerae</i> , <i>Enterococcus faecalis</i> , <i>Staphylococcus aureus</i> , <i>Staphylococcus epidermidis</i>
<i>Musa sapientum</i> (Musaceae)	Chloroform, ethyl acetate, aqueous	<i>Staphylococcus aureus</i> , <i>Bacillus subtilis</i> , <i>Bacillus cereus</i> , <i>Salmonella enteritidis</i> , <i>Escherichia coli</i>

Source: Chanda et al., 2010.

The role of antioxidant phytochemicals in the prevention of these diseases has been mainly attributed to the prevention of LDL oxidation through a scavenging activity against peroxy and hydroxyl radicals. Apple contains many different dietary phytonutrients with strong antioxidant capacities, such as phenolics, carotenoids, and vitamins, which may protect against free radicals. Apple peels have high concentrations of phenolic compounds and may assist in the prevention of chronic diseases. Phenolics are a much diversified group of secondary plant metabolites, which includes simple phenolic, phenolic acids (benzoic and cinnamic acid derivatives), lignans, lignins, coumarins, flavonoids, stilbenes, flavonolignans and tannins. Many of phenolic compounds have shown strong antioxidant properties as oxygen scavengers, peroxide decomposers, metal chelating agents, and free radical inhibitors. Besides antioxidant activity, phenolic compounds have a wide range of action which includes antitumoral, antiviral, antibacterial, cardioprotective, and antimutagenic activities. The conventional apple juice production (straight pressing of apple pulp or pressing after pulp enzyming) resulted in a juice poor in phenolics and with only 3–10% of the antioxidant activity of the fruit they were produced from. Polyphenols are one of the phytochemical groups whose “protective” properties include antioxidant, antimicrobial, anticancer and cardiovascular-protective activities. Different model systems were employed to evaluate the antioxidant properties of apple pomace polyphenols. The DPPH and superoxide ion radical scavenging activities of apple pomace polyphenols, and also their antioxidant property in the α -carotene/linoleic acid system were determined. The polyphenols examined were epicatechin, its dimer (procyanidin B2), trimer, tetramer and oligomer, quercetin glycosides, chlorogenic acid, phloridzin and 3-hydroxy-phloridzin. All the compounds showed strong antioxidant activities, and their DPPH-scavenging activities were 2-3 times and superoxide anion radical-scavenging activities were 10-30 times better than those of the antioxidant vitamins C and E. The total phenolics, total flavonoids, total flavan-3-ols, and some individual phenolic compounds contributed significantly to the antiradical activities of apple pomace. Flavonoids are polyphenolic antioxidants naturally present in vegetables, fruits, and beverages such as tea and wine. *In vitro*, flavonoids inhibit oxidation of low-density lipoprotein and reduce thrombotic tendency, but their effects on atherosclerotic complications in human beings are unknown (Hertog *et al.*, 1993). The peel of *Citrus* fruit is a rich source of flavanones and many polymethoxylated flavones, which are very rare in other plants. Flavonoids in regularly consumed foods may reduce the risk of death from coronary heart disease in elderly men. The contents of polyphenols and tannins in fruit seed and peel are shown in Table 8.

Grape seed extract is a by-product derived from the grape seeds (*Vitis vinifera*) (from grape juice and wine processing) that is extracted, dried and purified to produce a polyphenolic compound rich extract (Lau and King, 2003). The extraction of crushed grape pomace with a mixture of ethyl acetate and water yielded phenolic compounds displaying antioxidant activities comparable to BHT in the Rancimat test. Catechin, picatechin, epicatechin gallate and epigallocatechin were the major constitutive units of grape skin tannins. Recent literature has evidenced antioxidant properties of GSE both *in vivo* and *in vitro* (Yilmaz and Toledo, 2004). The antioxidant properties of GSE are primarily due to flavonoids that can perform scavenging action on free radicals (superoxide, hydroxyl, and 1,1-diphenyl-2-picrylhydrazyl (DPPH)), metal chelating properties, reduction of hydroperoxide formation and their effects on cell signalling pathways and gene expression (Jacob *et al.*, 2008; Sato *et al.*, 1996; Soobrattee *et al.*, 2005). The presence of the functional group “–OH” in the structure and its position on the ring of the flavanoid molecule determine the antioxidant capacity. Addition of “–OH” groups to the flavanoid nucleus will enhance the antioxidant activity, while substitution by –OCH₃ groups diminishes the antioxidant activity (Majo *et al.*, 2008). Degree of polymerization of the procyanidins may also determine the antioxidant activity as the higher the degree of polymerization, the higher the antioxidant activity. Among the different parts of grape plant, grape seeds exhibit highest antioxidant activity followed by the skin and the flesh (Pastrana-Bonilla *et al.*, 2003). The antioxidant potential of GSE is twenty and fifty fold greater. A new class of compounds, aminoethylthio-flavan-3-ol conjugates, has been obtained from grape pomace by thiolysis of polymeric proanthocyanidins in the presence of cysteamine. The antioxidant activity of the extracts obtained from grape by-products was analyzed by different *in vitro* tests: scavenging of the stable DPPH radical reactive •OH, O₂•- and of authentic peroxy nitrite (ONOO-). The content of five phenolic constituents of biological interest: catechin and epicatechin in seeds and quercetin, rutin and resveratrol in skin extracts was investigated. All the five phenols investigated possessed strong antiradical activity. Quercetin, catechin and epicatechin showed maximum activity (respectively, IC DPPH• 50 5.5, 6.7 and 6.8 M, and IC ONOO• 50 48.8, 55.7 and 56.7 M). Recent reports indicate a wide range of biological activities, e.g. radioprotective effects, the prevention of cataract antihyperglycemic effects the enhancement of postprandial lipemia, the modulation of the expression of antioxidant enzyme systems, the inhibition of the protein kinase activity of the epidermal growth factor receptor, protective effects against oxidative damage in mouse brain cells, and anti-inflammatory effects. The high efficiency of natural phenolic extracts obtained from grape seeds as potent antioxidants was confirmed by the fact which encourages the

prospect of their commercialization as natural powerful antioxidants in foods in order to increase the shelf-life of food by preventing lipid peroxidation and protecting from oxidative damage. Many of the grape seed products are commercially available. Flavonoids from citrus that have been extensively studied for anti-oxidative, anti-cancer, anti-viral, and anti-inflammatory activities, effects on capillary fragility, and an observed inhibition of human platelet aggregation. The citrus fruits possess another health benefit phytochemicals called limonoids, highly oxygenated triterpenoid. Citrus limonoids appear in large amounts in citrus juice and citrus tissues as water soluble limonoid glucosides or in seeds as water insoluble limonoid aglycones. Currently, limonoids are under investigation for a wide variety of therapeutic effects such as antiviral, antifungal, antibacterial, antineoplastic and antimalarial.

Anthocyanins exhibit antioxidant activities and therefore, may contribute to the prevention of heart disease (Hou, 2003; Bagchi *et al.*, 2004). Berries have been known to contain anthocyanin pigment abundantly and thus, have been used globally as a medicine or a source of health food/dietary supplement. Consequently, the antioxidant activity may be different among various berry extracts, in particular, the berry anthocyanin extracts in the commercial market (Nakajuma *et al.*, 2004).

Extraction of anthocyanin from waste of different fruits, vegetables and flowers

Waste from industrial processes, such as wine or juice production is an excellent source of anthocyanin pigment which could possibly be utilized as a food colourant. An

overview of the techniques employed in extracting the anthocyanin is made in Table 9. Absolute ethanol was used to facilitate subsequent concentration steps. Citric acid chelates techniques employed in extracting the anthocyanin. Metals may have an added protective effect throughout the processing of the spray dried powder. It is less corrosive than HCl and would still act to stabilize the anthocyanin structure in the cationic form by maintaining a low acid pH. The citric acid was added to the single strength extract such as that a 10 to 1 concentrate would have a pH of 3-3.2. This pH was chosen because the dried powders were used in colouring low acid food products.

Different varieties of grapes, as well as different extraction techniques, are used for the production of grape skin extract. Grape skin extract is available as a liquid or power and both versions are water soluble. The hue of the extract is pH and concentration dependent. While using methanol as a solvent, the methanol in the pigment solution can be removed by distillation and the resulting aqueous solution absorbed on an Amberlite C G-50 resin. The resin absorbs the anthocyanin and many of the impurities can be rinsed-off the column with water.

Solvent extraction time, size of ground hulls, pH of extracting solvent, hull/ solvent ratio and concentration of SO₂ in water were significant factors affecting yield of extracted anthocyanins. Ethanol-acetic acid-water was more effective extractant than acetic acid.

Lycopene is much more widely distributed in fruits and vegetables than other pigments. It is predominantly found in

Table 8: Total polyphenols and tannin content in fruits seed and peel (mean ± SD)

Fruit variety	Total polyphenols mg catechin × 100 g ⁻¹ d.w.		Tannins mg catechin × 100g ⁻¹ dw	
	Seeds	Peels	Seeds	Peels
Gooseberry	800.7±30	698.7±11.9	260.5±4.1	282.5±32.5
Watermelon	969.3±16.4	335.7±20.8	11.0±10.0	0
Apple (Idared)	345.0±32.1	1790.5±27.5	647.3±14.8	7420.7±90.2
Apple (Sampion)	702.5±8.3	1613.7±11.3	20.5±0.7	1053.5±20.4
Plum (Renkloda Ulena)	436.8±4.2	334.0±8.7	24.0±0.5	0
Plum (W [^] gicrka zwykla)	147.3±3.0	578.8±13.8	13.25±0.4	41.5±17.0
Melon (Galia)	57.2±2.6	466.5±8.8	0	0
Red grapes (Alphonsc Lavallec)	9207.5±46.0	5159.2±19.6	5577.2±26.1	1410.3±88.0
White grapes (Uva da Tavola)	8220.2±60.3	3794.5±32.9	3860.0±367.0	937.2±35.0
Lemon (Primofiori)	158.8±0.7	966.2±16.5	0	0
Red grapefruit (Slar Ruby Citra)	222.5±14.5	557.7±10.9	70.25±26.5	0
White grapefruit (Apemar) Grejprut biaty	205.5±6.1	528.8±12.5	77.3±26.5	61.25±17.3
Kiwi fruit (Hayward)	102.0±2.5	1161.0±13.1	0	136.0±4.0
Orange (Midnight)	212.0±84	849.3±21.8	0	0

Source: Chodak *et al.*, 2007.

chromoplast of the plant tissues and occur as the major pigment in red, fresh tomatoes, as well as in canned, condensed or processed tomato preparation.

The waste from fruits and vegetable processing industries being rich in polysaccharides (cellulose, hemicellulose and lignin) can be subjected to solid state fermentation (SSF) for the production of ethanol which has several uses (Badger and

Ethanol

Table 9: Comparison of anthocyanin extraction techniques from different waste

Type of waste	Extraction Techniques	Source
Fruits		
Grape	Absolute ethanol (100%) with 0.1% HCl	Heidari <i>et al.</i> , 2006
Grape	Methanol with citric acid (0.01%)	Clydesdale <i>et al.</i> , 1978
Concord grape	10L Absolute ethanol (100%) with 0.01% citric acid	Main <i>et al.</i> , 1978
<i>Vitis vinifera</i> var Grenache noir	Methanol 1-M with HCl (99:1 v/v).	Sarni-Manchado <i>et al.</i> , 1996
Blue grapes	Acidified methanol (75ml 3M HCl + 425 ml methanol)	Thakur and Arya, 1989
Concord grape	Methanol acidified with 0.01 % citric acid	Calvi and Francis, 1978
Blueberries	MeOH/ Formic acid/ Water (70/2/28)	Gao and Mazza, 1994
Bilberry, rabbiteye, blueberry and blackcurrant	90% ethanol with 0.1% H ₂ SO ₄ Filtrates collected after centrifugation were applied to non-ionic polymeric absorbent. Then, elution with acidified ethanol (0.05% citric acid)	Jun – ichiro Nakajima <i>et al.</i> , 2004
Blackberries	Methanol acidified with 0.1 % HCl	Julin <i>et al.</i> , 1992
Blackberries	Liquid nitrogen powder + acetone: water (70/30 v/v) + acetone: chloroform at 1:2 v/v	Chiang and Wrolstad, 2005; Rodriguez-Soana and Wrolstad, 2001
Black raspberry	Dichloromethane-methanol (1:1 v/v)	Tian <i>et al.</i> , 2005.
Tart cherry (<i>Prunus cerasus</i>)	Homogenized with water at 10,000g for 10 min at 4°C	Amitabh <i>et al.</i> , 1992
Strawberry	Polyvinyl-pyrrolidone (PVPP) resins with water were used for isolating anthocyanin. Then, anthocyanin were extracted from the resin by methanol with 0.1% HCl	Skrede <i>et al.</i> , 1992; Wrolstad and Putnam, 1969
Cranberry	Methanol with 0.03% HCl at a 5:1 solvent:pomace ratio	Jackman <i>et al.</i> , 1996
Lychee	Acidified ethanol (1.5 N HCl: 95% ethanol; 15:85 v/v)	Lee and Wicker, 1991
Fig (<i>Ficus carica</i> L.)	Acetone and 0.1N NH ₄ OH (9:1 v/v). Re-extraction with 1:1 (v/v) acetone + diethyl ether	Antoine <i>et al.</i> , 1976
Vegetables		
Sweet potato	1% HCl in water	Bassa and Francis, 1987
Red radish cv. Fuego and Red fleshed potato tuber	1. Chemical purification : Acetone/ Chloroform 2. Juice processing	Guisti and Wrolstad, 1996a; Rodriguez-Soana <i>et al.</i> , 1999
Red radish	Samples added with liquid nitrogen powder then added to 2L acetone/ Kg of skins. Further re-extraction with acetone 30:70 (v/v). Filtrates combined with chloroform (1:2 acetone: chloroform v/v)	Hong and Wrolstad, 1990.
Flower		
<i>Tradescantia pallida</i>	0.1% HCl with water. Acid extract was purified using a cation exchange resin. Then, pigment were eluted from column with 0.1% HCl in methanol	Zulin <i>et al.</i> , 1992
Purple sunflower seeds	3 solvents system used i.e. (50:1:49) ethanol-acetic acid-water (EAW), 0.01M acetic acid (AAc) or water containing SO ₂	Gao and Mazza, 1996
Zebrina	0.1% HCl with water	Teh and Francis, 1988
Roselle (<i>Hibiscus sabdariffa</i>)	Water extraction	Esselen and Sammy, 1973

Broder, 1989, Jarosz, 1988). It can be used as a liquid fuel or liquid fuel supplement and as a solvent in many industries.

Traditionally, alcohol is produced from liquid or liquid mash *via* submerged microbial fermentation. In recent years, there has been a considerable interest in the production of alcohol from food processing wastes such as apple pomace because of 1.) the rising energy costs of molasses ii) the negative cost of values of wastes as substrates. Apple pomace is not readily amenable to submerged microbial fermentation due to its nature. But solid state fermentation of apple pomace offers several advantages for ethanol production such as higher yield but has one difficulty of ethanol extraction from the solid materials. Different microorganisms have been used for the production of ethanol, predominantly yeast belonging to *Saccharomyces cerevisiae* has been a micro-organism of choice (Joshi and Sandhu, 1996). A detailed process for production of ethanol from apple pomace has been illustrated by Joshi *et al.* (1999). Natural fermentation of apple pomace was inferior to the yeast inoculated fermentation for ethanol, crude and soluble proteins. The production of ethanol in natural fermentation was almost half than that of *Saccharomyces cerevisiae* fermented apple pomace. Partial aseptic and anaerobic conditions were provided to the solid state fermentation of apple pomace by addition of SO₂ and found that addition of SO₂ upto 200 ppm increased the ethanol content by *Saccharomyces cerevisiae* while it was 150 ppm for *Candida utilis* and *Torula utilis* (Hang *et al.*, 1981). The original pH and the initial moisture content of apple pomace was found to be suitable for ethanol production, decreasing the pH or increasing the moisture content reduced the ethanol content. Fermentation time increased the ethanol production upto 96 hrs at 30°C and among the different nitrogen sources tried ammonium sulphate gave the highest ethanol production and *Saccharomyces cerevisiae* giving better response to it than *Candida utilis* and *Torula utilis* (Joshi and Devrajan, 2008). Addition of 0.4% of ammonium sulphate increased the ethanol yield. The combined effect of AMS and ZnSO₄, however was detrimental to ethanol production but AMS alone gave better ethanol yield. It was found that all the yeast fermented apple pomace distillates contained methyl and butyl alcohols, and aldehyde. *Saccharomyces cerevisiae* fermented distillate had more desirable characteristics than those obtained from fermentation with other yeasts and thus, had potential for conversion into potable alcohol. The distillate obtained from *Saccharomyces* fermented apple pomace had more desirable characteristics. After fermenting apple pomace with *Saccharomyces cerevisiae* for ethanol production, four methods i.e. hydraulic pressing, direct distillation, steam distillation and vacuum distillation of fermented apple pomace were applied for separation of ethanol. Out of these, the steam

distillation was found to be the best as it induced minimum alteration in the fermented apple pomace (Devrajan, 1997, Joshi and Devrajan, 2008). Apple, pear and cherry wastes have also been utilized for production of ethanol. Waste from processing of orange can be employed for production of ethanol. Orange peels after enzymatic hydrolysis was found suitable to SSF by using *Saccharomyces cerevisiae* for ethanol production (Converti *et al.*, 1989).

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