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# Effect of Processing on Phenolic Antioxidants of Fruits, Vegetables, and Grains—A Review

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*Understanding the influence of processing operations such as drying/dehydration, canning, extrusion, high hydrostatic pressure, pulsed electric field, and ohmic heating on the phytochemicals of fruits, vegetables, and grains is important in retaining the health benefiting properties of these antioxidative compounds in processed food products. Most of the previous investigations in the literature on the antioxidants of fruits, vegetables, and grains have shown that food-processing operations reduced the antioxidants of the processed foods, which is also the usual consumer perception. However, in the last decade some articles in the literature reported that the evaluation of nutritional quality of processed fruits and vegetables not only depend on the quantity of vitamin C but should include analyses of other antioxidant phytochemicals and antioxidant activity. Thermal processing increased the total antioxidant activity of tomato and sweet corn. Most importantly, analysis also depends on the condition, type, and mechanism of antioxidant assays used. This review aims to provide concise information on the influence of various thermal and nonthermal food-processing operations on the stability and kinetics of health beneficial phenolic antioxidants of fruits, vegetables, and grains.*

**Keywords** Anthocyanin, antioxidant activity, flavonoids, food processing, phenolics, phytochemicals

## INTRODUCTION

Phytochemicals are bioactive non-nutrient plant compounds in fruits, vegetables, grains, and other plant foods with health benefits (Liu, 2004). These compounds prevent many chronic diseases associated with cancer, inflammation, atherosclerosis, and aging caused by free radicals (Liu, 2003, 2004; Zern et al., 2005). Consumption of phytochemical rich foods maintains higher antioxidant levels in blood serum (Cao et al., 1998a, 1998b). Consumption of fruits and vegetables has been linked to lower risks of lung, colon, breast, cervical, esophageal, oral cavity, stomach, bladder, pancreatic, and ovarian cancers (Block et al., 1992). However, most of the food items have to be processed before consumption for better digestion and metabolism in the human digestive system.

Food-processing operations are primarily focused to inactivate disease causing microorganisms (pathogens) and enzymes to reduce moisture content to concentrate the processed foods, or to soften the outer tissue to separate out fruit/vegetable

skin. However, several changes including appearance, composition, nutrition, and sensory properties can occur during processing in the processed food in terms of color, texture, and flavor. Generally, food-processing procedures are recognized as one of the major factors on the destruction or changes of natural phytochemicals, which may affect the antioxidant capacity in foods (Nicoli et al., 1999). Vitamin C has been commonly considered as a marker of nutritional quality of processed fruits and vegetables (Dewanto et al., 2002a). It was reported that vitamin C contributed less than 0.4% of the total antioxidant activity in apples (Eberhardt et al., 2000). The above study suggested that phytochemicals present in free and conjugated/bound forms contribute toward the total antioxidant activity of fruits and vegetables. Recent research has now established that food processing operations have positive effects that improve the quality and health benefits of foods. For example, processed tomato and sweet corn exhibited higher antioxidant activities than fresh ones due to the increased release of bound phenolic compounds in the food matrices (Dewanto et al., 2002a, 2002b) despite loss in vitamin C content. Similarly, both hydrophilic and lipophilic-ORAC values in cooked tomato are significantly higher than raw tomato (Wu et al., 2004a, 2004b). Significant increases in

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hydrophilic-ORAC but decreases in lipophilic-ORAC values are reported in baked russet potatoes compared to raw ones (Wu et al., 2004a, 2004b). However, in some studies, lipophilic and hydrophilic-ORAC values of raw broccoli and carrots are significantly higher than that of cooked broccoli and carrots (Wu et al., 2004a, 2004b). Interestingly, cooking in boiling water decreases the radical scavenging activity of peppers, whereas microwave heating without water increases the activity (Chuah et al., 2005). Study of antioxidant activity of grains during processing is somewhat different than fruits and vegetables because phenolics are localized in outer layers (husk, pericarp, testa, and aleuronic cells) of grains more than in any other component. About 80% of the trans-ferulic acid in rye and wheat was observed in the bran (White and Xing, 1997). Similarly, freeze-dried fractions of durum wheat (*Triticum durum*) bran exhibit stronger antioxidant activity than extracts from other milling fractions (Onyeneho and Hettiarachchy, 1992). Based on the above literatures food processing can alter antioxidant activity in both positive and negative ways. In the last decade, several reviews by Nicoli et al. (1999), Klein and Kurilich (2000), and Kaur and Kapoor (2001) provided brief information on the antioxidant activity as influenced by food processing operations. Some authors also reported that complex mixtures of phytochemicals in whole foods are responsible for their health benefits and are better than single antioxidants due to a combination of additive and/or synergistic effects (Eberhardt et al., 2000; Liu, 2004).

Antioxidant activity is correlated with the occurrence of phytochemicals including phenolics, flavonoids, and anthocyanins in foods (Sun et al., 2002). In the last decade, scientific literature has critically evaluated and summarized the health benefits of anthocyanins in fruits and vegetables (Clifford, 2000; Prior, 2003; Stintzing and Carle, 2004). Anthocyanins have many applications for regulatory authorities to check adulteration as well as in food industries. For example, prune juice if adulterated with other fruit juices will show increased levels compared to anthocyanins present in prune juice alone (Van Gorsel et al., 1992). Adulteration of blackberry jams with strawberries can be detected with the profile analysis of pelargonidin and cyanidin-3-glucoside (Garcia-Viguera et al., 1997). In red raspberry juices, it was found that underprocessed samples had higher levels of polymeric color instead of the monomeric anthocyanin pigments, which is considered as a poor quality product (Altamirano et al., 1992).

From the available literatures, it is apparent that genetics, environment (location), and growing conditions (moisture, fertilization, pests and disease burden, etc.) affect the level of antioxidant activity of phytochemicals besides processing methods and storage in fruits and vegetables (Dewanto et al., 2002a; Reyes et al., 2004; Blessington, 2005). For example, potatoes grown in sandy soils have highest intensity of color in their skin and flesh (Burton, 1989). Connor et al. (2002) reported that there is a significant interaction between the antioxidant capacity and year-wise genotype of blueberry cultivars. Similarly, Alsaikhan et al. (1995) reported that the

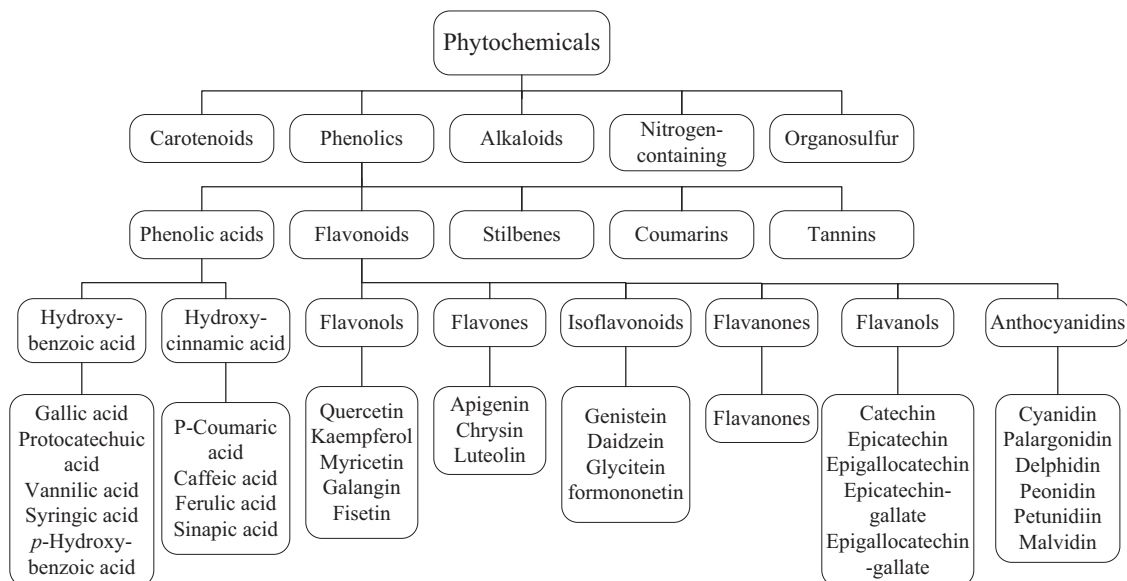
phenolic content and antioxidative activity of four potato cultivars was genotype-dependent and not related to flesh color.

Thus, the evaluation of food-processing operations influencing the antioxidant activity in processed foods is imperative in optimizing the conditions to increase or retain their availability and functionality. Over the past few decades, there has been an increased concern for nutritional values of processed foods in addition to preservation and microbiological safety. Application of kinetic models in thermal processing of foods is important to assessing and predicting the influence of operations/processing on critical quality parameters to minimize the undesirable changes and to optimize quality of specific foods. This review provides concise information on the effect of thermal and nonthermal food-processing operations on the stability and kinetics of phenolic antioxidants of processed fruits, vegetables, and grains.

### **PHENOLIC ANTIOXIDANTS OF FRUITS, VEGETABLES, AND GRAINS**

Antioxidants are the compounds, when present at low concentrations compared to those of an oxidizable substrate significantly delay or inhibit oxidation (process of losing electrons) of that substrate (Halliwell and Gutteridge, 1990; Liu, 2004; Liu and Finley, 2005). These antioxidants reduce localized oxygen concentration, prevent initiation of oxidation, inhibit radical oxygen species by directly scavenging free radicals such as superoxide (an oxygen-centered radical), thiyl (a sulfur-centered radical), trichloromethyl (a carbon-centered radical), and nitric oxide, singlet oxygen quenchers, peroxide decomposers, enzyme inhibitors, or synergists such as metal chelating agents or reducing agents (Namiki, 1990; Liu and Finley, 2005). The mechanism for the protective effect of these phenolic antioxidants to chronic diseases has been recently reviewed (Kinsella et al., 1993; Liu and Finley, 2005). Antioxidative phytochemicals in plants are broadly classified as carotenoids, phenolics, alkaloids, nitrogen-containing, and organosulfur compounds (Figure 1) (Liu, 2004). Lachman et al. (2005) reported that antioxidant activities in colored potatoes are mainly due to the presence of polyphenols/flavonoids, carotenoids, ascorbic acid, tocopherols, alpha-lipoic acid and selenium. Fruits (Sun et al., 2002; Shin et al., 2006; Wolfe et al., 2008), vegetables (Chu et al., 2002; Jiratanan and Liu, 2004; Yang et al., 2004; Song et al., 2010), and grains (Adom and Liu, 2002; Adom et al., 2003, 2005; de la Parra et al., 2007; Okarter et al., 2010; Zhang et al., 2010) with their quantity of phytochemicals are summarized in Tables 1–3.

Phenolics are products of secondary metabolism in plants that protect against pathogens, parasites, and predators besides helping in reproduction and growth of plants (Liu, 2004; Liu and Felice, 2007). Structures of some of the plant phenolic compounds with antioxidant activity are shown in Figure 2. Many of these phenolic compounds have been found to be



**Figure 1** Classification of Phytochemicals. Adapted and modified from Liu and Felice (2007).

more powerful antioxidants than vitamins C, E, and  $\beta$ -carotene using an in vitro model for heart disease, namely, the oxidation of low density lipoproteins. Hydroxybenzoic acid and hydroxycinnamic acid derivatives are major phenolic acids present in bound form in plant cells (Figure 3) (Liu, 2007). Hydroxybenzoic acid derivatives occur as sugar derivatives and organic acids in plant foods besides present in lignins and hydrolysable tannins, whereas hydroxycinnamic acid derivatives are present in cellulose, lignin, and proteins through ester bonds. Food-

processing operations such as thermal processing, fermentation, and freezing release these bound phenolic acids (Dewanto et al., 2002a). Alsaikhan et al. (1995) reported that antioxidant activity of broccoli was highest followed by potato, carrot, onion, and bell pepper using  $\beta$ -carotene/linoleic acid assay. Major antioxidant compounds in some common fruits, vegetables, and grains are shown in Table 4. Studies on 23 vegetables showed highest total phenolic content and antioxidant activity (LDL oxidation) per fresh weight

**Table 1** Phytochemicals and antioxidant activity of common grains

Item	Phenolics content ( $\mu\text{g}$ gallic acid eq/g)			Ref.	Antioxidant activity ( $\mu\text{mol}$ Vit C/100 g)			Ref.	Anthocyanin content ( $\mu\text{g/g}$ )	Ref.
	Free	Bound	Total		Free	Bound	Total			
Barley	—	—	450–1,346	a	—	—	—		4 (blue)	b
Rice	2.1	3.4	5.56 $\mu\text{mol/g}$	c	1,600	3,900	5,500	c	2,283 (black)	b
	—	—	197–376	a					944 (black)	g
Sorghum			385–746	h	—	—	—		93 (pink)	b
Maize	—	—	601	a	—	—	—		558 (red)	b
									225 (blue)	b
									965 (purple)	b
Wheat	1.9	6.1	7–10 $\mu\text{mol/g}$	c; e; f	800	6,800	7,600	c	106–153 (blue)	b
	—	—	1,342	a					13–139 (purple)	b
Corn	2.12	13.4	15.5 $\mu\text{mol/g}$	c	2,400	15,700	18,100	c	5 $\mu\text{g/g}^*$ (yellow)	d
	347–500	2,050–2,700	2,430–3,200 $\mu\text{g/g}^*$	d					13 $\mu\text{g/g}^*$ (white)	d
									97 $\mu\text{g/g}^*$ (red)	d
									368 $\mu\text{g/g}^*$ (blue)	d
Oat	1.77	4.7	6.5 $\mu\text{mol/g}$	c	3,100	4,300	7,400	c	—	
	—	—	472	a						
Rye	—	—	1,362–1,366	a	—	—	—		—	

Adapted from a: Mattila et al. (2005); b: Abdel-Aal et al. (2006); c: Adom and Liu (2002); d: de la Parra et al. (2007); e: Adom et al. (2003); f: Okarter et al. (2010); g: Dyke and Rooney (2007); h: Hahn et al. (1983).

\*Dry weight basis.

**Table 2** Phytochemicals and antioxidant activity of common fruits

Item	Phenolics content (mg gallic acid eq/100 g)			Ref.	Total antioxidant activity			Anthocyanin content (mg/100 g)	Ref.
	Free	Bound	Total		$\mu\text{mol Vit C}/100 \text{ g}^a$	$\mu\text{mol TE}/100 \text{ g}$ (ORAC assay)	$\mu\text{mol TE}/100 \text{ g}$ (DPPH assay <sup>c</sup> )		
Cranberry	507	20	527	a	177	8,394 <sup>b</sup>	—	140	e
	—	—	287	b				66	f
Apple	272	24	296	a	97	4,592 <sup>b</sup>	1,400 (red)	1.3–12	e
	—	—	156	b		218 <sup>d</sup>		1.7 (red)	f
Grape	182	19	201 (red)	a	64 (red)	2,605 <sup>b</sup> (red)	1,700 (red)	27 (red)	e
	—	—	161 (red)	b		739 <sup>d</sup> (red)	1,200 (green)	120 (Concord)	e
						446 <sup>d</sup> (white)		37 (red)	f
Strawberry	147	13	160	a	64	8,348 <sup>b</sup>	—	21	e
	—	—	235	b					
Lemon	66	15	81	a	42	1,848 <sup>b</sup>	—	—	
	—	—	51	b					
Peach	65	19	84	a	49	2,235 <sup>b</sup>	—	4.8	e
	—	—	73	b				4.2	f
Orange	56	25	81	a	31	2,887 <sup>b</sup>	600		
	—	—	57	b		750 <sup>d</sup>			
Banana	56	34	90	a	32	565 <sup>b</sup>	1,100	—	
	—	—	54	b		221 <sup>d</sup>			
Pear	53	17	70	a	34	1,759 <sup>b</sup>	600	—	
	—	—	95	b		134 <sup>d</sup>			
Pineapple	40	54	94	a	17	1,055 <sup>b</sup>	—	—	
	—	—	78	b					
Grapefruit	30	19	49	a	24	1,640 <sup>b</sup>	-	5.9 (red)	f
	—	—	71	b					
Wild Blueberry	—	—	429	b	—	9,621 <sup>b</sup>	—	486	e
Wild Blackberry	—	—	412	b	—	6,221 <sup>b</sup>	—	245–300	e
Pomegranate	—	—	338	b	—	4,479 <sup>b</sup>	—	—	
Blueberry	—	—	285	b	—	4,826 <sup>b</sup>	—	386	e
								218	f
Plum	—	—	239	b	—	5,661 <sup>b</sup>	2,200 (red)	19	e
						949 <sup>d</sup> (red)	2,000 (black)	124 (black)	e
								25 (dark)	f
Raspberry	—	—	239	b	—	5,292 <sup>b</sup>	—	687 (black)	e
								92 (red)	e
								39–54	f
Cherry	—	—	151	b	—	5,945 <sup>b</sup>	800	122	e
								66	f
Nectarine	—	—	66	b	—	1,586 <sup>b</sup>	1,200	6.8	e
								2.4	f
Mango	—	—	62	b	—	1,164 <sup>b</sup>	—	—	
Kiwifruit	—	—	60	b	—	1,262 <sup>b</sup>	1,000	—	
						602 <sup>d</sup>			
Avocado	—	—	24	b	—	1,343 <sup>b</sup>	—	—	
Cantaloupe	—	—	16	b	—	237 <sup>b</sup>	200	—	
Honeydew	—	—	15	b	—	274 <sup>b</sup>	—	—	
Watermelon	—	—	14	b	—	1,385 <sup>b</sup>	100	—	
						97 <sup>d</sup>			
Elderberry	—	—	—	—	—	—	—	1,375	e
Blackcurrant	—	—	—	—	—	—	—	476	e
								201	f

Adapted from a: Sun et al. (2002); b: Wolfe et al.(2008); c: Miller et al. (2000); d: Wang et al. (1996); e: Wu et al. (2006); f: Koponen et al. (2007).

in beans (kidney and pinto) (Vinson et al., 1998). The main antioxidative components in cereal grain are classified as phenolic compounds such as anthocyanins, tannins, and ferulic acid, and other substances (Liu, 2007). White and Xing

(1997) reported the presence of *p*-hydroxybenzoic, protocatechuic, vanillic, trans-*p*-coumaric, (*p*-hydroxyphenyl)-acetic, syringic, trans-sinapic, caffeic, and ferulic acids, with ferulic acid as the most abundant phenolic acid in oat flour. In

**Table 3** phytochemicals and antioxidant activity of common vegetables

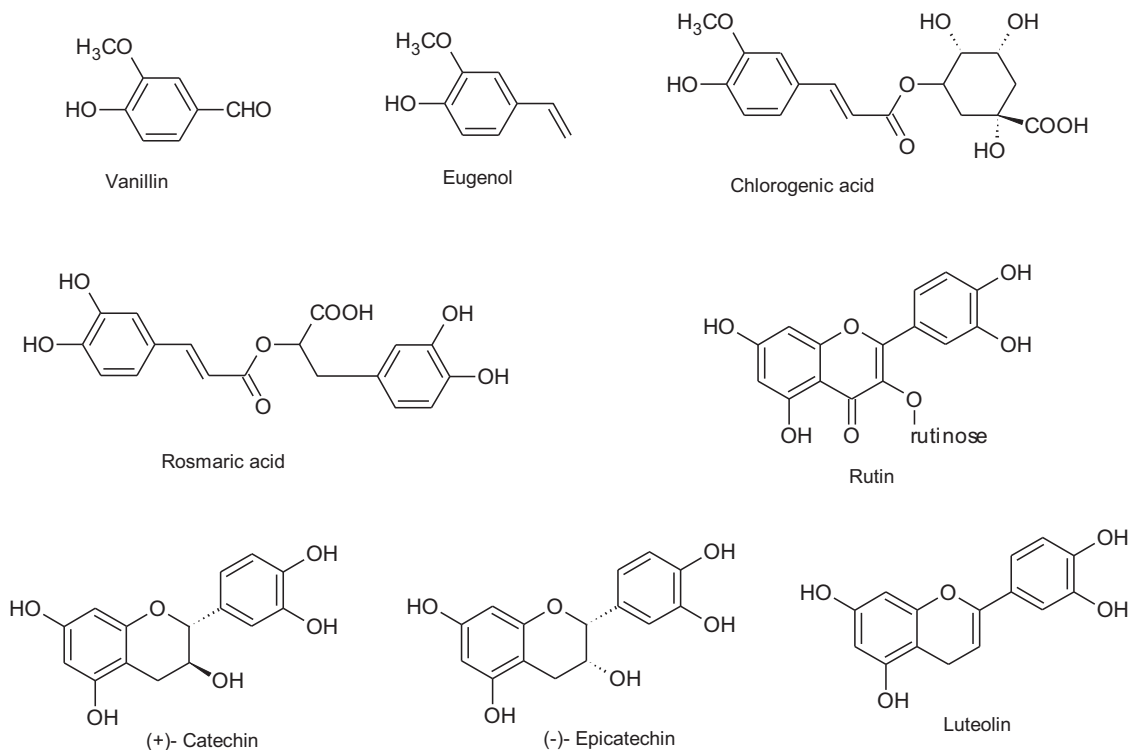
Item	Phenolics content (mg gallic acid eq/100 g)			Ref.	Total antioxidant activity			Anthocyanin content (mg/100 g)	Ref.
	Free	Bound	Total		$\mu\text{mol Vit C}/100 \text{ g}^{\text{a}}$	$\mu\text{mol TE}/100 \text{ g}$ (ORAC assay)	$\mu\text{mol TE}/100 \text{ g}$ (DPPH assay <sup>d</sup> )		
Broccoli	81	21	102	a	4,403	1,631 <sup>c</sup>	600	—	
			126	c		1,290 <sup>e</sup>			
Spinach	80	11	91	a	4,220	2,605 <sup>c</sup>	500	—	
			151	c		1,700 <sup>e</sup>			
Onion	69	7	76 (yellow)	a	1,409	736 <sup>c</sup> (yellow)	200 (yellow)	48 (red)	f
			17–115 (all)	b	500–4,550 <sup>b</sup>	404 <sup>c</sup> (white)			
			52 (yellow)	c		560 <sup>e</sup>			
			24 (white)	c					
Red sweet pepper	59	6	65	a	4,695	802 <sup>c</sup>	—	—	
			138	c					
Cabbage	37	18	55	a	1,797	1,359 <sup>c</sup>	1,400 (red)	322 (red)	f
			45	c		480 <sup>e</sup>	140 (green)		
Carrot	35	21	56	a	4,256	677 <sup>c</sup>	200	—	
			31	c		340 <sup>e</sup>			
Potato	23	15	38	a	486	397 <sup>c</sup>	400 (white)	11–60 (blue)	g
			24	c		460 <sup>e</sup>	305 (red)		
Lettuce	22	6	28	a	273	202 <sup>c</sup>	150	2.2 (red)	f
			13	c		410 <sup>e</sup>			
Cucumber	14	6	20	a	128	152 <sup>c</sup>	100	—	
			9	c		110 <sup>e</sup>			
Celery	15	6	21	a	508	223 <sup>c</sup>	50	—	
			14	c		110 <sup>e</sup>			
Beet	—	—	131	c	—	1,909 <sup>c</sup>	800	—	
						1,170 <sup>e</sup>			
Asparagus	—	—	83	c	—	1,879 <sup>c</sup>	—	85	f
Brussels sprout	—	—	109	c	—	1,859 <sup>c</sup>	500	100 (red)	f
						1,580 <sup>e</sup>			
Eggplant	—	—	87	c	—	1,755 <sup>c</sup>	—	—	
Radish	—	—	42	c	—	1,442 <sup>c</sup>	—	—	
Squash	—	—	24	c	—	1,107 <sup>c</sup>	—	—	
Sweet potato	—	—	31	c	—	732 <sup>c</sup>	—	—	
Cauliflower	—	—	48	c	—	700 <sup>c</sup>	200	—	
						510 <sup>e</sup>			
Green pea	—	—	21	c	—	619 <sup>c</sup>	—	—	
Green bean	—	—	14	c	—	219 <sup>c</sup>	—	—	
Tomato	—	—	21	c	—	271 <sup>c</sup>	—	—	

Adapted from a: Chu et al. (2002); b: Yang et al. (2004); c: Song et al. (2010); d: Miller et al. (2000); e: Cao et al. (1996); f: Wu et al. (2006).

another study, (Li et al., 2005) reported that Chinese black-grained wheat has higher antioxidant activity compared to white and blue wheat genotypes.

Flavonoids are found as conjugates in glycosylated or esterified forms in fruits and vegetables (Liu, 2004). These types of compounds can also occur as aglycones as a result of food processing (Liu and Felice, 2007). The generic structure of flavonoids consists of 2 benzene rings (A and B rings) linked by three carbons that are usually in an oxygenated heterocycle ring or C ring. Anthocyanins are one of the most commonly found flavonoids in fruits and vegetables that attract consumers due to their beautiful colors (Figure 4). The compounds are water-soluble pigments responsible for the orange red through deep purple colors produced by chemical combination of its C6-C3-C6 structure with glycosides, acyl groups, and

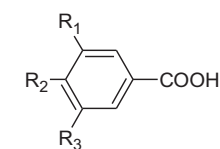
other molecules in flowers, fruits, and vegetables. Mostly, anthocyanins occur as glycosides such as 3-monoglycosides and 3, 5-diglycosides in nature. Table 5 summaries common anthocyanins present in fruits, vegetable and grains. In addition, many anthocyanins have acylations (i.e., ester bonds between sugars and organic acids) with coumaric, caffeic, ferulic, *p*-hydroxybenzoic, synapic, malonic, acetic, succinic, oxalic, and malic acids (Francis, 1989). Substitution of hydroxyl and methoxyl groups influences the color of the anthocyanins. For example, more hydroxyl groups provide bluish color, whereas methoxyl groups increase the redness of the anthocyanins. Anthocyanins are present in the vacuole of the plant cell and the molecules are protected by unique mechanisms such as self-association and copigmentation in the cell. Self-association is to form helical stacks through the



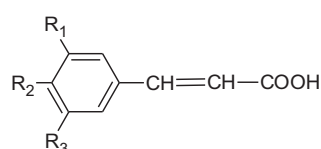
**Figure 2** Common dietary phenolics in foods.

hydrophobic attraction and hydrogen bonding between the flavylium nuclei. The stacking protects the chromophores behind the sugar groups from the hydration reaction (Hoshino et al., 1981). Copigmentation occurs through hydrogen bonding of the phenolic groups between anthocyanin and flavone molecules (Francis, 1989). Flavonols, amino acids, benzoic acids, coumaric, and cinnamic acids also react with anthocyanins due to interaction known as intermolecular copigmentation. Intramolecular copigmentation occurs in the anthocyanins

because of the acylation by organic acids. A spectral characteristic of glycosylation and acylation in potato anthocyanin is shown in Figure 5. Antioxidant activity and stability of the anthocyanin depends on the number and position of free hydroxyl groups attached to the structure and type of acylation (Patras et al., 2010). Hamouz et al. (1999) and Lachman et al. (2000) reported that purple/blue coloration of the purple potato is due to acylation of cinnamic acid to anthocyanidin giving high antioxidant activities, whereas glycosidic attachment at



Benzoic acid derivatives



Cinnamic acid derivatives

Benzoic acid Derivatives	Substitutions		
	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
Benzoic acid	H	H	H
<i>p</i> -Hydroxybenzoic acid	H	OH	H
Protocatechuic acid	H	OH	OH
Vanillic acid	CH <sub>3</sub> O	OH	H
Syringic acid	CH <sub>3</sub> O	OH	CH <sub>3</sub> O
Gallic acid	OH	OH	OH

Cinnamic acid derivatives	Substitutions		
	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
Cinnamic acid	H	H	H
<i>p</i> -Coumaric acid	H	OH	H
Caffeic acid	OH	OH	H
Ferulic acid	CH <sub>3</sub> O	OH	H
Sinapic acid	CH <sub>3</sub> O	OH	CH <sub>3</sub> O

**Figure 3** Structures of common phenolic acids.

**Table 4** Major antioxidants in some common fruits, vegetables, and grains

Source	Major antioxidants
<b>Fruits</b>	
Apple	Benzoic acid, cinnamic acid, flavan-3-ols, anthocyanidin, flavonols, dihydrochalcones
Berry	Anthocyanins, flavonols, flavanols, proanthocyanidins, ellagitannins, gallotannins, stilbenoids, phenolic acids
Grape	Resveratrol, catechin, anthocyanins, gallic acid
Grapefruit	Narirutin, hesperetin, hesperidin, ascorbic acid
<b>Vegetables</b>	
Asparagus	Rutin, chlorogenic acid, ascorbic acid
Bean	Quercetin, kaempferol glucoside, ascorbic acid
Beet	Ferulic acid
Broccoli	Caffeic acid, ferulic acid, quercetin, kaempferol, ascorbic acid
Cabbage	Chlorogenic acid, caffeic acid, kaempferol, lutein, ascorbic acid
Carrot	Chlorogenic acid, caffeic acid, $\beta$ -carotene
Cauliflower	Cinnamic acid, quercetin, ascorbic acid
Celery	Chlorogenic acid, apigenin, apigenin glucoside, luteolin glucoside, $\alpha$ -tocopherol
Corn	Caffeic acid, cinnamic acid, coumaric acid
Cucumber	Ascorbic acid
Garlic	Myricetin, apigenin, ascorbic acid
Lettuce	Chlorogenic acid, caffeic acid, quercetin
Mushroom	Ferulic acid, ascorbic acid
Onion	Quercetin glucoside
Bell Pepper	Caffeic acid, quercetin glucoside, luteolin glucoside, $\alpha$ -tocopherol, ascorbic acid
Potato	Caffeic acid, cinnamic acid, <i>p</i> -hydroxybenzoic acid, ascorbic acid
Squash	$\beta$ -carotene
Spinach	Quercetin, ascorbic acid, patuletin glucoside, spinacetin glucoside, 5, 3'-hydroxy-3-methoxy-6,7-methylenedioxyflavone-4'-glucuronide methyl ester, 5-hydroxy-3,3'-dimethoxy-6,7-methylenedioxyflavone-4'-glucuronide methyl ester
Sweet potato	Caffeic acid, cinnamic acid, $\alpha$ -tocopherol
Tomato	Chlorogenic acid, caffeic acid, quercetin, ascorbic acid
<b>Grains</b>	
Barley	Protocatechuic acid, <i>p</i> -hydroxybenzoic acid, salicylic acid, vanillic acid, syringic acid, ferulic acid, coumaric acid, sinapic acid
Corn	Lutein, $\alpha$ - and $\beta$ -carotene, $\beta$ -cryptoxanthin, zeaxanthin, tocopherols, phytosterols
Oat	Tocopherols, avenanthramides, <i>p</i> -hydroxybenzoic acid, vanillic acid, phytosterols
Rice bran	Tocopherols, $\gamma$ -oryzanol,
Rye	Protocatechuic acid, <i>p</i> -hydroxybenzoic acid, vanillic acid, syringic acid, ferulic acid, <i>p</i> -coumaric acid, caffeic acid, sinapic acid
Soybean	Isoflavones, tocopherols, tocotrienols
Sorghum	Gallic acid, Protocatechuic acid, <i>p</i> -hydroxybenzoic acid, gentisic acid, salicylic acid, vanillic acid, syringic acid, ferulic acid, caffeic acid, sinapic acid, sinamic acid
Wheat	Carotenoids, tocopherol, ferulic, vanillic, caffeic, coumaric and syringic acid, phytosterols

position 3 and 5 reduces antioxidant activities. In another study, Wang et al. (1997) reported that cyanidin glycosides tend to have higher antioxidant capacity than peonidin or malvidin glycosides because of the free hydroxyl groups on the 3' and 4' positions. Understanding anthocyanins in fruits and

vegetables such as blueberries, grape (Camire et al., 2002), and red and purple fleshed potatoes could provide alternative sources of natural colorants from their rich anthocyanins (Rodriguez-Saona et al., 1998; Lachman et al., 2005). Pericarp of the pigmented varieties of cereals such as barley, maize, wheat, rice, and rye contains most of the flavonoids. Sorghums have 3-deoxyanthocyanins, a unique anthocyanin, which is without the hydroxyl group in the 3 position of the C-ring (Dykes and Rooney, 2007).

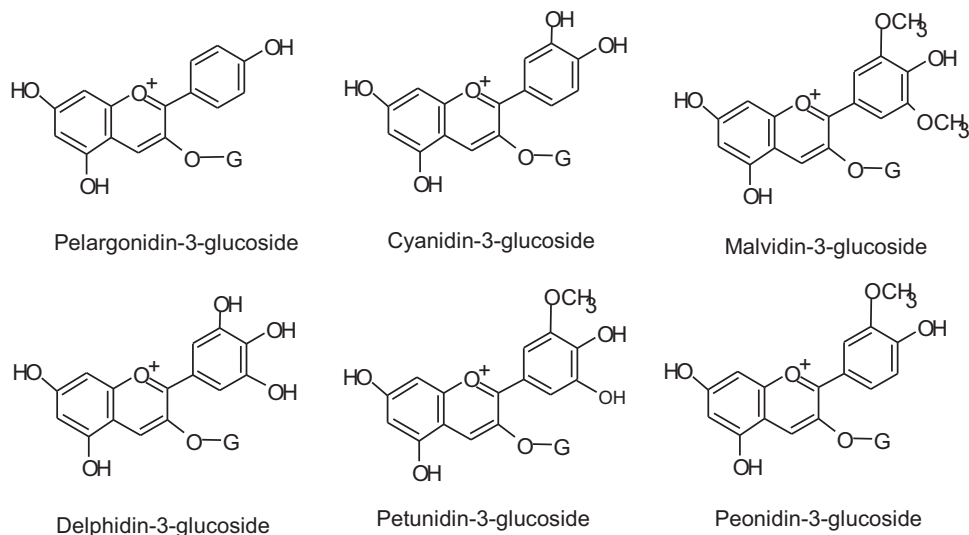
### EFFECT OF PROCESSING ON PHYTOCHEMICALS

Processing of foods involve heating with different energy transfer media such as water, air, oil, and electromagnetic waves (Figure 6). In addition, storage can also be classified as passive processing with no energy applied directly to foods. Polyphenolic compounds including anthocyanins and proanthocyanidins are not completely stable during processing (Talcott et al., 2003). Physical and biological factors such as temperature increase and enzymatic activity may result in destruction of phenolic antioxidants such as phenolic acids and anthocyanins. After harvest, these compounds can change during food processing and storage (Kader et al., 2002; Rossi et al., 2003), which may reduce related bioactivity. During processing of foods, various transformations of phenolics occur to produce yellowish or brownish pigments (Clifford, 2000). The following section discusses the effect of important food-processing operations on the phytochemicals of fruits, vegetables, and grains.

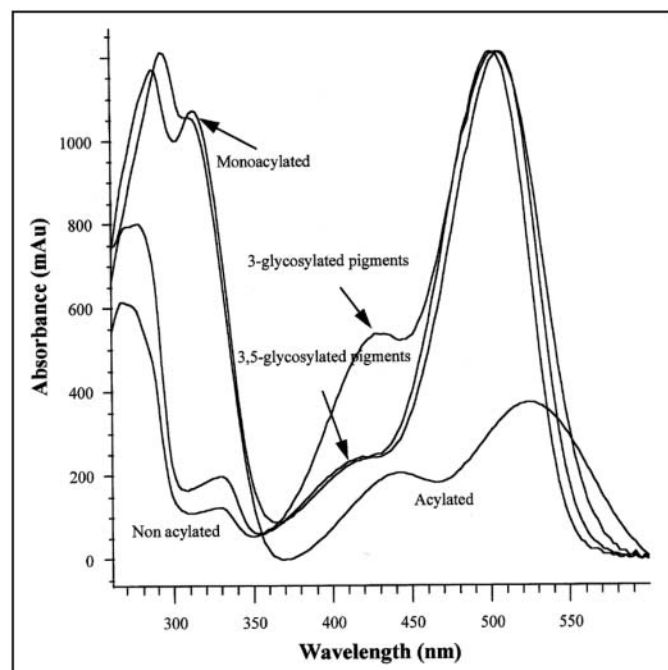
### Blanching

Blanching is an important food-processing step applied to soften the product as well as to inactivate the enzymes that otherwise could cause browning or other possible reactions in fruits and vegetables. Phenolic compounds are degraded by a number of enzymes found in plant tissue such as glycosidases, polyphenoloxidases (PPO), and peroxidases. Glycosidases produce anthocyanidins and sugars, and anthocyanidins are very unstable and rapidly degraded. PPO catalyzes the oxidation of *o*-dihydrophenols to *o*-quinones that further react to brown polymers. The effectiveness of blanching is indicated by the complete inactivation of peroxidase. The most commonly used method for blanching is by steam or hot water, where the resistance to heat transfer at the surface is negligible compared to the internal resistance to heat transfer (mechanism of heat penetration is by conduction). Thus, blanching time depends on the dimension of food matrix. For example, blanching time for small size products, such as peas, are 1–2 minutes, while for larger products, such as corn on the cob, is 11 minutes (Feinberg et al., 1968). The longer time required for the temperature rise at the cold spot or slowest heating point, normally the geometric center, of a relatively



**Figure 4** Common anthocyanins in fruits and vegetables.**Table 5** Common anthocyanins present in fruits, vegetables, and grains

Fruit/veg/grain	Major anthocyanin	Minor anthocyanins	Reference
Blackberry	Cyanidin-3-glucoside	Cyanidin-3-rutinoside; Cyanidin-3-dioxalylglucoside; Cyanidin-3-xyloside Cyanidin-3-malonylglucoside	Fan-Chiang and Wrolstad (2005); Rommel et al. (1992)
Strawberries	Pelargonidin-3-glucoside	Cyanidin-3-glucoside	Jackman et al. (1987)
Litchi pericarp	Cyanidin-3-rutinoside	Malvidin-3-acetylglucoside; Quercetin-3-rutinoside; Cyanidin glucoside; Quercetin glucoside	Lee and Wicker (1991); Sarni-Manchado et al. (2000)
Blood orange	Cyanidin-3-glucoside	Delphinidin-3,5-diglucoside; Cyanidin-3,5-diglucoside; Delphinidin-3-glucoside; Peonidin-3,5-diglucoside; Cyanidin-3-(acetyl)-glucoside; Cyanidin-3-(feruloyl)-glucoside	Krifi et al. (2000)
Acai	Cyanidin-3-glucoside	Pelargonidin-3-glycoside	Del Pozo-Insfran et al. (2004)
Bilberry	Cyanidin galactoside	Delphinidin arabinoside; Cyanidin glucoside; Delphinidin galactoside; Delphinidin glucoside; Petunidin glucoside; Malvidin galactoside; Cyanidin arabinoside; Peonidin glucoside; Malvidin arabinoside	Yue and Xu (2008)
Elderberry	Cyanidin-3-sambubioside	Cyanidin-3-glucoside; Cyanidin 3,5-diglucoside; Cyanidin-3-sambubioside-5-glucoside	Stintzing et al. (2002)
Red radish	Pelargonidin-3-sophoroside-5-glucoside		Matsufuji et al. (2007)
Black carrot	Cyanidin-3-xylosylgalactoside	Cyanidin-3-xylosylglucosyl-galactoside	Stintzing et al. (2002)
Cherry	Cyanidin-3-glucosyl-rutinoside	Cyanidin-3-glucoside; Cyanidin-3-rutinoside; Peonidin-3-rutinoside	Kim and Padilla-Zakour (2004)
Purple carrot	Cyanidin 3-(sinapoylxylosyl-glucosylgalactoside)	Malvidin 3-monoglucoside; Peonidin 3-monoglucoside	Glassgen et al. (1992)
Red cabbage	Cyanidin-3-sophoroside-5-glucoside	Cyanidin-3,5-diglucoside	Giusti et al. (1999)
Purple-fleshed potato	Petunidin-3-( <i>p</i> -coumaroyl-rutinoside)-5-glucoside	Malvidin-3-( <i>p</i> -coumaroyl-rutinoside)-5-glucoside	Lewis et al. (1998)
Red-fleshed potato	Pelagonidin-3-( <i>p</i> -coumaroyl-rutinoside)-5-glucoside	Peonidin-3-( <i>p</i> -coumaroyl-rutinoside)-5-glucoside	Lewis et al. (1998)
Sorghum	Apigeninidin	Apigeninidin 5-glucoside, 7-methoxyapigeninidin	Wu and Prior (2005)
Blue wheat	Delphinidin-3-glucoside	Delphinidin-3-rutinoside, Cyanidin-3-glucoside, cyanidin-3-rutinoside	Abdel-Aal et al. (2006)
Purple wheat	Cyanidin-3-glucoside	Peonidin-3-glucoside	Abdel-Aal et al. (2006)

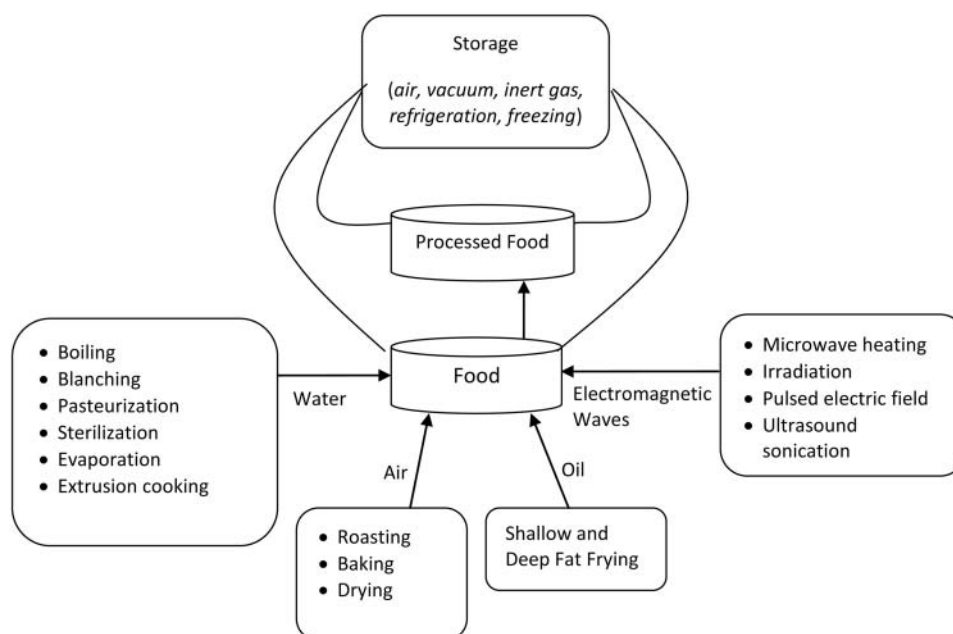


**Figure 5** Spectral characteristics of potato anthocyanins, indication of glycosylation, and acylation patterns. Modified from Rodriguez-Saona et al. (1998).

large object such as corn on the cob could damage the quality of the kernels, whereas shortening the length of blanching time could reduce the degree of enzyme inactivation and thus result in shorter shelf-life, nutritional and functional value. Thus, optimal blanching time is necessary for a particular fruit or vegetable to preserve its overall nutritional and health-promoting components.

Wolfe and Liu (2003) reported that blanching of Rome beauty apple peels in boiling water for 10–20 seconds increased the total phenolics and flavonoid contents compared to untreated samples. A blanching time of one minute in boiling water has been recommended for green leaves of sweet potato to retain antioxidant activity (Chu et al., 2000). Similarly, Nayak et al. (2011c) reported that eight minutes of saturated steam blanching prior to drum drying increase total antioxidant capacity (175%) of dry flakes when compared to raw purple potato (“Purple Majesty” cv). Blanching opens up the cell matrix and, therefore, could increase the polyphenols yield during extraction that may either enhance or reduce the antioxidant activity. Blueberry juice has higher recovery of phenolic compounds and strong radical-scavenging activity to DPPH and hydroxyl radicals after steam blanching for three minutes than the nonblanched juice (Rossi et al., 2003). But it has also been reported that blanching in water at 98°C for two minutes diminishes the antioxidant capacity of purple carrots (Uyan et al., 2004). Amin and Lee (2005) observed that five to ten minutes of blanching in hot water at 98°C reduced ( $p < 0.05$ ) antioxidant activities and phenolics content of all vegetables except for cabbage and mustard cabbage. After 15 minutes of blanching, the loss of antioxidant activity ( $\beta$ -carotene bleaching assay) was highest in Chinese cabbage (40%) followed by Chinese white cabbage (19%), mustard cabbage (9%), and red cabbage (4%). However, the total phenolic content of Chinese cabbage increased ( $p < 0.05$ ) after 15 minutes of blanching compared with other vegetables.

Mizrahi (1996) observed that two minutes of ohmic blanching of large whole vegetables had similar effects as 4 min of water blanching. The investigator also reported that the energy dissipated by the electric current passing through the samples



**Figure 6** Heating/energy-transfer medium in various processing methods.

was capable of heating it uniformly and very quickly regardless of its shape or size. In another study, ohmic blanching (25–40 V/cm) of artichoke by-product was faster at inactivating the peroxidase enzyme, without producing blanching waste water compared to hot water blanching at 85°C, thus retaining higher total phenolic content (Icier, 2010). The same investigator reported that ohmic blanching (40 V/cm) at 85°C had similar peroxidase inactivation times ( $310 \pm 2$  seconds) as water blanching at 100°C ( $300 \pm 2$  seconds). Increase in the mass transfer and effective diffusion rate has been reported after ohmic blanching of strawberries during osmotic dehydration (Allali et al., 2010).

Peeling of fruits and vegetables followed by blanching could have detrimental effects on the phenolic antioxidants. For example, peach puree containing periderm tissue blanched in boiling water (20 minutes) and pasteurized (boiling water for 30 minutes) was 7–11% higher in antioxidant activity ( $\beta$ -carotene/linoleic acid assay) than peeled samples (Talcott et al., 2000). Shorter blanching of puree with periderm in boiling water (two minutes) yielded the lowest initial antioxidant activity, but the level of retention was greater during storage than that of peeled samples. The same investigators also reported that total water-soluble phenolic compounds were higher in the case of longer blanching time than that of shorter blanching time, which could be due to increased tissue softening and enhanced chemical extraction with the additional heat applied prior to pasteurization.

Understanding the mechanism and critical points of anthocyanins destruction by enzymatic activity could be important in the design of an extraction procedure and perhaps in the final formulation in a food (Francis, 1989; Rossi et al., 2003). Juice prepared from blanched blueberry-pulp extract degrades anthocyanins, whereas unblanched extract cause 50% loss of anthocyanins (Skrede et al., 2000). Similarly, blanching of blueberry for three minutes using steam induce higher anthocyanin retention, i.e., 23% instead of 12% (unblanched) when processed into juice (Rossi et al., 2003). Anthocyanin content of blanched (98°C for two minutes) purple carrots increase 27% compared with the fresh sample (Uyan et al., 2004). Steam blanching of purple- and red-fleshed potatoes reduces the peroxidase activity by 98–99% to retain the anthocyanins (Reyes and Cisneros-Zevallos, 2007). Similarly, 90% of total anthocyanins in purple potatoes (“Purple Majesty” cv) are retained after saturated steam blanching for eight minutes (Nayak et al., 2011c). Wolfe and Liu (2003) reported that blanching (boiling water for 10 seconds) followed by oven drying retains or increases anthocyanin content of apple peels. Inactivating enzymes to reduce oxidation by using SO<sub>2</sub> to fruits and vegetables has also been reported in the literature. Cemeroglu et al. (1994) reported that addition of SO<sub>2</sub> inhibits phenolase activity in sour cherry. Similarly, heat and SO<sub>2</sub> treatments of high bush blueberries increase the recovery of anthocyanins but not of other polyphenols in the juices (Lee et al., 2002). Introduction of the blanching step has also a positive effect on the recovery of individual anthocyanin. For

example, in blueberry juice processing, the percent recovery increase by 71–2672% for monoglucosides of cyanidin, malvidin, petunidin, peonidin, and delphinidin (Rossi et al., 2003). However, in some studies, blanching applied alone or in combination with other treatments has reduced anthocyanins depending on the type of fruit or vegetable. Blanching (95°C for three minutes) in combination with pasteurization during preparation of blueberry purees reduced 43% total monomeric anthocyanins compared to fresh fruit (Brownmiller et al., 2008). Volden et al. (2008) observed 59% loss in anthocyanin content of red cabbage after blanching. Kader et al. (1999) proposed that cyanidin 3-glucoside (*o*-diphenolic) is degraded by a mechanism of coupled oxidation involving the enzymatically generated *o*-quinone with partial regeneration of the *o*-diphenolic cosubstrate confirming the role of PPO in anthocyanin degradation. In another study, pelargonidin-3-glucoside, were degraded by a mechanism involving a reaction between the *o*-quinone and/or secondary products of oxidation formed from the quinone and the anthocyanin pigment (Kader et al., 2001).

Blanching is an important step to inactivate enzymes in fruits and vegetables before applying to further processing or storage. It is apparent that increase in the anthocyanin retention in fruits/vegetables is attributed to two main factors; (1) reduction of enzyme-mediated anthocyanin degradation, i.e., complete inactivation of native PPO and (2) greater extraction yield linked to the increase of fruit skin permeability caused by the heat treatment (Kalt et al., 2000). Blanching time and temperature based on a particular heating media depend on the size and volume of food products. Exposure of fruits/vegetables to insufficient time of heating may not inactivate enzymes causing undesirable problems in downstream processes, whereas overexposure reduces the nutritional and health benefiting functional properties of treated items. Optimization of this important step using thermal and nonthermal processing techniques required to retain most of the phenolic antioxidants in fruits and vegetables.

### Cooking/Thermal Processing

Cooking of vegetables, fruits, and grains has mixed effects on phenolic antioxidants of cooked foods. For example, Sablani et al. (2010) reported that canning of raspberries (100°C, 28 minutes) and blueberries (100°C, 22 minutes) increases the phenolic content and antioxidant activity by 50% and 53%, respectively. Processing and heating during jam making (at 104–105°C) reduces the content of total phenolics of some varieties of cherries and plums, whereas no significant change ( $p < 0.05$ ) occurred in raspberries, plums, and some varieties of cherries (Kim and Padilla-Zakour, 2004). The same study also reported unpredictable changes in antioxidant activity (ABTS assay) of cherries, plums, and raspberries, depending on variety during jam processing. In contrast, the total phenolic content reduced after dehydration, while

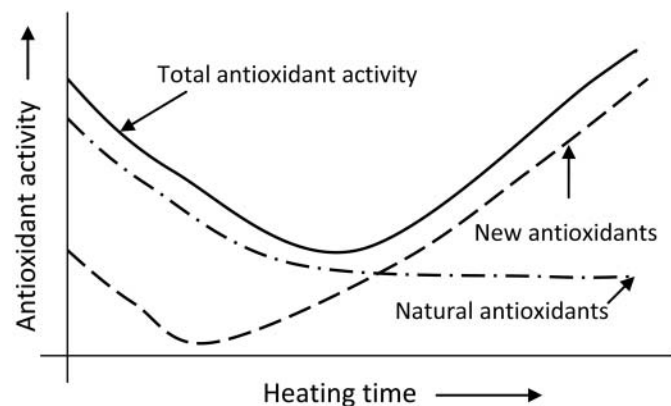
antioxidant capacity in dried plums increase when compared to that of fresh plums (Piga et al., 2003). During baking, the outer layer of food items is usually heated to over 120°C, while the inner temperature remains lower than 95–100°C. At these high temperatures of 90–120°C, chemical oxidation of phenolic antioxidants to quinones and their polymers occurs in addition to caramelization (between reducing sugars and ascorbic acid), Maillard reaction (between reducing sugar and amino acids), Strecker degradation (dicarbonyl compounds with amino acids), and hydrolysis of esters and glucosides of antioxidants. Baking of purple wheat bran at 177°C for 20 minutes has not altered the total phenolic content in the processed samples (Li et al., 2007). It was also reported that the total phenolics and total antioxidant activity of sweet corn has increased by 54 and 44%, respectively, after thermal processing at 100–121°C for 10–50 minutes (Dewanto et al., 2002b). In other studies, antioxidant activities in processed tomatoes (Re et al., 2002; Dewanto et al., 2002a; ) and coffee (Nicoli et al., 1997) were retained or higher than their fresh equivalents. The increase or retention of antioxidant activities in processed foods could be due to the development of new compounds with potential antioxidant capacity (Figure 7), although the content of naturally occurring antioxidants has significantly decreased due to the heat processing (Nicoli et al., 1997; Anese et al., 1999; Nicoli et al., 1999).

Although a decrease in the antioxidant potential is found for short heat treatments, a recovery of these properties has been reported during prolonged heat treatment. Jiratanan and Liu (2004) observed 12% reduction in the phenolic content of beets at initial application of heat (115°C for 15–30 minutes), but further processing raised its content back to the equivalent of unprocessed beets and eventually increased by 14% after processing at 115°C for 45 minutes. Similar results in the antioxidant capacity of aged citrus juice and orange juice has been reported as they became more discolored (Lee, 1992). The initial reduction in the antioxidative activity can be attributed not only to thermal degradation of naturally occurring antioxidants but also to formation of early Maillard reaction products

(MRPs) with pro-oxidant properties. The antioxidant activity of MRPs can be mainly attributed to the high molecular weight brown compounds, which are formed in the advanced stages of reaction. Antioxidant compounds depletion in thermally treated fruits and vegetables may also be attributed to consumption of ascorbic acid and polyphenols as reactants in the Maillard reaction (Kaanane et al., 1988).

Phenolic contents, including in the free and bound forms, and antioxidant activity during processing also depend on the type of fruit or vegetable. Heat treatment of table beets at 105–125°C for 15–45 minutes either retain or increase free, bound and total phenolic content, total flavonoids, and total antioxidant activity (Jiratanan and Liu, 2004). The same investigators also observed reductions in the antioxidant activity, phenolic contents, and total flavonoids (majority comes from free flavonoids) in green beans at similar processing conditions of 100–121°C for 10–40 minutes. The authors hypothesized that the processed beets and green beans would contribute little to release of bound phytochemicals in the colon by intestinal microflora to induce a site-specific reinforcement of antioxidants (Andreasen et al., 2001; Adom and Liu, 2002). It is important to understand the area and application of various antioxidant assays used to access the antioxidant activity of cooked fruit/vegetables. For example, after being heated at 90°C for 147 hours, the radical scavenging capacity (DPPH) of the Roselle pigment model system increased from 18% to 43%, and FRAP decreased from 980 to 640 mol/l, while TEAC (ABTS assay) remained around 2.2 Trolox (mmol/l) (Tsai and Huang, 2004).

Microwave cooking of vegetables do not follow a specific trend on the phenolic antioxidants. Microwave cooking (800 Watt) either retains or decreases a small quantity of the total phenolic content in cauliflower, peas, spinach, and Swiss chard, whereas significant reduction occurs after boiling (6–13 minutes) (Natella et al., 2010). The same investigators observed significant increase or retention of total antioxidant capacity in cauliflower, peas, spinach, Swiss chard, potatoes, tomatoes, and carrots despite reduction in phenolic content. (Jiménez-Monreal et al., 2009) studied the effects of microwave cooking on different radical scavenging capacity (lipoperoxyl, hydroxyl, and ABTS radicals) of vegetables. The researchers observed 30–50% losses of lipoperoxyl radical scavenging capacity in most of the vegetables; whereas artichoke, asparagus, garlic, onion, and spinach retained and eggplant, maize, pepper, and Swiss chard increased their antioxidant activity after microwave cooking (2–4.5 minutes at medium power). Microwave cooking (218°C, 30 minutes) retained 55% of the chlorogenic acids followed by 30 minutes boiling (35%) of potatoes (*cv* NDA 1725), whereas oven-baking (212°C, 45 minutes) potatoes lost all of them (Dao and Friedman, 1992). In the same study, commercially processed french-fried potatoes, mashed potato flakes, and potato skins contained no chlorogenic acid. The nature of the heat and their harshness in different cooking methods is attributed to relative loss of chlorogenic acid in the potato. Microwave cooking



**Figure 7** Changes in total antioxidant activity in vegetable matrix subjected to different processing conditions. Adapted and modified from Nicoli et al. (1999).

(600 Watt, 0.5–5 minutes) of broccoli severely degrades phenolic content and antioxidant capacity in florets and stems (Zhang and Hamauzu, 2004), with similar findings for potatoes cooked at 1000 Watt for four minutes (Tudela et al., 2002). Studies on green vegetables and herbs found that the phenolic content and antioxidant capacity increased or remained unchanged after microwave irradiation at 1000 Watt for 1–1.5 minutes (Turkmen et al., 2005). Microwave heating (1500 Watt, 4–16.2 minutes) of apple mash from 40–70°C results in an increase in phenolic and flavonoid compounds in the juice with increase in temperature (Gerard and Roberts, 2004).

At high temperatures, the structure of anthocyanin is opened to form chalcone, which is degraded further to brown products (Francis, 1989). However, it has been observed that optimal conditions permit regaining of color on cooling if there is sufficient time (several hours) for the reversion. Processing of black raspberries in canned-in-water or syrup (87.8–93.3°C for four minutes) losses 42% and 51% of total anthocyanin, respectively (Hager et al., 2008a), whereas blackberry products losses 17.8% and 10.5%, respectively, in similar condition (Hager et al., 2008b). In another study, only 50% of the total anthocyanin content of elderberry was lost after three hours of heating at 95°C (Sadilova et al., 2006). Degradation of anthocyanins occurs in strawberry (Garzon and Wrolstad, 2002), raspberry (Kim and Padilla-Zakour, 2004), and sour cherry (Cemeroglu et al., 1994) as the fruits are processed into juice, concentrate, or jam and continue to degrade during storage. The investigators also reported that the degradation of anthocyanins in concentrates was greater compared to juices. Patras et al. (2009) reported significant loss ( $p < 0.05$ ) in anthocyanin content of blackberry (3%) and strawberry (28%) puree processed at 70°C for two minutes. Anthocyanin degradation in processed berry products is attributed to indirect oxidation by phenolic quinones generated by polyphenol oxidase (PPO) and peroxidase (Kader et al., 1997; Skrede et al., 2000). In contrast, anthocyanins in Bing cherries increase slightly after canning (100°C for 12 minutes) (Chaovalikit and Wrolstad, 2004b). The increase in the anthocyanin content could be due to the increased extraction efficiency in the softened fruits. Increase in membrane permeability at high temperatures in the macerated peel tissue facilitate phenolic extraction (Spanos et al., 1990) and release of bound phenolic compounds by breakdown of the cellular constituents has also been reported in the literature (Dewanto et al., 2002a).

Boiling (94–96°C, three minutes) and steaming (10 minutes), respectively, reduce 41% and 29% anthocyanin content of red cabbage (Volden et al., 2008). Similar losses were reported in pasteurized (90°C, 1.5 minutes) blueberry products (Lee et al., 2002). In contrast, Kirca et al. (2007) reported that anthocyanins from black carrots were reasonably stable during heating at 70–90°C due to diacylation of anthocyanin structure. According to the same investigators, besides temperature, the stability of monomeric anthocyanins in black carrot juice and concentrates also depends on solids content and pH. Similarly, cyanidin-3-rutinoside has the highest stability to the

effect of thermal treatment at 70–95°C in blackcurrants (Rubinskiene et al., 2005). Greater thermal stability (25–80°C, 15 minutes to 6 hours) of anthocyanins present in red cabbage compared to blackcurrants, grape skins, and elderberries in a soft drink model system is attributed to the protection of flavylum system through copigmentation (Dyrby et al., 2001). In another study, purple-fleshed potato and grape extracts showed lower color stability than red-fleshed potatoes and purple carrots at 98°C (Reyes and Cisneros-Zevallos, 2007). Maccarone et al. (1985) studied the stabilization of anthocyanins in blood orange juice and found that microwave pasteurization and addition of tartaric acid and glutathione improved the stability. The investigators reported that complexation of anthocyanins with rutin and caffeic acid provided the highest stability.

Phenolic antioxidant activity in vitro sometimes has a different effect on the bioavailability of the compounds. For example, although heat treatments including moderate heating (IQF and freeze-drying), high temperature heating (cooking at 100°C for five minutes, canning and spray-drying) and jam preparation retain most of the phenolic content and antioxidant activity (FRAP and DPPH) of wild or cultivated blueberries found in unprocessed whole fruit, processing diminished antiproliferation activity on heap-1c1c7 cells (Schmidt et al., 2005). The changes in the antiproliferation activity on heap-1c1c7 cells are thought to be the breakdown of the active proanthocyanidin oligomers ranging from monomeric catechin units to hexamers in blueberries (Kayano et al., 2003).

### Drying/Dehydration

Drying/dehydration is one of the oldest techniques of preserving foods for future use. With this technique, water is removed to reduce water activity that diminishes the bacterial activity in the dried/dehydrated food. In addition to the safety of food during preservation, many researchers focused on the changes of phytochemicals during drying or dehydration. Saskatoon berries (“Thiessen” and “Smoky” cv.) processed using freeze-drying, vacuum microwave-drying, air-drying, and a combination of air-drying and vacuum microwave-drying caused reduction ( $p < 0.05$ ) in the total phenolics, antioxidant activities and anthocyanin contents as compared with fresh frozen berries (Kwok et al., 2004). However, freeze-drying render maximum antioxidant activity in the berries followed by vacuum microwave-drying. In another study, Vacuum microwave-drying produce dehydrated cranberry products with higher ORAC antioxidant activity followed by freeze-drying and air-drying (Leusink et al., 2010). However, the antioxidant activity of freeze dried samples measured using ABTS assay was higher than vacuum microwave and air dried samples in the same study. One possible explanation for this discrepancy could be due to the differences in the action of antioxidants in the assay. The ORAC assay measures the affinity of antioxidative compounds to neutralize the free radicals over a period of time accounting for any potential lag phases

in antioxidant activity rather than providing a measurement of only fast acting antioxidants, whereas the ABTS assay neutralize free radicals at a particular point of time without accounting for slow acting antioxidants. In the above case of drying of cranberry, freeze-drying degraded cranberry antioxidants could have contributed to the lag phase in antioxidant activity more than vacuum microwave-drying. During microwave-drying, water molecules transmit and absorb energy because of volumetric heat generation in the wet sample resulting in higher interior temperature that helps water rapidly reach its boiling point compared to other convective drying methods (Khraisheh et al., 1995). Microwave-drying in combination with vacuum also reduce the drying temperature, oxygen exposure, and heating time because of enhanced penetration of heat that provides a constant internal temperature and lasts until the final stage of drying has been reached (Oliveira and Franca, 2002). However, the mechanism of slow rate of drying and moisture removal during freeze drying that is different than vacuum-microwave drying has no severe effect on the phytochemicals of cranberries. Increase in the antioxidant activity after drying has also been observed in vegetables. Nindo et al. (2003) reported that the total antioxidant activity of asparagus, using ABTS assay, is significantly higher ( $p < 0.05$ ) after refractance window and freeze-drying than tray-drying, spouted bed, and combined microwave and spouted bed drying compared to raw material. Similar results were observed in combined microwave and hot air-drying of purple carrots (Uyan et al., 2004).

Steaming (100°C, one hour) and flaking (100°C, 20 minutes) reduce tocotrienols, caffeic acid, and some avenanthramides, but increase ferulic acid and vanillin, whereas autoclaving (100–120°C, 2.4 bar, 16 minutes) increases contents of tocopherols, tocotrienols, and acids of vanillin, ferulic and *p*-coumaric acids of oat groats (Bryngelsson et al., 2002). However, autoclaving in the same study degraded avenanthramides in the oat groats. Drum-drying (8 bar steam pressure) of whole meal or rolled oats decreases all tocopherols and phenolic compounds but avenanthramides are unaffected (Bryngelsson et al., 2002). When compared with conventional drying/dehydration methods, high temperature short time food-processing operations such as microwave drying exposes the food matrix for a small time, thereby reducing the detrimental effects of high heat, oxidation on the antioxidant compounds of dehydrated foods.

Depending on the severity of heat application, dehydration reduces moisture content of fruits and vegetables, which is important to maintaining the equilibrium media for stability of anthocyanins. The rate of evaporation of water might have influence on the water soluble anthocyanin pigments. Air-drying and freeze-drying increase the anthocyanin content of apple peels, whereas oven-drying has similar amount compared to fresh peels (Wolfe and Liu, 2003). Interestingly, the blanched freeze dried peels contain more anthocyanins than the fresh apple peels in the same study (Wolfe and Liu, 2003). Increase in the anthocyanin contents of freeze dried apple

peels is because of blanching effect, which inactivated enzymes to reduce degradation of anthocyanins. The retention of total anthocyanins in Saskatoon berries is higher (~30%) when dried by combining the air with microwave vacuum method than with air-drying (15%) compare to fresh berries (Kwok et al., 2004). Better retention of anthocyanin is attributed to the reduced time of exposure of berries to heat during microwave vacuum-drying. The same investigators reported that the combination of heat and atmospheric oxygen in air-drying favored enzymatic browning activity of PPO, whereas less enzymatic browning was observed because of reduced heat and oxygen exposure during vacuum microwave-drying. Similar results were observed during drying of cranberry using microwave vacuum, freeze-drying, and air-drying methods. Microwave vacuum-drying and freeze-drying produce dehydrated products containing relatively higher level of total anthocyanins per gram dry solid compared to air-drying (Leusink et al., 2010). Uyan et al. (2004) observed a twofold increase in the anthocyanin content during hot air dehydration of purple carrots, whereas combining microwave and hot air-drying increased the quantity by 1.75-fold. However, jam processing (105°C) significantly reduces ( $p < 0.05$ ) the anthocyanin contents by 21–89% of cherries, plums, and raspberries (Kim and Padilla-Zakour, 2004). Ersus and Yurdagel (2007) studied microencapsulation of black carrot anthocyanins by spray-drying (drying air inlet: 160–200°C; outlet: 107–131°C) using different maltodextrins as carrier and coating agents. The same investigators observed an increase in the anthocyanin contents (7.9–36.83%) with different maltodextrins at a constant air inlet with varying outlet temperatures, whereas higher inlet temperatures (>160–180°C) caused more anthocyanin losses in the powders.

### Extrusion

Extrusion cooking is a high temperature, short-time continuous process in which food materials are plasticized and cooked by the combination of temperature under pressure and mechanical shear, resulting in molecular transformation and chemical reactions in the processed foods. Extrusion cooking increases the phenolic content of oats (Zielinski et al., 2001), cauliflower by-products (Stojceska et al., 2008). It also increases the antioxidant activity in sweet potatoes (Shih et al., 2009), cauliflower by-products (Stojceska et al., 2008), and retains in the value-added potato products (Nayak et al., 2011a), and fruit powders from blueberries, cranberries, concord grapes and raspberries (Camire et al., 2007). Recently, Nayak et al. (2011d) reported that value-added extruded products prepared using purple colored potatoes have increased the total phenolics, antioxidant activity (ORAC values), total flavonoids, and cellular antioxidant activity assayed using HepG2 liver cancer cells when compared to unprocessed formulations. The increase in the phenolic compounds and antioxidant activity of the extrudates may have been the

consequence of high temperature, water-stress, and wounding (Reyes et al., 2007) and partly accounted for by the presence of higher molecular weight MRPs, which are formed at higher temperatures and act as antioxidants. In contrast, reduction in phenolic content has been reported for extruded bean (19–21%), oat cereals (24–46%), and oat extrudates (50%) (Zadernowski et al., 1999; Viscidi et al., 2004; Korus et al., 2007). The antioxidant activities (DPPH) and total phenolics in extrudates was reduced by 60–68% and 46–60%, respectively, compared to the unprocessed barley flour (Altan et al., 2009). Similar losses in the antioxidant activity during extrusion cooking were observed in sorghum (Dlamini et al., 2007) and grass peas (Grela et al., 1999). The loss of natural antioxidants during extrusion over 80°C has been attributed to their low resistance to heat (Zadernowski et al., 1999), evaporation and decomposition (Hamama and Nawar, 1991) at these elevated temperatures. It has also been reported that high temperature during extrusion alter molecular structure of phenolic compounds and can reduce their chemical reactivity, decrease their extractability due to a certain degree of polymerization (Alonso et al., 2000) causing loss of antioxidant properties (Zadernowski et al., 1999). Interestingly, the effect of screw speed using a corotating twin screw extruder did not follow a specific trend on the losses of antioxidant activity and total phenolics content of extrudates prepared from barley (Altan et al., 2009). Similar results have been reported by Ozer et al. (2006) on the preparation of the extrudates from dry-mix of chick peas, corn, oats, carrot powder, and hazelnuts. The investigators hypothesized that increased shearing effects at increased speed are more dominant than the effect of residence time on the destruction of antioxidant activity over the extrusion condition, even though increased screw speeds associates with decreased residence times.

Extrusion cooking of corn meal with grape juice and blueberry concentrate at a die temperature of 130°C degrades 74% of anthocyanin compared to the unheated formulation (Camire et al., 2002). Similarly, extrusion cooking of corn meal with dehydrated fruit powder from blueberry, cranberry, concord grape, and raspberry at similar die temperature reduces up to 90% of anthocyanin content for all the dehydrated fruits used except raspberries (Camire et al., 2007). However, in another study Li et al. (2007) reported that heating at 177°C for 20 minutes compared to unheated does not alter total anthocyanin content of purple wheat bran. The same investigators observed that baking of muffins prepared from purple wheat bran with other ingredients at a similar temperature for 7–12 minutes did not retain any anthocyanin.

High shearing action with high temperature inside the extruder barrel changes the structure of food matrices there by affecting the functionality of phytochemicals in the extruded products. Most of the researchers observed that reduction in the antioxidant activity and anthocyanin contents understandably due to a decrease in the natural phenolic antioxidants in the extrudates. However, retention of anthocyanins in baked wheat bran at such a high temperature is surprising.

## ***Irradiation***

Irradiation is aimed to (i) preserve foods by destroying or inactivating organisms that cause spoilage, (ii) sterilize for storage without refrigeration, (iii) provide an alternative to chemical use to control sprouting, ripening, and insect damage. Being a cold process, it is important to study the impact of irradiation on the structure and function of phytochemicals in irradiated foods. An increase in the intensity of irradiation treatment (1–3 kGy) reduces the total antioxidant activity compared to nonirradiated kiwi fruits during storage of one to three weeks (Kim and Yook, 2009). The same investigators reported that electron-donating ability (DPPH) decreases slightly for control, whereas samples irradiated at 1 and 2 kGy doses are not significantly different during storage. Song et al. (2006) reported that the total phenolic content and antioxidant capacity (FRAP) of the irradiated (3 and 5 kGy) are higher than that of the nonirradiated carrot juice. However, irradiation (5 kGy) reduces the antioxidant activity of kale juice in the same study. Breitfellner et al. (2002) studied the effect of gamma radiation (1–10 kGy) on phenolic acids such as 4-hydroxybenzoic acid, gallic acid, cinnamic acid, *p*-coumaric acid, and caffeic acid and their hydroxylation products in strawberry. The investigators observed a significant increase in 4-hydroxybenzoic acid with the gamma radiation doses. Similarly, an increase in the antioxidant activities due to irradiation (0.85–2.57 kGy) was observed in fruit juice and alfalfa sprouts (Fan and Thayer, 2001). Increased antioxidants in irradiated lettuce could be due to the fact that free radicals generated during irradiation act as stress signals and trigger stress-responses for their synthesis (Fan et al., 2003). Fan et al. (2003) hypothesized that irradiation stimulates the synthesis of phenolics but not that of vitamin C, although both phenolics and vitamin C are antioxidants. Gamma radiation of citrus fruit induces accumulation of 4-(3-methyl-2-butenyloxy) isonitrosoacetophenone that exhibits both antioxidant and antifungal activities (Dubery et al., 1999). In another study, seeds of kidney bean, cabbage and beets exposed to ultraviolet (UV) irradiation (460–760  $\mu\text{W}/\text{cm}^2$  for 30, 60, and 90 minutes) stimulated synthesis of anthocyanins in leaves of kidney bean varieties (3–6%) and white beet (14–21%) (Kacharava et al., 2009). It is reported that low doses of irradiation were effective (9–20%) in stimulating synthesis of anthocyanins in cabbage and red beet in the same study. Biosynthesis of antioxidants such as anthocyanin is attributed to the free radicals produced by UV irradiation of seeds that change cell membrane permeability and electric potential, presumably initiating diverse metabolic responses during irradiation in addition to some stress factors (Barka et al., 2000).

## ***Nonthermal Processing***

Conventional heat processing of fruits and vegetables remains the most widely accepted technology for food safety

and shelf-life. In the last decade, nonthermal technologies such as high hydrostatic pressure, pulsed electric field (PEF), and ultrasound have emerged as alternative techniques in food processing to improve nutritional qualities of processed foods. High-pressure treatments on fruit and vegetable products are dependent on the food matrices and processing parameters (pressure, time, and temperature), and antioxidant activity assay methods (de Ancos et al., 2000; Fernandez-Garcia et al., 2001a, 2001b; Sanchez-Moreno et al., 2009). Slight modification in the nutritional composition (vitamins C, A, E, B1, B2, and folic acid), bioactive compounds and the antioxidant capacity contributed by vitamins C, A, and E, carotenoid compounds, and flavonoids has been reported in purees (persimmons, tomatoes, strawberries, and kiwifruit), juices (lemon, orange, carrot, apple, and broccoli) and a mix of vegetable soup (*gazpacho*) (Donsi et al., 1996; Quaglia et al., 1996; Sanchez-Moreno et al., 2009). Cano et al. (1997) reported that peroxidase activity is decreased with high pressure up to 300 MPa for a treatment carried out under 20°C for 15 minutes, whereas above 300 MPa, the activity increased slightly at this temperature. However, complete loss of enzymatic activity was achieved at 900 MPa. The same investigators also observed strongly diminishing PPO activity with high-pressure treatments up to 400 MPa. On the contrary,  $\beta$ -glucosidase has highest activity after a high-pressure treatment at 400 MPa, and the activity decreased as the pressure treatment increased up to 800 MPa (Zabetakis et al., 2000a). (Sanchez-Moreno et al., 2009) reported that long time high pressurization (600 MPa/30 minutes) combined with thermal treatment (60°C) causes 25% reduction, whereas thermal treatment alone (60°C/30 minutes) reduces 10% in antioxidant capacity (linoleic acid/ $\beta$ -carotene assay) of freshly squeezed apple juice compared to untreated samples. Similarly, the antioxidant capacity of tomato puree (DPPH) is significantly reduced after high pressure treatments (400 MPa/25°C/15 minutes), although no difference is observed between pressurized tomato puree and low and high pasteurized products (70°C/30 seconds and 90°C/1 minutes). In contrast, the antioxidant capacity (ABTS) of fruit juices (orange, apple, peach, and citrus) and vegetable purees (carrots and tomato) has not been affected after high pressure treatment (600 MPa/20°C/60 minutes) (Butz et al., 2003). Similar retentions on the antioxidant capacity (DPPH) of freshly squeezed orange juice with combined treatments of high pressure/temperature (100 MPa/60°C/5 minutes, 350 MPa/30°C/2.5 min, 400 MPa/40°C/1 minute) have been reported (Plaza et al., 2006b; Sanchez-Moreno et al., 2003). Zabetakis et al. (2000b) reported high hydrostatic pressure processing (200–800 MPa for 15 minutes) has minimal effect on the anthocyanin content of strawberry juice.

PEF treatment (35 kV/cm in bipolar mode, 800 Hz pulse frequency, 4  $\mu$ s pulse width, 750  $\mu$ s total treatment time, and temperature  $\leq$ 50°C) of freshly squeezed orange juice does not alter the contents of naringenin, hesperetin, or total flavanone, whereas pasteurization using heat only (90°C/1 minute) reduces naringenin content of squeezed orange juice

(Sanchez-Moreno et al., 2005). Similarly, apple juice pasteurized using a PEF treatment (35 kV/cm, bipolar pulses of 4  $\mu$ s 1200 pulses per second) has a 14.5% reduction in the total phenolic content, whereas thermal pasteurization (90°C, 30 seconds) reduced it by 32.2% (Aguillar-Rosas et al., 2007). Plaza et al. (2006a) reported that a low pasteurization treatment (70°C, 30 seconds) or a PEF treatment of 35 kV/cm for 750  $\mu$ s (4- $\mu$ s bipolar pulses, 800 Hz) does not significantly modify the antioxidant activity of treated compared to untreated orange juice. Odriozola-Serrano et al. (2009a) reported that more than 80% anthocyanin content of strawberry juice processed with high intensity pulsed electric field (HIPEF) is retained. It has been well documented that electric field strength, pulse width, pulse frequency, pulse polarity, treatment time, or pulse shape are among the most important HIPEF processing parameters affecting microbial, enzymatic inactivation, antioxidant activity, and level of anthocyanins (Elez-Martínez and Martín-Belloso, 2007; Marsellés-Fontanet and Martín-Belloso, 2007; Odriozola-Serrano et al., 2009a). Zhang et al. (2007) observed that processing of cyanidin-3-glucoside in methanolic solution by PEF (at 1.2, 2.2, and 3.0 kV/cm, 300 numbers of pulses, and temperature  $\leq$ 47°C) degrades anthocyanin to colorless chalcones. In contrast, Odriozola-Serrano et al. (2009a) reported higher anthocyanin content of strawberry juice submitted to bipolar pulses than in monopolar mode.

Ultraviolet (UV) rays induce an increase in enzymes that are responsible for the biosynthesis of secondary metabolites such as flavonoids, which act as screens preventing UV-induced damage in the genetic material of plant cells (Cantos et al., 2000). Ultrasound (US) releases enzymes from the cells for secondary metabolite biosynthesis due to mechanical stresses and microstreaming induced by acoustic cavitations at low US intensity levels (Lin et al., 2001). Prolonged US pretreatment combined with air-drying conditions reduces the total phenolics, flavonoid contents and the antioxidant capacity of dried apples (Opalic et al., 2009). The same investigator observed that drying time has no significant effect on the contents of the total phenolics, flavonoid, or antioxidant activity. However, ultraviolet (UV) rays and ultrasound increase the total phenolics, total antioxidant capacity (TEAC and ORAC) of peanuts and US is more effective than UV in increasing total antioxidants (Sales and Resurreccion, 2010).

Application of sonication using ultrasound decreases anthocyanin content of blackberry juice by 5% (Tiwari et al., 2009). Degradation of compounds responsible for color and anthocyanins during ultrasound processing is attributed to (i) oxidation reaction that is promoted by the interaction with free radicals formed during sonication (Portenlanger and Heusinger, 1992); (ii) extreme physical conditions occur during sonication (temperature up to 5000 K and pressure up to 500 MPa at micro-scale) (Suslick, 1988); and (iii) sonochemical reactions including generation of free radicals, enhancement of polymerization/depolymerization reactions, and other reactions (Floros and Liang, 1994).



## Storage

The effects of storage on the polyphenols have been reported in apples (Price et al., 1999), broccoli (Price et al., 1998), berries (Hakkinen et al., 2000), and onions (Price et al., 1997). The ORAC values of black raspberries remained stable after IQF and over three months storage, and increased by 18% after six-month storage. The ORAC values of berries canned-in-syrup remained stable during storage, while the values for berries canned-in-water remained stable up to one month of storage and increased by 32% and 27% after three and six-month storage at 25°C. The ORAC values of puree, nonclarified, and clarified juices remained stable over the six-month storage at 25°C (Hager et al., 2008a). Similar results were observed in the storage of processed blackberries at 25°C (Hager et al., 2008b). However, in some cases the phytochemicals reduce during storage following treatments. For example, Hakkinen et al. (2000) observed that domestic cooking and cold storage (−20°C for three to nine months) decreased myricetin and kaempferol more than quercetin. Song et al. (2006) reported that during the storage (at 10°C for one to three days) of irradiated (3 and 5 kGy) kale juice, the antioxidant capacity decreases in spite of increase in total phenolic content. The same authors reported that the antioxidant activity and total phenolic content of carrots increase during the same storage period. The stability of the antioxidant activity in carrots is attributed to the presence of  $\beta$ -carotene and its synergistic effect with other antioxidants to protect against oxidation during storage. It could be possible that the presence of vitamin C in kale juice contributes more to its antioxidant activity, which decreases during irradiation, than the irradiation-induced phenolic compounds (Yun-Zhong et al., 2002; Wrona et al., 2003).

The total flavonoid contents of fresh-cut spinach do not change during seven-day storage at 10°C in both air and modified atmosphere packaging with 6% oxygen plus 14% carbon dioxide on day 7 (Gil et al., 1999). The ORAC and percent polymeric color values of canned cherries (100°C, 12 minutes) increase after five-month storage at 22°C (Chaovanalikit and Wrolstad, 2004a). This suggests that polymeric compounds form during storage compensated for the loss of antioxidant capacity due to degradation of monomeric anthocyanins. Friedman (1997) observed greater polyphenol content at lower temperature storage of potatoes and attributed this to less PPO activity at lower temperatures. The investigator further hypothesized that enzymatic activity is indirectly related to monomeric polyphenol content, which means greater the enzymatic activity, more the transformation of monomeric polyphenols to polymeric ones. The higher PPO activity at the higher storage temperature may account for the greater discoloration due to transformation of polyphenols to polymeric pigments at that temperature. In another study, the DPPH radical scavenging ability in new mulberry wine has reported to be 71% and increased to 78% after a year's storage at 20°C. At the same time, the FRAP reducing power decreased from 5720

to 4630 m mol/L (Tsai et al., 2004). These changes support the hypotheses of Friedman (1997) on the conversion of monomeric anthocyanins to the copigmented and polymeric forms during storage.

Blessington et al. (2007) reported that each day of storage after gamma irradiation (75–200 Gy) increases 0.21 units  $\mu\text{g TE/g FW}$ , whereas 0.17 units  $\mu\text{g TE/g FW}$  in antioxidant activity (DPPH) with a unit increase of dosage in potato (Atlantic cv). The same investigator observed that increase in the phenolic content with storage was reported to be 1.98 units  $\mu\text{g TE/g FW}$  per day and 0.16 units  $\mu\text{g TE/g FW}$  for 1 Gy increase in radiation. The gain in phenolic content could be due to dehydration, leading to concentration of solids at the end of the storage period. Additionally, stimulation of synthesis of both antioxidants and polyphenols is known to occur with stress, which may have increased at the end of the storage period due to dehydration (Friedman, 1997; Kang and Saltveit, 2002). For example, the activity of PAL (phenylalanine ammonia lyase), which produces a precursor to phenolic compounds, has been reported to increase under stressful conditions, and this is associated with the accumulation and synthesis of phenolic compounds (Kang and Saltveit, 2002).

There is no particular trend reported in the effects of storage on the antioxidant capacity of nonthermally treated fruits and vegetables. Three different high pressure treatments (100 MPa/60°C/5 minutes, 350 MPa/30°C/2.5 minutes, and 400 MPa/40°C/1 minutes), do not change the antioxidant capacity of orange juice compared to freshly squeezed and stored for 10 days, whereas a 17–23% reduction occurred after 40 days of storage at 4°C (Sanchez-Moreno et al., 2003; Plaza et al., 2006b). In contrast, no significant differences in antioxidant capacity (ABTS) of treated orange juice packaged in glass, polypropylene, teflon, borax flasks, or polypropylene pouches are observed at more extreme conditions of 500 and 800 MPa for five minutes at 20°C and stored for 21 days at 4°C (Fernandez-Garcia et al., 2001a). In another study, no difference ( $p > 0.05$ ) in the antioxidant capacity of combined treated apple juice (600 MPa/60°C/30 minutes) and nontreated samples observed after one-month storage at 4°C (Sanchez-Moreno et al., 2009). Dede et al. (2007) reported that antioxidant capacity of carrot and tomato juices was unaltered after treatment (250 MPa/35°C/15 minutes) and storage for 30 days at 4°C. On the contrary, the antioxidant capacity of a tomato product known as “gazpacho” in Spain has not altered after high pressure treatments (150 MPa/60°C/15 minutes and 350 MPa/60°C/15 minutes) but decreased 39–46% after storage for 40 days at 4°C (Plaza et al., 2006a). However, reports on storage following high pressure treatments for whole fruits and vegetables are limited. Doblado et al. (2007) reported that HPP (400 MPa/25°C/2 minutes) has no effect on the antioxidant activity of broccoli, whereas a reduction in carrots and an increase in green beans are observed.

Individual quick freezing (−70°C/12 hours) and storage (−20°C/6 months) of black raspberries increase the total anthocyanins (Hager et al., 2008a). Retention of anthocyanins

in red raspberries has also been observed during frozen storage (Mullen et al., 2002). The retention or increase in the anthocyanin contents is attributed to moisture loss and enhanced extraction of anthocyanins due to tissue softening. However, anthocyanin content of raspberries declines linearly during storage (25°C/6 months) with losses of 76% and 75% in canned-in water or syrup, respectively (Hager et al., 2008a). Similarly, 65.8%, 60.6%, and 58.4% losses have been observed in blackberry canned-in-syrup products, canned-in-water products, and purees, respectively, stored at 25°C for six months. In another study, total anthocyanins in strawberries canned in 20°Brix syrup declined 69% over two months at room temperature (Ngo et al., 2007). Residual PPO activity is hypothesized to cause a decrease in color characteristics in partially processed peach puree during storage (Bian et al., 1994), while nonenzymatic browning in pasteurized peach purees has attributed to reducing the extent of sugar degradation and HMF formation (Garza et al., 1999). During storage of canned products, pelargonidin-3-glucoside, the main anthocyanin in strawberries, is hydrolyzed by acid to pelargonidin and further breaks into hydroxybenzoic acid (Stintzing and Carle, 2004). Losses of anthocyanins and increase in percent polymeric color in fruits are attributed to residual enzyme activity and/or condensation reactions of anthocyanins with other phenolics (Chaovanalikit and Wrolstad, 2004b; Ngo et al., 2007; Brownmiller et al., 2008; Hager et al., 2008a, 2008b). Ersus and Yurdagel (2007) observed 33% and 11% loss of anthocyanins in encapsulated black carrot anthocyanin powders with different maltodextrins at 25 and 4°C, respectively, stored for 64 days. In contrast, phenolic acids such as ferulic and syringic acid have also been shown to complex with anthocyanins in strawberry and raspberry juices to improve the color (Rein and Heinonen, 2004). Condensation reactions have been reported by the reaction of acetaldehyde, anthocyanins, and flavan-3-ols, producing an increment of color (up to seven times). It is believed that acetaldehyde forms a bridge between the two flavonoids, and consequently condensation reactions could proceed and contribute to the polymeric color (Francis, 1989).

Higher stability of anthocyanins can be achieved by using lower temperature and short-time heating during processing and storage (Rodriguez-Saona et al., 1999; Krifi et al., 2000). For example, cyanidin and delphinidin-rutinosides in blood orange juice are the most stable anthocyanins found during storage for 12 months at -18°C, whereas transformations at 4°C under nitrogen storage induced a slow degradation process of anthocyanins (Krifi et al., 2000). The investigators proposed that the condensation reactions in the presence of carbonyl derivatives formed by sugar and ascorbic acid degradations under acidic conditions are responsible for the transformation of anthocyanins into high molecular weight brown compounds.

In model systems of red radish and red-fleshed potato anthocyanins, radish extracts have higher stability during storage (2°C and 25°C for 65 weeks) than potato extracts (Rodriguez-Saona et al., 1999). The presence of diacylation in

red radish anthocyanin as compared to monoacylated anthocyanins in red-potatoes is responsible for its enhanced stability. Diacylated anthocyanins are stabilized by a sandwich-type stacking caused by hydrophobic interactions between the planar aromatic residues of the acyl groups and the positively charged pyrylium nucleus, which prevents the addition of nucleophiles such as water to the C2 and C4 position of the anthocyanin, reducing the formation of pseudobase (Brouillard, 1981; Goto and Kondo, 1991). In case of monoacylated anthocyanins, only one side of the pyrylium ring can be protected against the nucleophilic attack (Brouillard, 1983).

Water activity is another important factor influencing the stability of anthocyanins during storage. Markaris et al. (1957) observed that strawberry anthocyanins are stable when stored in dry crystalline form or on dry paper chromatograms. Brønnum-Hansen and Flink (1985) observed that at water activity ( $a_w$ )  $\leq 0.31$ , water uptake had no effect on the anthocyanin extracts from freeze dried elderberries, whereas at  $a_w \geq 0.5$ , a significant increase in anthocyanin degradation rate during storage occurred. The investigators also reported that storage at high temperature (50°C) and high water activity (0.5  $a_w$ ) had a most pronounced effect on the stability of elderberry anthocyanins, i.e., half-life was two months. Thakur and Arya (1989) found that an increase in  $a_w$  produced loss of grape anthocyanins adsorbed onto microcrystalline cellulose. In contrast, degradation of strawberry anthocyanin increases with increasing  $a_w$  in fruit and in a model system (Erlandson and Wrolstad, 1972; Garzón and Wrolstad, 2001). In another study, anthocyanin extract in a model system heated at 43°C for 160 minutes was relatively stable at different water activities (Kearsley and Rodriguez, 1981).

### CO<sub>2</sub> Treatment

Carbon dioxide is a nontoxic, nonflammable gas generally considered as safe by the food industries. Supercritical CO<sub>2</sub> can easily penetrate complex structures and porous food materials because of its low viscosity and zero surface tension (Zhang et al., 2006). Plaza et al. (2010) reported unchanged antioxidant activity using DPPH assay of CO<sub>2</sub> treated guava puree (30.6 MPa, 8% CO<sub>2</sub>, 6.8 minutes, 35°C) when compared to thermal treatments at 90°C for 1 minutes. Reduction in red color intensity of the internal tissue in some fruits (Kader, 1986) and change in external color from red to red-purple in strawberries with CO<sub>2</sub> treatment (Ke et al., 1991) has been reported. Gil et al. (1997) reported no significant change ( $p > 0.05$ ) in external anthocyanin content of fresh strawberries stored under CO<sub>2</sub> treated atmosphere compared to the initial fruit, whereas internal anthocyanin content decreases significantly ( $p < 0.05$ ). The discrepancy of opposite behavior of anthocyanin in external and internal tissues is attributed to higher concentrations of cyanidin 3-glucoside in external tissue providing higher color stability, while pelargonidin glycosides are present in the internal tissue. In addition, the

accumulation of phenolic compounds was greater in external tissue which provides more stability to the color.

### *Influence of Other Ingredients/factors During Processing*

Methods including addition of external ingredients have been proposed to retain anthocyanins to reach higher color intensity. In frozen strawberries, it is shown that sugar addition has a stabilizing effect on the total monomeric anthocyanins, enhancing the shelf life of colored products (Wrolstad et al., 1990). Similarly, litchi is a tropical fruit and within two to three days after harvest its pericarp becomes desiccated and turns brown. To reduce the color degradation, litchis are coated with chitosan (1.0–2.0%) and stored (4°C/90% relative humidity). The use of chitosan delay changes of contents of anthocyanin, flavonoid, and total phenolics. Interestingly, the activities of PPO and peroxidase, which have been involved in anthocyanin degradation, are inhibited (Zhang and Quantick, 1997). The same investigators also suggested that a plastic coating forms a protective barrier on the surface of the fruit and reduces the supply of oxygen for enzymatic oxidation of phenolics.

Light has effect on the phenolic acid content of vegetables during storage. For example, chlorogenic acid levels of dark-stored potatoes increases, but less compared to light-stored potatoes (Griffiths et al., 1995; Friedman, 1997). In general, it is also considered that light has deleterious effects on anthocyanin stability and exposure of natural colored fruit/vegetable beverages to light must be avoided. Francis (1989) reported that the presence of other flavonoids, flavone, isoflavone, and aurone sulfonates increase the photostability of anthocyanins. The mechanism is true in the fact that light intensity has a profound effect on apple color, because the light-exposed peel contains twice as much anthocyanin as a shaded peel (Ju et al., 1995). Results similar to that of apple peel has been reported in the anthocyanin extract heated at 43°C for 160 minutes in a model system (Kearsley and Rodriguez, 1981). Interestingly, no difference ( $p > 0.05$ ) in the color changes of raspberries, sweet, and sour cherries during light or dark storage, although the reaction rate of color degradation in light is higher than that in dark storage (Ochoa et al., 2001). Light intensity has no affect ( $p > 0.05$ ) on the stability of freeze dried elderberry anthocyanin stored at different water activities (Brønnum-Hansen and Flink, 1985).

Oxygen has a negative effect on anthocyanin stability depending on the media conditions (Markakis, 1982). Additionally, it has been suggested that flavonoids act as free radical scavengers to protect anthocyanin molecules (Sarma et al., 1997). The presence of oxygen can accelerate the degradation of anthocyanins either through a direct oxidative mechanism and/or through the action of oxidizing enzymes (Jackman et al., 1987). In contrast, the presence or absence of oxygen has no influence on the stability of freeze dried elderberry anthocyanins during storage at lower moisture content (Brønnum-Hansen and Flink, 1985).

### **KINETICS OF CHANGES OF PHYTOCHEMICALS IN PROCESSED PRODUCTS**

Degradation of phenolic compounds is primarily caused by oxidation, cleavage of covalent bonds, or enhanced oxidation reactions due to thermal processing. Although chemical or enzymatic oxidations have been widely proven to cause a progressive decrease in polyphenol antioxidant properties, processing, or prolonged storage times can promote or enhance the rate of oxidation of phenolic compounds depending on intrinsic properties of the food matrix as well as on processing conditions such as water activity, pH, time, temperature, and oxygen availability (Nicoli et al., 1999).

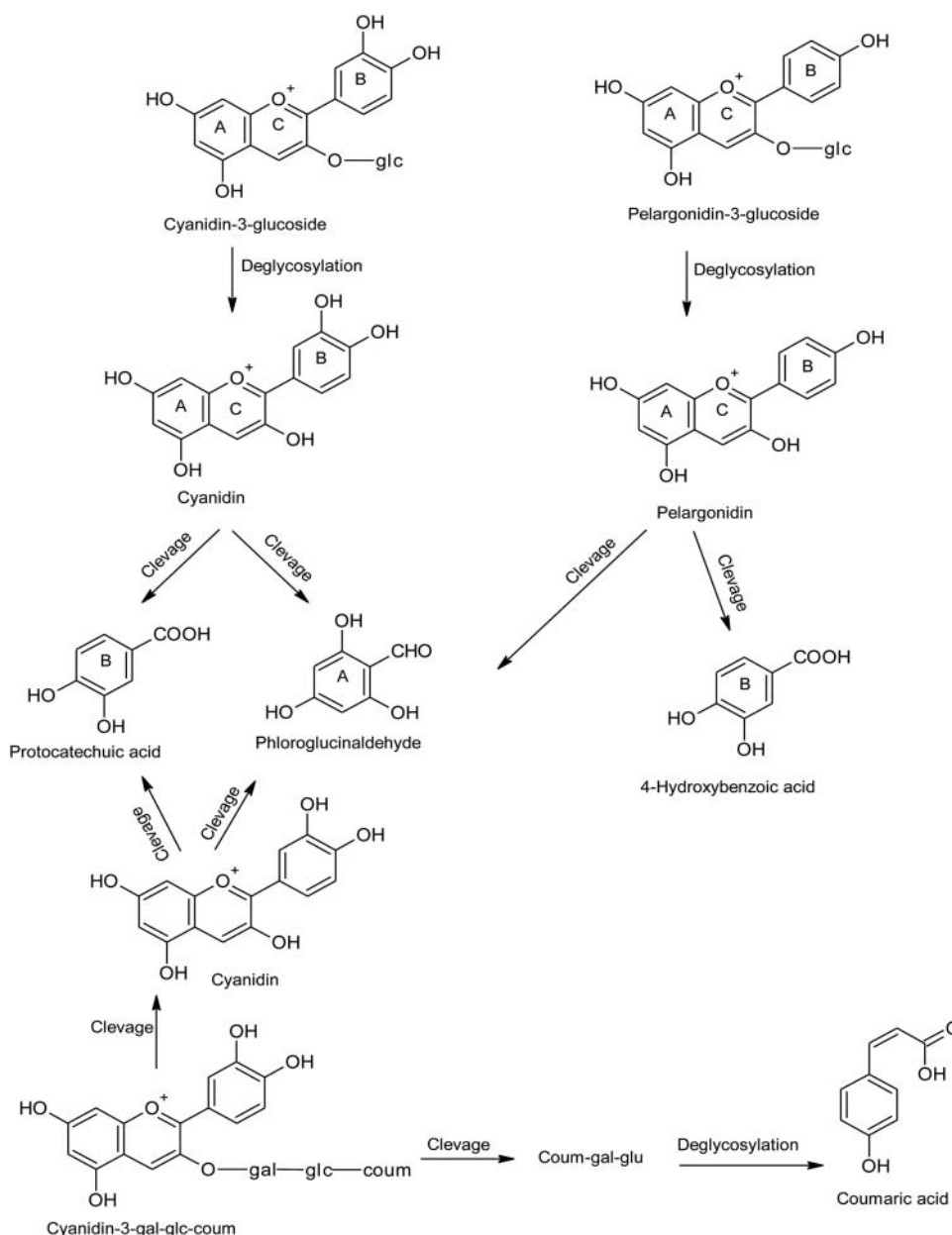
Phytochemicals such as phenolic acid and flavonoids present in the fruits, vegetables, and grains in free and bound states degrade/modify their structural form during thermal and non-thermal processing. As the treatment progresses, naturally occurring antioxidants degrade and new compounds with potential antioxidant activity form. Nayak et al. (2011d) support that high molecular weight phytochemicals break down into a number of smaller compounds with low molecular weight thereby increasing the total phenolic contents, antioxidant activities and flavonoid contents in the processed when compared to unprocessed foods. Nicoli et al. (1999) reported that polyphenols with an intermediate oxidation state exhibit higher radical scavenging efficiency than non-oxidized ones. The higher antioxidant properties of partially oxidized polyphenols is attributed to their increased ability to donate a hydrogen atom from the aromatic hydroxyl group to a free radical and/or to the capacity of their aromatic structures to support the unpaired electron through delocalization around the  $\pi$ -electron system. Thus, phenolic antioxidants do not follow a specific reaction order. However, almost all the literature data report that the reduction of anthocyanins follow a specific pattern in foods.

Several studies have been reported on the thermal degradation of various anthocyanins and their degradation products (Jackman and Smith, 1992; Seeram et al., 2001; Sadilova et al., 2006; Sadilova et al., 2007; Patras et al., 2010). At first, Markaris et al. (1957) hypothesized that opening of the heterocycle pyrylium ring and chalcone formation is a first step of anthocyanin degradation and heating shifts the equilibrium toward the chalcone while the chalcone-flavylium reversion is very slow. Hrazdina (1971) reported that heating decomposed anthocyanin into a chalcone structure, the latter being further transformed into a coumarin glucoside derivative with a loss of the B-ring. Adams (1973) proposed hydrolysis of sugar moiety and aglycone formation as initial degradation steps possibly due to the formation of cyclic-adducts. Markakis and Jurd (1974) reported two major pathways for the degradation of anthocyanins. The first proceeds through the carbinol pseudobase to the chalcone and coumarin glycoside, whereas the second pathway involves hydrolysis of the glycosidic bond as the first step in anthocyanin degradation to form the anthocyanidin. The aglycon, which is more unstable than its glycosides,

proceeds through a highly unstable  $\alpha$ -diketone intermediate to form aldehydes and benzoic acid derivatives. Tanchev and Ioncheva (1976) identified quercetin, phloroglucinaldehyde, and protocatechuic acid in addition to four other compounds by paper chromatography following thermal degradation of anthocyanins.

Von Elbe and Schwartz (1996) suggested that coumarin 3, 5-diglycosides are common thermal degradation products of anthocyanin 3, 5-diglycosides. In another study on thermal degradation of strawberries, elderberries, and black carrots at pH 1, deglycosylation was proposed as the first step of anthocyanin degradation into their respective aglycones yielding a

phenolic acid (protocatechuic acid) and a phenolic aldehyde (phloroglucinaldehyde) (Sadilova et al., 2006). However, the same investigator reported that opening of the pyrylium ring and chalcone glycoside formation was the first step rather than deglycosylation during the thermal degradation at pH 3.5 for the same anthocyanins (Sadilova et al., 2007). A proposed mechanism of thermal degradation of acylated and nonacylated anthocyanin is shown in Figure 8. High temperatures in combination with high pH caused degradation of cherry anthocyanins resulting in three different benzoic acid derivatives (Seeram et al., 2001). Sarni et al. (1995) studied oxidative degradation of *o*-diphenolic cyanidin-3-glucoside and



**Figure 8** Possible thermal degradation of nonacylated (Cyanidin-3-glucoside and Pelargonidin-3-glucoside) and acylated (cyanidin glucosides with coumaric acid) anthocyanins. Modified from Sadilova et al. (2007, 2006).

non-*o*-diphenolic malvidin-3-glucoside in the presence of caffeoyltartaric acid and grape polyphenoloxidase in model solutions. The same investigators reported that both anthocyanins reacted with the enzymatically generated caffeoyltartaric acid *o*-quinone. They also indicated that cyanidin-3-glucoside was degraded mostly by coupled oxidation, whereas malvidin-3-glucoside formed adducts with caffeoyltartaric acid quinone. Jam making of cherries (Kim and Padilla-Zakour, 2004) and plums (Donovan et al., 1998) generated 5-(hydroxymethyl) furfural as a MRP.

### Degradation Kinetics of Anthocyanins

Thermal degradation of anthocyanins has been studied for various fruits and vegetables such as strawberries (Markaris et al., 1957; Garzón and Wrolstad, 2001), raspberries (Tanchev, 1972; Ochoa et al., 2001), Concord grapes (Calvi and Francis, 1978), plums (Ahmed et al., 2004), pomegranates (Marti et al., 2002), sour cherries (Cemeroglu et al., 1994), radishes (Giusti and Wrolstad, 1996b), purple potato (Nayak et al., 2011b), and red cabbage (Dyrby et al., 2001). Table 6 provides detailed degradation kinetics and estimated parameters of various fruits and vegetables. A varied number of possible inactivation/degradation reactions in food matrices occur during processing, which may involve several reaction mechanisms. The rate of inactivation/degradation is reflected by the numerical values of the kinetic parameter estimates such as order of the reaction, rate constants, activation energy, *D*-values, and *z*-value. The order of the reaction in the thermal degradation of anthocyanins, which is dependent on the concentration and time, could be predicted by the following relationship:

$$\frac{dC}{dt} = -k(C)^n \quad (1)$$

where *k* is the rate constant, *n* is the reaction order, *C* is the concentration of the total anthocyanins, and *t* is the reaction time.

Order of reaction is determined by comparing the coefficient of regression, where exponent *n* in Equation (1) was set to zero, half, one, and two in zero-, half-, first, and second-order reactions, respectively. The integrated form of zero-, half-, first, and second-order kinetic models is given in Equations (2)–(5).

$$\text{Zero-order : } C_t = C_0 - kt \quad (2)$$

$$\text{Half-order : } 2\sqrt{C_t} - \sqrt{C_0} = kt \quad (3)$$

$$\text{First-order : } \ln \frac{C_t}{C_0} = -kt \quad (4)$$

$$\text{Second-order : } \frac{1}{C_t} - \frac{1}{C_0} = kt \quad (5)$$

Degradation of anthocyanins under isothermal conditions are reported to follow a first order kinetics (Equation (4)) in strawberries, elderberries and black carrots at pH 3.5 (Sadilova et al., 2007), purple-fleshed potatoes (Nayak et al., 2011b), red-fleshed potatoes, grapes, and purple carrots (Reyes and Cisneros-Zevallos, 2007) and delphinidin-3-rutinoside isolates (from eggplant skin) and malvidin-3-glucoside (from grapes) (Tanchev and Yoncheva, 1974). However, degradation of anthocyanins from red radishes and red-fleshed potatoes follows a quadratic model during storage at 25°C for 65 weeks (Rodriguez-Saona et al., 1999). For first-order degradation kinetics, estimation of half-lives is important. The half-lives (*t*<sub>1/2</sub>) of the anthocyanins were calculated as:

$$t_{1/2} = \frac{\ln 2}{k} \quad (6)$$

Acylation of anthocyanins in black carrots prolongs their half-life (*t*<sub>1/2</sub> ~ 2.8 hours) compared with nonacylated derivatives from strawberry (*t*<sub>1/2</sub> ~ 1.9 hours) and elderberry (*t*<sub>1/2</sub> ~ 1.9 hours) isolates at pH 3.5 (Sadilova et al., 2007). However, higher half-life values of 4.1 and 3.2 hours are observed for black carrots and strawberries, respectively, at similar temperature conditions (Sadilova et al., 2006). In vegetables, diacylated anthocyanins have higher half-lives compared to monoacylated anthocyanin. For example, red radish juice with diacylated anthocyanin has half-lives of 16 weeks compared to red-fleshed potato juice with a half-life of 10 weeks at pH 3.5 (Rodriguez-Saona et al., 1999). The same researchers reported that the half-lives of prepared anthocyanin extracts from red radishes (24 weeks) were higher than those of red-fleshed potatoes (11 weeks) at pH 3.5. However, purified anthocyanins free from salts, sugars, and colorless phenolic compounds degrade at a faster rate compared to anthocyanins in the crude or unpurified extracts directly from the food material; which, could be due to inter- and intramolecular copigmentation reactions in the color's pigments and cofactors such as colorless nonanthocyanin phenolic compounds in the extracts (Nayak et al., 2011b).

Thermal processing (blanching, pasteurization, and sterilization) and storage of foods involve temperature as the major extrinsic factor for food safety and nutritional quality. Parameters to estimate the influence of temperature on the quality of food such as activation energy (*E*<sub>a</sub>), *z*-value and *Q*<sub>10</sub> (Equations (7)–(10)) which have been reported for acai (Del Pozo-Insfran et al., 2004), blood orange juice (Kirca and Cemeroglu, 2003; Cisse et al., 2009), blackberry juice, Roselle extract (Cisse et al., 2009), purple potato (Nayak et al., 2011b), and black carrot juice (Kirca et al., 2007). The thermal death time method (*D*-*z* model) is used to estimate the decimal reduction time (*D*-value), i.e., heating time required to reduce the anthocyanins concentration by 90% and *z*-value, i.e., temperature change necessary to alter the thermal death time by one log cycle (Holdsworth, 2000). *Q*<sub>10</sub> is the factor by which the reaction

**Table 6** Degradation kinetic parameters of anthocyanins. (d: days; h: hour)

Fruit/vegetable	Processing condition	Degradation kinetics	Kinetic parameter	Reference
Acai	10–30°C; H <sub>2</sub> O <sub>2</sub> (0–30 mmol/L)	First order	$k = (7.7\text{--}13.9) \times 10^3 \text{ min}^{-1}$ ; $t_{1/2} = 90\text{--}50 \text{ min}$ ; $Q_{10} = 1.5$ (10–20°C); $Q_{10} = 1.2$ (20–30°C)	Del Pozo-Insfran et al. (2004)
Grapes	25–98°C; pH 3.0	First order	$k = 0.0006\text{--}0.2853 \text{ h}^{-1}$ ; $t_{1/2} = 47 \text{ d--}2.4 \text{ h}$ ; $D\text{-value} = 157 \text{ d--}8.1 \text{ h}$ ; $z\text{-value} = 28^\circ\text{C}$ ; $Q_{10} = 2.28$ ; $E_a = 75.03 \text{ kJ/mol}$ ;	Reyes and Cisneros-Zevallos (2007)
Grape skin (in McIlvaine buffer (B) and carbonated soft drink (SD))	25–80°C for 0.25–6 h; pH 3.0	First order	$k = 3.6\text{--}15 \times 10^{-3} \text{ h}^{-1}$ (B); $k = 2.4\text{--}320 \times 10^{-3} \text{ h}^{-1}$ (SD); $E_a = 58 \text{ kJ/mol}$ (B); $E_a = 77 \text{ kJ/mol}$ (SD)	Dyrby et al. (2001)
Concord grapes pomace	Retort (126.7°C) for >30 min	Nonlinear regression (nonisothermal)	$k_{110}^\circ\text{C} = 0.0607 \text{ min}^{-1}$ ; $E_a = 65.32 \text{ kJ/mol}$	Mishra et al. (2008)
Raspberries	4–40°C for five months	Nonlinear regression	$k = (2.00\text{--}7.10) \times 10^3 \text{ d}^{-1}$ ; $E_a = 26 \text{ kJ/mol}$	Ochoa et al. (2001)
Sweet cherries	4–40°C for five months	Nonlinear regression	$k = (1.28\text{--}6.95) \times 10^3 \text{ d}^{-1}$ ; $E_a = 32.49 \text{ kJ/mol}$	Ochoa et al. (2001)
Sour cherries	4–40°C for five months	Nonlinear regression	$k = (1.10\text{--}5.37) \times 10^3 \text{ d}^{-1}$ ; $E_a = 34.8 \text{ kJ/mol}$	Ochoa et al. (2001)
Plum puree	50–90°C for 0–20 min	First order	$E_a = 37.48 \text{ kJ/mol}$	Ahmed et al. (2004)
Blood orange juice	5–37°C and 70–90°C	First order	$E_a = 73.2\text{--}89.5 \text{ kJ/mol}$ (11.2–69 °Brix)	Kirca and Cemeroğlu (2003)
Blood orange juice	30–90°C	First order	$D\text{-value} = (13\text{--}158) \times 10^3 \text{ s}$ ; $Z\text{-value} = 36^\circ\text{C}$ ; $E_a = 66 \text{ kJ/mol}$ ; $\Delta H = 63 \text{ kJ/mol}$ ; $\Delta S = -149 \text{ J/mol}\cdot\text{K}$	Cisse et al. (2009)
Blueberry juice	40–80°C	First order	$k = (0.064\text{--}2.25) \times 10^3 \text{ min}^{-1}$ ; $t_{1/2} = 180.5\text{--}5.11 \text{ h}$ ; $Q_{10} = 4.27$ (40–50°C); $Q_{10} = 1.67$ (50–60°C); $Q_{10} = 2.95$ (60–70°C); $Q_{10} = 1.67$ (70–80°C); $\Delta H = 77.8 \text{ kJ/mol}$ ; $\Delta S = -43.07 \text{ J/mol}\cdot\text{K}$	Kechinski et al. (2010)
Strawberries	Stored at 25°C	First order	$t_{1/2} = 56\text{--}934 \text{ d}$	Garzon and Wroldstad (2001)
Strawberries (fresh-cut)	5–20°C with 80 kPa O <sub>2</sub> flushing	Nonlinear regression (Weibull model)	$k_a = 4.4 \times 10^{-3}\text{--}4.4 \times 10^{-2} \text{ d}^{-1}$	Odriozola-Serrano et al. (2009b)
Strawberry concentrate	95°C for 6–7 h	First order	$t_{1/2} = 1.95 \text{ h}$ (pH 3.5)	Sadilova et al. (2007); Sadilova et al. (2006)
Elderberry concentrate	95°C for 6–7 h	First order	$t_{1/2} = 1.96 \text{ h}$ (pH 3.5)	Sadilova et al. (2007); Sadilova et al. (2006)
Elderberries (in McIlvaine buffer (B) and carbonated soft drink (SD))	25–80°C for 0.25–6 h; pH 3.0	First order	$t_{1/2} = 1.9 \text{ h}$ (pH 1.0)	Sadilova et al. (2001)
Blackcurrant juice (model system)	Heating (4–100°C)	First order	$k = 31\text{--}90 \times 10^{-3} \text{ h}^{-1}$ (B); $k = 5.5\text{--}180 \times 10^{-3} \text{ h}^{-1}$ (SD); $E_a = 89 \text{ kJ/mol}$ (B); $E_a = 56 \text{ kJ/mol}$ (SD)	Dyrby et al. (2001)
Blackcurrants (in McIlvaine buffer (B) and carbonated soft drink (SD))	110°C for 1–30 min	Nonlinear regression (nonisothermal)	$k = (0.16\text{--}3.10) \times 10^{-3} \text{ h}^{-1}$ ; $t_{1/2} = 180 \text{ d--}2.18 \text{ h}$ ; $E_a = 73 \text{ kJ/mol}$ (21–100°C)	Harbourne et al. (2008)
Blackberry juice and concentrate	110–140°C for 1 min	Nonlinear regression (nonisothermal)	$k_{110}^\circ\text{C} = 1.02 \text{ h}^{-1}$ ; $E_a = 81.5 \text{ kJ/mol}$	Harbourne et al. (2008)
	25–80°C for 0.25–6 h; pH 3.0	First order	$k = 9.954 \text{ h}^{-1}$ ; $E_a = 91.09 \text{ kJ/mol}$	Harbourne et al. (2008)
	60–90°C (for 8.9°Brix)	First order	$k = 2.4\text{--}43 \times 10^{-3} \text{ h}^{-1}$ (B); $k = 4.5\text{--}87 \times 10^{-3} \text{ h}^{-1}$ (SD); $E_a = 69 \text{ kJ/mol}$ (B); $E_a = 50 \text{ kJ/mol}$ (SD)	Dyrby et al. (2001)
			$k = 0.69\text{--}3.94 \times 10^3 \text{ min}^{-1}$ ; $t_{1/2} = 16.7 \text{ h--}2.9 \text{ h}$ ; $E_a = 58.95 \text{ kJ/mol}$	Wang and Xu (2007)

(Continued on next page)

**Table 6** Degradation kinetic parameters of anthocyanins. (d: days; h: hour) (*Continued*)

Fruit/vegetable	Processing condition	Degradation kinetics	Kinetic parameter	Reference
Blackberry juice (65°Brix)	5–37°C	First order	$k = 2.0\text{--}59.1 \times 10^3 \text{ min}^{-1}$ ; $t_{1/2} = 330.1 \text{ h--}11.7 \text{ h}$ ; $E_a = 75.5 \text{ kJ/mol}$	Wang and Xu (2007)
Blackberry concentrate (65°Brix)	5–37°C	First order	$k = 5.2\text{--}89.9 \times 10^3 \text{ min}^{-1}$ ; $t_{1/2} = 133.3 \text{ h--}7.7 \text{ h}$ ; $E_a = 65.06 \text{ kJ/mol}$	Wang and Xu (2007)
Blackberry juice	100–180°C	Nonlinear regression (nonisothermal)	$E_a = 92 \pm 8 \text{ kJ/mol}$ (100–140°C); $E_a = 44 \pm 40 \text{ kJ/mol}$ (140–180°C);	Jimenez et al. (2010)
Blackberry juice	100–180°C	First order (isothermal)	$E_a = 74 \text{ kJ/mol}$	Jimenez et al. (2010)
Blackberry juice	30–90°C	First order	$D\text{-value} = (30\text{--}341) \times 10^3 \text{ s}$ $Z\text{-value} = 56\text{--}57^\circ\text{C}$ $E_a = 37 \text{ kJ/mol}$ $\Delta H = 34 \text{ kJ/mol}$ ; $\Delta S = -232 \text{ to } -233 \text{ J/mol-K}$	Cisse et al. (2009)
Roselle extract	30–90°C	First order	$D\text{-value} = (30\text{--}2280) \times 10^3 \text{ s}$ $Z\text{-value} = 34\text{--}44^\circ\text{C}$ $E_a = 47\text{--}61 \text{ kJ/mol}$ $\Delta H = 44\text{--}58 \text{ kJ/mol}$ $\Delta S = -205 \text{ to } -165 \text{ J/mol-K}$	Cisse et al. (2009)
Black Carrot juice	70–90°C; pH 4.3 and pH 6.0; 11–64°Brix 70–90°C; pH 2.5–7.0 Storage at 4–37°C; pH 4.3	First order First order First order	$E_a = 62.5\text{--}95.1 \text{ kJ/mol}$ ; $Q_{10} = 1.7\text{--}2.8$ (70–80°C); $Q_{10} = 2.0\text{--}2.2$ (80–90°C) $E_a = 78.1\text{--}47.4 \text{ kJ/mol}$ $E_a = 62.1\text{--}86.2 \text{ kJ/mol}$ ; $Q_{10} = 2.3\text{--}3.1$ (4–20°C); $Q_{10} = 2.5\text{--}3.6$ (20–37°C)	Kirca et al. (2007)
Black carrot concentrate	95°C for 6–7 h	First order	$t_{1/2} = 2.81 \text{ h}$ (pH 3.5) $t_{1/2} = 4.1 \text{ h}$ (pH 3.5)	Sadilova et al. (2007); Sadilova et al. (2006)
Red radish juice concentrate	Stored (2 or 25°C for 65 weeks)	Second order	$t_{1/2} = 16 \text{ weeks}$ (25°C) $t_{1/2} > 65 \text{ weeks}$ (2°C)	Rodriguez-Saona et al. (1999)
Red-fleshed potato juice concentrate	Stored (2 or 25°C for 65 weeks)	Second order	$t_{1/2} = 10 \text{ weeks}$ (25°C)	Rodriguez-Saona et al. (1999)
Purple-fleshed potatoes	25–98°C; pH 3.0	First order	$k = 0.0007\text{--}0.3259 \text{ h}^{-1}$ ; $t_{1/2} = 41 \text{ d--}2.1 \text{ h}$ ; $D\text{-value} = 137 \text{ d--}7.1 \text{ h}$ ; $z\text{-value} = 28.4^\circ\text{C}$ ; $Q_{10} = 2.25$ ; $E_a = 72.49 \text{ kJ/mol}$ ;	Reyes and Cisneros-Zevallos (2007)
Red-fleshed potatoes	25–98°C; pH 3.0	First order	$k = 0.0003\text{--}0.0725 \text{ h}^{-1}$ ; $t_{1/2} = 89 \text{ d--}9.6 \text{ h}$ ; $D\text{-value} = 297 \text{ d--}32 \text{ h}$ ; $z\text{-value} = 31.5^\circ\text{C}$ ;	Reyes and Cisneros-Zevallos (2007)
Purple carrots	25–98°C; pH 3.0	First order	$Q_{10} = 2.08$ ; $E_a = 66.7 \text{ kJ/mol}$ ; $k = 0.0001\text{--}0.1004 \text{ h}^{-1}$ ; $t_{1/2} = 216 \text{ d--}6.9 \text{ h}$ ;	Reyes and Cisneros-Zevallos (2007)
Red cabbage (in McIlvaine buffer (B) and carbonated soft drink (SD))	80°C; pH 3.0	First order	$D\text{-value} = 717 \text{ d--}23 \text{ h}$ ; $z\text{-value} = 26^\circ\text{C}$ $Q_{10} = 2.44$ ; $E_a = 81.34 \text{ kJ/mol}$ ; $k = 9.0 \times 10^{-3} \text{ h}^{-1}$ (B); $k = 3.6 \times 10^{-3} \text{ h}^{-1}$ (SD)	Dyrby et al. (2001)

rate is increased if the temperature is raised by 10°C.

$$D = \frac{\ln 10}{k} \quad (7)$$

$$k = k_{\text{ref}} \exp\left[\frac{E_a}{R} \left(\frac{1}{T_{\text{ref}}} - \frac{1}{T}\right)\right] \quad (8)$$

$$\log(D/D_{\text{ref}}) = -(T - T_{\text{ref}})/z \quad (9)$$

$$Q_{10} = \frac{k_{(T+10)}}{k_T} \quad (10)$$

$$\frac{C_t - C_{\text{eq}}}{C_o - C_{\text{eq}}} = \exp(-kt) \quad (11)$$

where  $D_{\text{ref}}$  is the  $D$ -value at temperature  $T_{\text{ref}}$ ,  $E_a$  is the activation energy ( $\text{kJmol}^{-1}$ ),  $T$  is the absolute temperature (K),  $R$  is the universal constant ( $8.135 \text{ Jmol}^{-1}\text{K}^{-1}$ ),  $k_T$  is the reaction rate at temperature  $T$  and  $k_{(T+10)}$  is the reaction rate at temperature  $T+10$ , and  $C_{\text{eq}}$  is the final equilibrium value of the concentration. Ochoa et al. (2001) used the concept of fractional conversion (Equation (11)) to study the effect of light and room temperature on the kinetics of color change in raspberries, sweet, and sour cherries. Calculation of activation energy using the two-step procedure has resulted in a relatively large standard deviation and particularly with a large confidence interval caused by the small number of degree of freedom (Arabshahi and Lund, 1985). Ochoa et al. (2001) used a nonlinear model (Equations (12) and (13)) to increase the degree of freedom, thus narrowing the confidence interval for kinetics of color in fruits.

$$C_{(t_{ij}, T_i)} = C_{0(T_i)} \exp(-k_{(T_i)} t_{ij}) \quad (12)$$

$$C_{(t_{ij}, T_i)} = C_{0(T_i)} \exp\left[-k_{0(T_i)} t_{ij} \exp\left(-\frac{E_a}{RT_i}\right)\right] \quad (13)$$

where  $C_{(t_{ij}, T_i)}$  is the concentration of  $C$  at time  $t_{ij}$  and temperature  $T_i$ , and  $C_{0(T_i)}$  is the initial concentration at time zero for temperature  $T_i$ .

The isothermal methods work well for kinetic studies of samples with rapid heat transfer rate, and where sufficient concentration remains when lag time ends. For example, for first-order isothermal reactions, “sufficient concentration” for accurate estimation of  $k$  is  $C/C_o \geq 25\%$  (Back and Arnold, 1977). Processing of solid or semi-solid foods enriched with anthocyanins at high temperature may be nonisothermal because of their varying water content. In addition the come-up-time, i.e., time to reach  $\pm 0.5^\circ\text{C}$  of the set temperature, will be different depending on the sample matrix. For a nonisothermal process, temperature of an individual sample changes with time. The non-isothermal model (Equations (14) and (15)) involves thermal history ( $\beta$ ) combining both individual time and temperature. The kinetic parameters such as reaction rate constant ( $k$ ) and activation energy ( $E_a$ ) can be estimated by minimizing the sum of square errors (Dolan, 2003). Recently, Mishra et al. (2008) and Harbourne et al. (2008) studied the nonisothermal

kinetic degradation of anthocyanins of grape pomace and blackcurrants, respectively, in model systems.

$$\frac{C_t}{C_o} = e^{-k_t \beta} \quad (14)$$

$$\beta = \int_0^t \exp\left[\frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right)\right] \quad (15)$$

The Weibull model (Equation (16)) has the potential to describe chemical degradation kinetics in addition to microbial and enzymatic kinetics. Recently, Oms-Oliu et al. (2009) used the Weibull model and accurately described the kinetics of phenolics and antioxidant changes (DPPH) in fresh-cut watermelon stored at 5–20°C for 14 days. In another study, Odriozola-Serrano et al. (2009b) reported the accuracy of the Weibull model to describe the changes in anthocyanins and antioxidant activity (DPPH) of fresh-cut strawberries stored at similar conditions under a high oxygen atmosphere (80 kPa).

$$\text{AOX} = \text{AOX}_0 \exp[-(t.k_\alpha)^\gamma] \quad (16)$$

where AOX is the percentage relative antioxidant property,  $\text{AOX}_0$  is the intercept of the curve,  $t$  (days) is the storage time,  $k_\alpha$  (per day) is the kinetic constant, which is the inverse of the scale factor ( $\alpha$ ) and  $\gamma$  is the shape parameter. Mean storage time ( $t_m$ ), i.e., the time for 100% depletion of the antioxidant property and reaction rate constant ( $k$ ) is expressed as Equations (17) and (18), respectively:

$$t_m = \left(\frac{1}{k_\alpha}\right) \cdot \Gamma \cdot \left(1 + \frac{1}{\gamma}\right) \quad (17)$$

$$k = \ln[1 + \exp\{c(T - T_c)\}]^m \quad (18)$$

where  $\Gamma$  is the gamma function,  $T$  (K) is the storage temperature,  $T_c$  (K) is the marker of the temperature range where the changes accelerate,  $c$  (per K), and  $m$  are dimensional constants.

The enthalpy of activation ( $\Delta H$ ) and entropy of activation ( $\Delta S$ ) of anthocyanins has been reported for blueberry juice (Kechinski et al., 2010) and blood oranges, blackberries, and Roselle (Cisse et al., 2009) using the Eyring-Polanyi model based on transition state theory:

$$k = \frac{k_b}{h} T \cdot e^{-\frac{\Delta H - T\Delta S}{RT}} \quad (19)$$

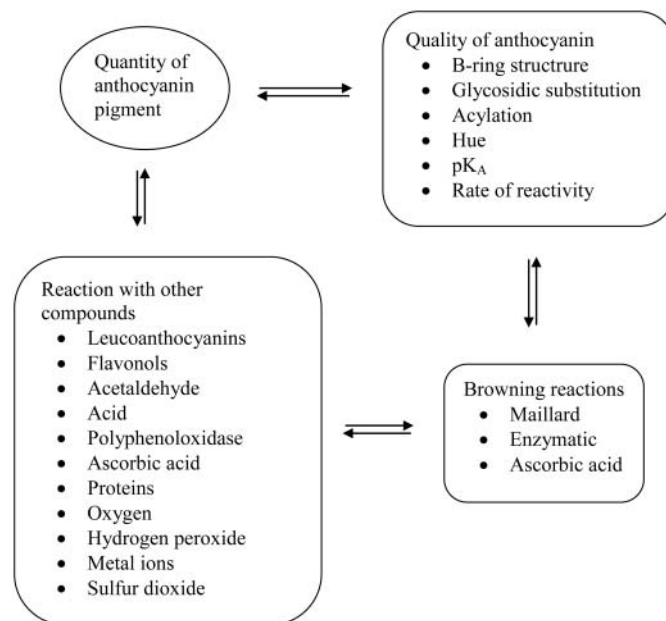
where  $T$  is the absolute temperature (K),  $k_b$  is the Boltzman constant ( $1.381 \times 10^{-23} \text{ J/K}$ ),  $h$  is the Planck constant ( $6.626 \times 10^{-34} \text{ Js}$ ), and  $R$  is the gas constant ( $8.31 \text{ J/mol K}$ ).

Retention of natural phytochemicals or phenolic antioxidants with antioxidant activity in the fruit, vegetables, and grains after thermal or nonthermal processing seems complicated when the food items are exposed to various conditions including temperature, pressure, and other factors such as light, oxygen and metal. In the reviewed literatures, it has



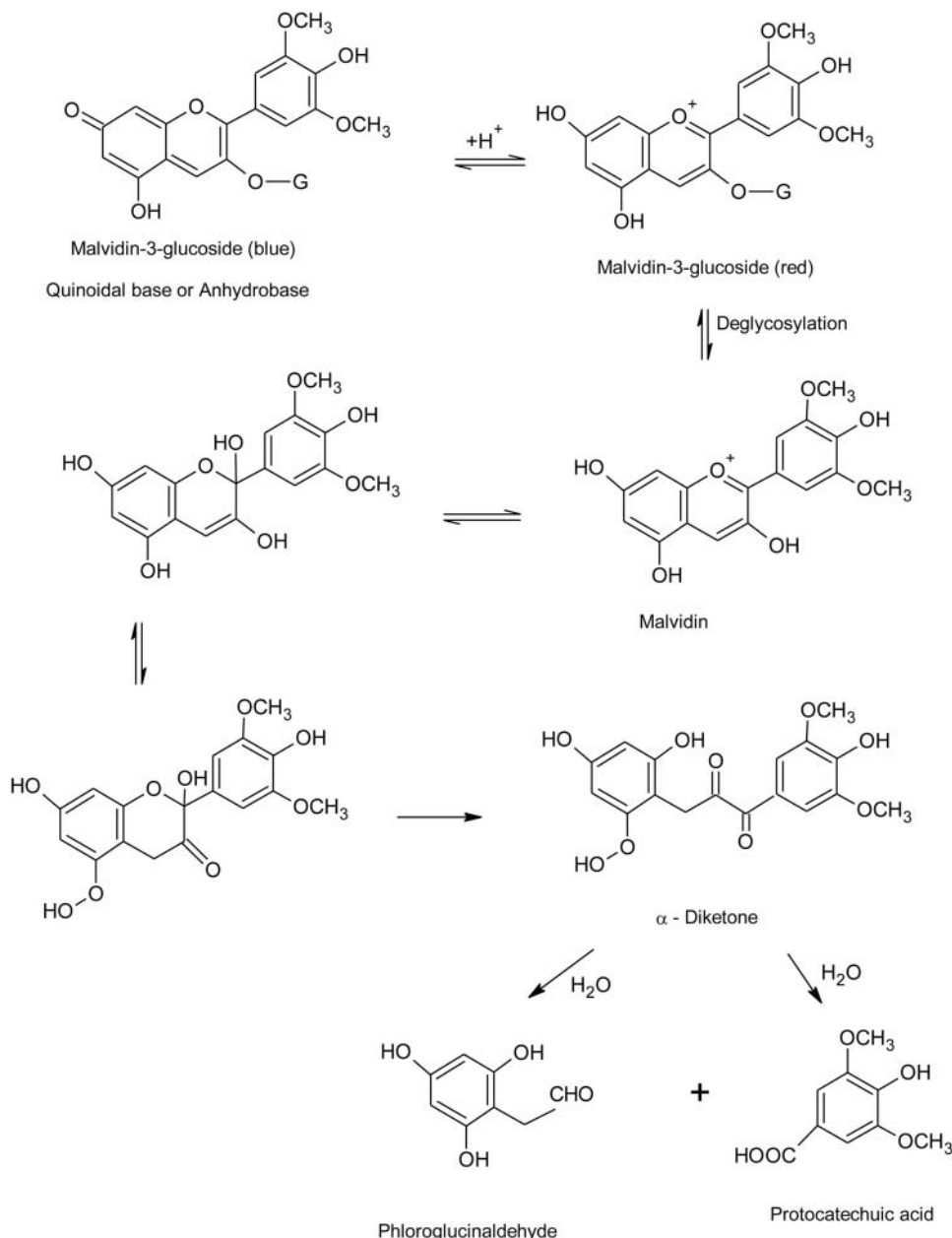
been reported that processing and storage can promote or enhance the stability and quantity of phenolic antioxidants in the processed foods. In most of the cases, processing diminishes the natural antioxidants, while forming new compounds with potential antioxidative properties. These newly formed antioxidative compounds compensate the total antioxidant capacity of processed foods. However, the assessment of antioxidant compounds in processed foods depends on many factors externally as well as internally in the food matrices. The antioxidant activity differs depending on measurement duration and mode, temperature, oxygenation, and medium in the model food systems used (Liu and Finley, 2005). The behavior of antioxidants also depends on the mediums such as bulk lipid, emulsified, and aqueous, which is called the polar paradox. According to the polar paradox, polar antioxidants are more effective in bulk lipids, whereas nonpolar antioxidants are effective in emulsified media (Porter et al., 1977; Cuvelier et al., 2000). The overall antioxidant activity would depend on the redox reactions occurring between different natural antioxidants and lipid oxidation products (Namiki, 1990; Halliwell et al., 1995; Mortensen and Skibsted, 1997). For example, when a small amount of olive oil is mixed with tomato puree, ascorbic acid decreased after a few hours of storage. This is because of the ability of ascorbate compounds to reduce the radical forms compared to  $\alpha$ -tocopherol contained in the lipid matrix (Nicoli et al., 1999). The investigators hypothesized that the above mechanism is due to the lower standard redox potential value of the ascorbyl radical forms of the  $\alpha$ -tocopherol radical. The interaction between the vegetable matrix and the lipid fraction becomes more evident when heated. Thus, when studying the antioxidant properties of complex foods, it must be taken into account that water-soluble antioxidants could protect lipid soluble antioxidants, because of the polar paradox (Porter et al., 1977).

Properly calculated and monitored processing and storage of processed foods is important for increasing shelf-life without altering their color attributes, nutritional value, and functionality. The overall color changes may be due to a number of factors such as pigment degradation, pigment polymerization, reactions with other components of the formulation, nonenzymatic browning, oxidation of tannins, and other reactions completely unrelated to the added colorant (Francis, 1989). A number of structural changes occur in the anthocyanins during food-processing operations. The factors affecting the stability and interrelationship between the quality and quantity of the anthocyanins have been reported in plants or food systems (Figure 9) (Jackman et al., 1987). However, the stability of anthocyanins is related to the presence of a number of hydroxyl groups and methoxyl groups attributed to the flavylium structure besides glycosylation (Francis, 1989). Francis (1989) reported that anthocyanins with diglucosides are more stable than monoglucosides although presence of more sugar molecules in the diglucosides accelerates browning in food matrices. In another thermal degradation of anthocyanin study, Tanchev and Yoncheva (1974) reported that the existence of



**Figure 9** Interrelationships between anthocyanin quantity and quality, and various factors affecting the stability of anthocyanins. Adapted from Jackman et al. (1987).

three hydroxyl groups leads to more rapid degradation of delphinidin-3-rutinoside than malvidin-3-glucoside. Besides position, understanding acylation of organic groups in the structure and influence of pH in fruits/vegetable and model food systems during processing is also important to analyze the stability of anthocyanins. The equilibrium between colored cationic form and colorless pseudobase and degradation is directly influenced by pH (Figure 10). Anthocyanins are stable at pH (< 4) and become colorless at high pH (Francis, 1989). In slightly alkaline solutions (pH 8–10) highly colored ionized anhydrous bases are formed. At pH 12, these ionized anhydrous bases hydrolyze rapidly to fully ionized chalcones (Bridle and Timberlake, 1997). Sadilova et al. (2006) reported that methoxylation of the acyl moiety improves the structural integrity towards heat. Acylated anthocyanins retain more color at the higher pH than nonacylated anthocyanins in addition to their resistance to heat, light, and SO<sub>2</sub>. Giusti and Wrolstad (1996a) observed that the C-5 acylation of anthocyanins in red radish provide high stability to the compounds. Acylation of anthocyanins with ferulic, sinapic and coumaric acids in black carrots prolongs their half-life compared with non-acylated derivatives from strawberry and elderberry isolates (Sadilova et al., 2007). Acylation has not been shown to increase the stability of anthocyanins in some model systems. For example, anthocyanins from grape, red cabbage and *Ajuga reptans* in pH 3.5 citrate buffer solutions has not showed any difference in their degradation rate in spite of their different chemical configurations (Baublis et al., 1994). Grape anthocyanins include mono and diglucosides of five different monoacylated aglycones (Lea, 1988), red cabbage contains



**Figure 10** Effect of pH on the possible degradation mechanism of anthocyanin (Malvidin-3-glucoside). Adapted from Wong (1989).

diacylated triglucosides of cyanidin (Mazza and Miniati, 1993), and *Ajuga reptans* contains glycosylated cyanidin, acylated with *p*-hydroxycinnamic acid, ferulic acid, and malonic acid (Callebaut et al., 1990). Similar findings have been reported on the acylated aglycones of pelargonidin from strawberry juice or concentrate (Garzon and Wrolstad, 2002) and a model system (Garzón and Wrolstad, 2001). Formation of new anthocyanins by the reaction of malvidin 3-monoglucoside and procyanidin B2 in the presence of acetaldehyde (15°C for four months) has been observed in a model system imitating wine. Three new pigments have been observed and their visible spectra show a bathochromic shift in relation to the

anthocyanin by the condensation of these compounds. Interestingly, these compounds have improved stability (Francia-Aricha et al., 1997).

In conclusion, based on the reviewed literature, the following points could be recommended for future research:

- It is apparent that complex mixtures of phytochemicals rather than a single antioxidant in foods are responsible for health benefits due to their additive and/or synergistic effects. It would be biased to measure the antioxidant activity of fruits, vegetables, and grains solely based on the presence of vitamin C or any individual antioxidative

compound in raw or processed foods. It has already been reported that vitamin C provides only 0.4% of total antioxidant activity of apples, whereas other phytochemicals including phenolics, flavonoids, and anthocyanins contribute significantly to the total antioxidant activity in apple (Eberhardt et al., 2000). In addition to the investigations focused on measuring total phytochemicals, possible synergistic effects among antioxidative compounds should also be taken into consideration in antioxidant activity of processed foods.

- The role and contribution of water in the processed foods has not been investigated thoroughly for the measurement of antioxidant activity. Depending on the type of processing technique with different mediums of heating, the total antioxidant activity could be underestimated in regards to the quantity of water present in the processed foods. For example, cooking of pepper in the presence of water decreases the radical scavenging activity while microwave cooking in the limited water condition increases it (Chuah et al., 2008). Rate of diffusion, location of water in the food matrix during processing, and final moisture content should be studied and reported to determine the overall antioxidant activity of processed foods.
- High temperature treatments can have detrimental effects on the phenolics and flavonoids thereby reducing the antioxidant activities of processed fruits, vegetables, and grains. However, in some studies antioxidant activity increases after processing which could be due to intrinsic properties of the food matrix. Therefore, special attention should be given to the outer layers of fruits, vegetables, and grains because of the presence of bran, husk, testa, and aleuronic cells along with other parameters while processing.
- Degradation of higher molecular weight phenolics to lower phenolic compounds with potential antioxidative activity during various food processing operations could be compensating the overall antioxidant activity of processed foods. Characterization of degradation products from phenolic antioxidants of fruits, vegetables, and grains on a case-by-case basis will provide the required knowledge base to understand the detailed mechanism taking place at the molecular level during processing and kinetics of antioxidant activity in processed foods.
- Research focusing on optimization of thermal and nonthermal food processing operations has potential to retain phytochemicals in the processed foods. Processing operations optimized for food safety may be combined with phytochemical study to analyze both the nutritional as well as safety aspects.

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