CHAPTER 2 BACKGROUND

This chapter reviews the pertinent theories and literatures including previous studies about general information of white cabbage, phytochemicals in fruits and vegetables, DF, production of DF powder, drying, pretreatment and effect of processing on phytochemicals. In addition, MAE is also described.

2.1 White Cabbage

White cabbage (*Brassica oleracea* L. var. *capitata*) is a leafy vegetable of *Brassica* family, round or oval in shape. The cabbage consists of soft light green or whitish inner leaves covered with harder and dark green outer leaves. It can be eaten either cooked in many ways such as boiling, frying and steaming or raw as salad.

The nutritional composition in white cabbage is shown in Table 2.1. The main composition of white cabbage is carbohydrate, which is approximately 90% of dry weight. The cabbage is also good source of dietary fiber (DF) and minerals. DF in white cabbage consists of 275 g/kg dry weight of total dietary fiber (TDF), which separates into 82 g/kg dry weight of soluble DF (SDF) and 171 g/kg dry weight of insoluble dietary fiber (IDF) (Wennberg and Nyman, 2004).

Table 2.1 Nutritional composition in white cabbage

Composition	Value (per 100 g)
Energy value (kJ)	113
Water (g)	90.7
Carbohydrate (g)	5.0
Total sugars (g)	4.9
Protein (g)	1.4
Total nitrogen (g)	0.23
Fat (g)	0.2
Starch (g)	0.1
Dietary fiber (g)	2.1
Mineral	
Sodium (mg)	7
Potassium (mg)	240
Calcium (mg)	49
Phosphorus (mg)	29
Iron (mg)	0.5
Zinc (mg)	0.2
Sulfur (mg)	54
Chloride (mg)	40
Manganese (mg)	0.2
Iodine (μg)	2
Copper (mg)	0.01

Source: Ghosh and Madhavi (1998)

It has been reported that consumption of white cabbage is associated with a reduced risk of coronary heart disease, cardiovascular disease, hypertension and especially cancer due to containing various phytochemicals, which possess antioxidants such as phenolic compounds, vitamin C and vitamin E and anticarcinogenic activities such as glucoisnolates as shown in Table 2.2. Table 2.3 shows the antioxidant contents in various *Brassica* vegetables. The major antioxidants in white cabbage are phenolic compounds and ascorbic acid. Only small contents of β-carotene and α-tocopherol are presented in white cabbage.

Table 2.2 Phytochemicals in white cabbage (edible part)

Phytochemicals	Value (per 100 g fresh weight)
Phenolics (mg)	18.14
Vitamin C (mg)	9.65
α-tocopherol (mg)	0.137
Lutein (mg)	0.107
β-carotene (mg)	0.05
Pantothenate (mg)	0.21
Thiamine (μg)	0.12
Riboflavin (mg)	0.01
Niacin (mg)	0.3
Vitamin B6 (mg)	0.18
Folate (μg)	34
Glucosinolates (μmol)	136.85

Source: Ghosh and Madhavi (1998); Singh et al. (2006)

Table 2.3 Variation of antioxidant contents in *Brassica* vegetables (edible part)

Vegetables	Phenolics[e]	β-carotene[f]	α-tocopherol[f]	Ascorbic acid[f]	Total glucosinolates[g]
Broccoli	63.4[c]	0.89[a]	1.62[a]	74.71[a]	10.7[b]
Cauliflower	19.1[c]	0.07[a]	0.17[a]	41.98[a]	11.7[b]
White cabbage	18.7[c]	0.08[a]	0.17[a]	27.32[a]	10.1[b]
Brussels sprouts	37.7[c]	0.14[c]	0.15[c]	15.8[c]	22.9[b]
Kale	359[d]	4.86[a]	1.92[a]	102[d]	14.3[b]
Chinese cabbage	9.93[c]	0.01[c]	0.08[c]	9.71[c]	4.6[b]

[a]Kurilich et al. (1999); [b]Kushad et al. (1999); [c]Singh et al. (2006) ; [d]Korus (2011)

[e]mg Gallic acid equivalent/100 g fresh weight, [f]mg / 100 g fresh weight, [g]μmol/g dry weight

2.2 Phytochemicals in Fruits and Vegetables

Fruits and vegetables contain various phytochemical compounds, which possess antioxidant and anticarcinogenic properties. These compounds play important role in the prevention of many diseases and maintaining good health (Huang et al., 1994; Kusznierewicz et al., 2007; Song and Thornalley, 2007). This section describs the details about the major phytochemicals found in *Brassica* vegetables.

2.2.1 Antioxidants

Antioxidants are substances that can protect cells from the damage caused by unstable molecules known as free radicals by donating one of their own electrons to end oxidative reaction. They can be classified into two groups, which are water-soluble antioxidants and lipid-soluble antioxidants (Pokorny et al., 2001). Examples of water-soluble antioxidants are ascorbic acid (vitamin C) and phenolic compounds. Lipid-

soluble antioxidants including carotenes and α-tocopherol (vitamin E) can protect cell from lipid peroxidation. Both types of antioxidant can be synthesized in human body or consumed from foods. Antioxidant activity depends on many factors such as antioxidant concentration, temperature, oxygen concentration (Podsędek, 2007).

Antioxidants can inhibit or retard the oxidation process in two ways. Firstly, scavenging free radicals is described as primary antioxidants. The primary antioxidant can react with free radicals and convert them to more stable product. Secondly, a mechanism that does not involve directly scavenging of free radicals is described as secondary antioxidants. The secondary antioxidants can retard the rate of chain reaction by various mechanisms including binding of metal ions, scavenging oxygen, converting hydroperoxides to non-radical species, absorbing Ultraviolet (UV) radiation or deactivation or deactivation singlet oxygen (Jadhav et al., 1996; Pokorny et al., 2001).

2.2.1.1 Phenolic Compounds

Phenolic compounds originate from one of the main classes of secondary metabolite, which is derived from phenyl alanine and tyrosine. These compounds contain a benzene ring with one or more hydroxyl group (Naczk and Shahidi, 2004). Phenolic compounds can act as antioxidants by several pathways, i.e., scavenging free radicals, donating hydrogen atoms or electron, or chelating metal cations. The most importance is likely to be by free radical scavenging that the polyphenol can break the free radical reaction (Croft, 1999). Several reports have shown that phenolic compounds have a high antioxidant activity and have antioxidant activity greater than other antioxidant compounds such as carotenoid, vitamin C and vitamin E (Naczk and Shahidi, 2004; Singh et al., 2006; Podsędek, 2007).

Phenolic compounds range from simple, low molecular-weight, single aromatic-ringed compound to large and complex tannins and derived polyphenols. These compounds can be classified into at least 10 different classes (Table 2.4) based on the number and arrangement of their carbon atoms (Vermerris and Nicholson, 2006; Cartea et al., 2011). Among phenolic compounds, flavonoids are the most numerous of the phenolics and are found throughout the plant kingdom (Cartea et al., 2011). The brief details of flavonoids are described below.

Flavonoids are polyphenolic compounds consisting of fifteen carbons with two aromatic rings connected by a three-carbon bridge (C6-C3-C6). They present in high concentrations in the epidermis of leaves and fruits (Cartea et al., 2011). Flavonoids may be grouped into three big classes based on their general structure (Pokorny, 2001; Naczk and Shahidi, 2004). The chemical structures of flavonoids are shown in Figure 2.1. Chalcones and dihydrochalcones, which are structurally related to other flavonoids consist of a linear C3-chain connecting the two rings. They are best known as the yellow pigment in flowers and fruits. An example of them is phloridzin (phloretin-2′-O-D-glucoside), which is a compound found in apple leaves and possesses anti-tumor activity. Flavones and flavonols comprise of double bond between C2 and C3, which occur as aglycones in foods. Approximately 200 flavonols and 100 flavones have been identified in plants (Vermerris and Nicholson, 2006). Anthocyanidins are typically not found as free aglycones, with the excepton of the following widely distributed, colored compounds. The most common anthocyanidin is cyanidin. These compounds are present in the vacuoles of colored plant tissues such as leaves or flower petals (Cartea et al., 2011). Anthocyanins are water-soluble glycosides of anthocyanidins. The most common glycoside is the 3-glycoside. (Vermerris and Nicholson, 2006).

Table 2.4 Classification of phenolic compounds

Structure	Classes
C6	Simple phenolics
C6-C1	Phenolic acids and related compounds
C6-C2	Acetophenones and phenylacetic acids
C6-C3	Cinnamic acids, cinnamyl aldehydes, cinnamyl alcohols
C6-C3	Coumarins, isocoumarins, and chromones
C15	Chalcones, aurones, dihydrochalcones
C15	Flavans
C15	Flavones
C15	Flavanones
C15	Flavanonols
C15	Anthocyanidins
C15	Anthocyanins
C30	Biflavonyls
C6-C1-C6, C6-C2-C6	Benzophenones, xanthones, stilbenes
C6, C10, C14	Quinones
C18	Betacyanins
Lignans, neolignans	Dimers or oligomers
Lignin	Polymers
Tannins	Oligomers or polymers
Phlobaphenes	Polymers

Source: Vermerris and Nicholson (2006)

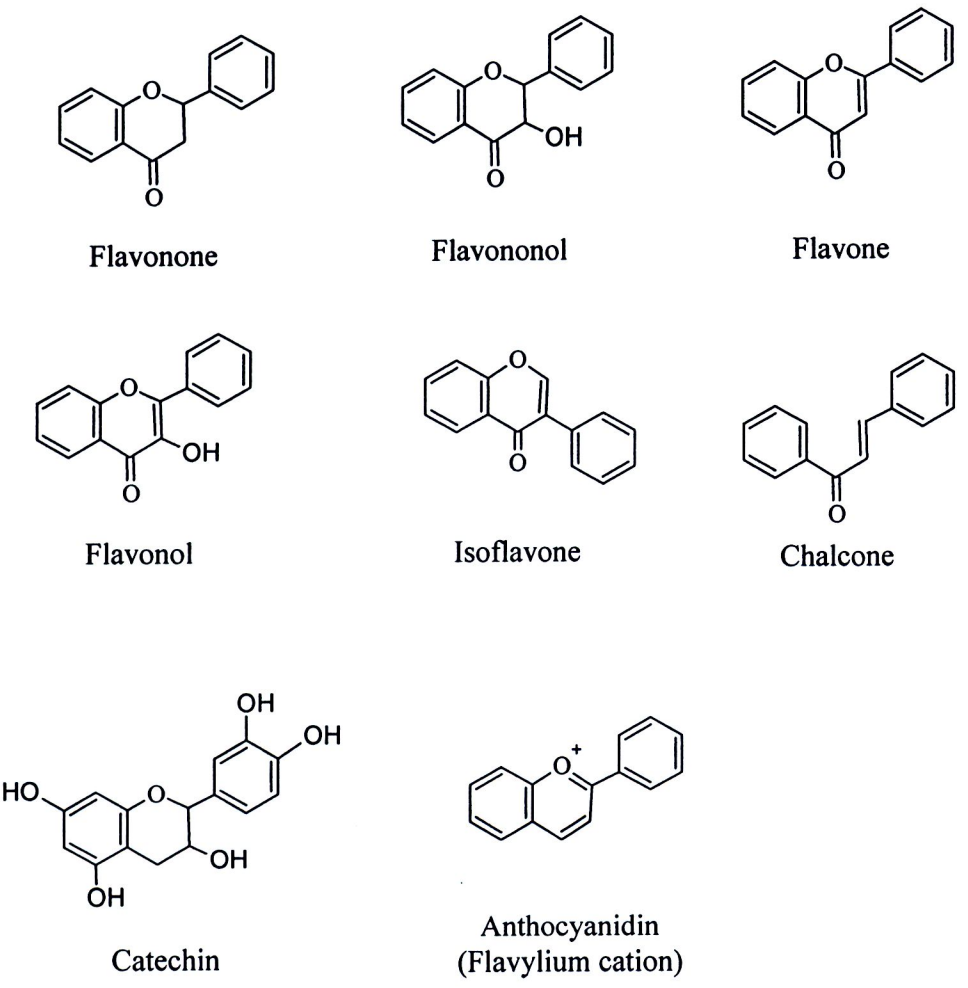
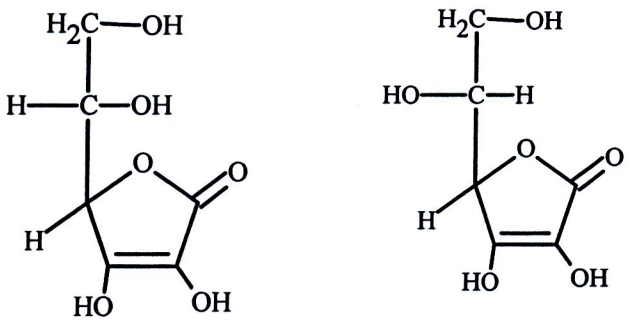


Figure 2.1 Chemical structures of flavonoids (Naczk and Shahidi, 2004)

Phenolic compounds associate with in almost all foods including fruits and vegetables. For example, flavones are often found in grains and vegetables such as celery, lemons, olives, peppers and red grapes (Ruiz-Rodriguez et al., 2008). Flavonols can be found in onions, apple peel, berries, black grapes, tea, broccoli, endives, leeks, grapefruit, pears and corn (Vermerris and Nicholson, 2006). Anthocyanins occur in several berry fruits, such as cherries and strawberries, plums and eggplants, cabbages and radishes (Ruiz-Rodriguez et al., 2008). Catechins mainly occur in considerable amounts in green tea and black grapes (Rice-Evans et al., 1997). Tannins can be found in fruits such as pomegranates, cranberries and blue berries (Vattem et al., 2005).

2.2.1.2 Ascorbic Acid

Ascorbic acid or vitamin C is a water-soluble vitamin. Vitamin C occurs as L-ascorbic acid and dehydroascorbic acid (Figure 2.2). L-ascorbic acid has stereoisomer, i.e. L- and D-ascorbic acid and L- and D-isoascorbic acid. However, stereoisomer of L-ascorbic acid is not found in natural. L-ascorbic acid is stable on exposure to air and daylight at ambient temperature for long periods of time when dry but it is unstable and easily oxidized in aqueous solution especially in the presence of trace amounts of copper, iron and alkali (Dahey et al., 2000). The first product of oxidation is the radical monodehydroascorbate (MDHA) or semidehydroascorbate. MDHA as intermediate can spontaneously be changed to dehydroascorbic acid. Dehydroascorbic acid possesses full vitamin C activity because it can be immediately changed to ascorbic acid in the animal body (Ball, 2006).



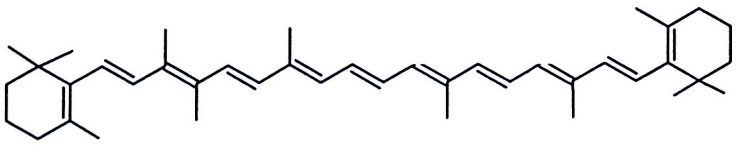
L-ascorbic Acid Isoascorbic Acid

Figure 2.2 Ascorbic acid and their related compounds (Pokormy et al., 2001)

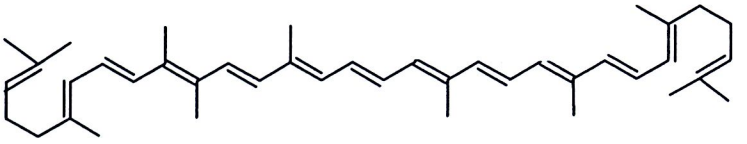
Vitamin C can be found in fruits particularly citrus fruits such as oranges, lemon and vegetables such as broccoli, cabbage, kale and carrot (Decuypere, 2007). The variation of their concentration depends on maturity, climate, sunlight, method of harvesting and storage (Dahey et al., 2000).

2.2.1.3 Carotenoids

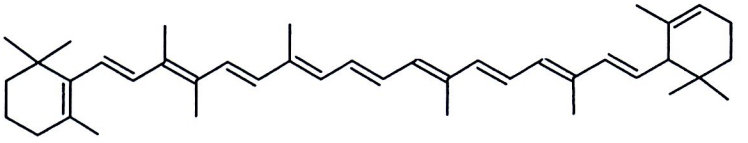
Carototenoids are yellow, orange, and red lipid-soluble pigments, which play important role in photosynthetic process (Pokorny, 2001). Their structures are a polyisoprenoid, which is a long conjugated chain of double bond and a near bilateral symmetry (Rao and Rao, 2007) (Figure 2.3). Carotenoids can be grouped into two classes, which are unoxygenated group and oxygenated group. The unoxygenated carotenoids include α-carotene, β-carotene and lycopene, which are known as carotenes. The oxygenated carotenoids are carotenoids that contain oxygen molecules such as lutein and zeaxanthin, which are known as xanthophylls (Pokorny, 2007).



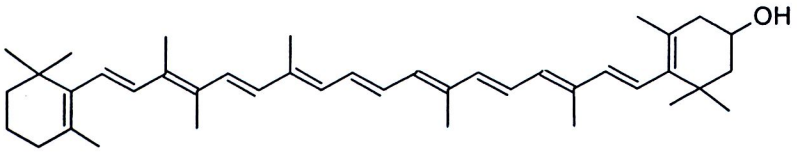
β-carotene



Lycopene



α-carotene



β-cryptoxanthin

Figure 2.3 Structure of carotenoids (Rao and Rao, 2007)

The presence of the conjugated double bonds in carotenoid structure can undergo isomerization to *cis-trans* isomers (Rao and Rao, 2007). *Cis-trans* isomerism is indicated by citing the double bond or bonds with a *cis* configuration. In nature, carotenoids occur primarily in form of the *all-trans* configuration but small amounts of 9-*cis*, 13-*cis* and 15-*cis* isomers of β-carotene have been found in fresh and processed fruits and vegetables (Pokorny, 2001; Rao and Rao, 2007). Carotenoids, which are asymmetrical structure such as α-carotene and β-cryptoxanthin present the number of *cis* isomers greater than symmetrical structure such as β-carotene (Rao and Rao, 2007).

Carotenoids act as antioxidants by quenching singlet molecular oxygen and can also react with free radicals. The reaction of carotenoid (CAR) with a free radical (R*) leading to electron transfer or possibly addition reactions shown below are expected (Pokorny, 2007).

Hydrogen abstraction: $R^* + CAR(H) \rightleftharpoons RH + CAR^*$

and

Electron transfer: $R^* + CAR \rightleftharpoons R^- + CAR^{*+}$

2.2.1.4 Vitamin E

Vitamin E is represented to biological activity of four tocopherols and four tocotrienols, especially α-tocopherol (Porkony et al., 2001). Tocopherols (Toc) are methyl-substituted derivatives of tocol, which consist of the group of chromanol ring connected at C-2 to a saturated isoprenoid side chain. Tocotrienols (Toc-3) are analogous structures whose side chains contain three *trans* double bond. Each of these groups have four isomers (β-, γ-, δ- and α-) but the most important form is α-tocopherol, which is the most active form to against of free radicals (Ball, 2006) (Figure 2.4).

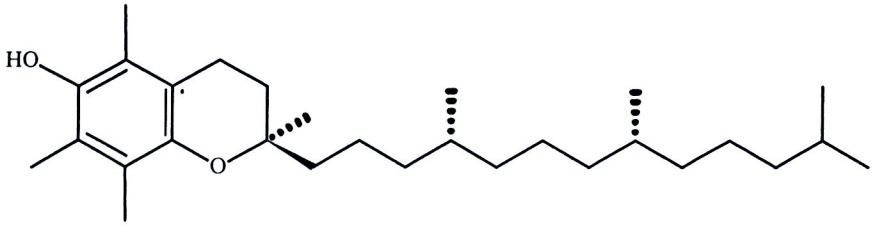


Figure 2.4 Structure of α-tocopherol (Pokormy et al., 2001)

Tocopherol and tocotrienols are destroyed by sunlight and artificial light containing wavelength in UV region (280-750 nm) but they are slowly atmospheric oxidized by oxygen. However the oxidation can be accelerated by light, heat, alkalinity and certain trace metal (Decuypere, 2007).

Vitamin E is lipid-soluble antioxidant vitamins. It can protect cell membranes by denoting the hydrogen of hydroxyl group to the lipid radicals, which is produced in lipid peroxidation. There are many diets that are a source of vitamin E such as nuts and vegetable oil whereas they are present in relatively low levels in fruits and vegetables (Decuypere, 2007).

2.2.2 Glucosinolates

Glucosinolates are thioglucosides, which are mostly found in *Brassica* vegetables (Chen and Andreasson, 2001; Cartea and Velasco, 2008). Glucosinolates and their breakdown products have been considered to be bioactive compounds, which possess cancer-protective properties (Fahey et al., 2006). They consist of β-thioglucoside N-hydroxysulfate containing a side chain (R) and a sulfur-linked β-D-glucopyranose moiety (Ruiz-Rodriguez et al., 2008). General structure of glucosinolates is shown in Figure 2.5.

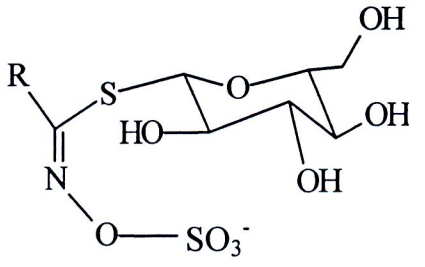


Figure 2.5 General structure of glucosinolate (Chen and Andreasson, 2001)

Glucosinolates are synthesized from amino acids and identified into three groups based on different amino acid precursors. There are aliphatic type (derived from methionine), aromatic or alkenyl type (derived from phenylalanine or tyrosine) and indolyl type (derived from tryptophane) (Cartea and Velasco, 2008). More than 110 classes of glucosinolates have been identified in *Brassica* plants (Chen and Andreasson, 2001). The variation in classes and concentration of glucosinolates attributes on types and varieties of plants, stage of plant development, part of plant, growing condition, post-harvest management and processing (Jones et al, 2006; Cartea and Velasco, 2008). Cartea and Velasco (2008) reported that the highest content of glucosinolates were found in seed and followed by leaves, roots and stems of rapeseed *(Brassica napus)*. In addition, Velasco et al. (2007) showed that the total and individual glucosinolate concentrations in kale changed during plant development. The concentration of aliphatic glucosinolates in kale leaves increased from the early stage until the prebolting stage while the concentration of indole glucosinolates increased until 5 months and then decreased.

Different *Brassica* vegetables show variation in classes and amount of glucosinolates (Table 2.5). Sinigrin and glucobrassicin are the major glucosinolates in cabbage, kale and cauliflower while glucoraphanin and glucobrassicin are abundant in broccoli (Kushad, 1999). Moreover, glucosinolate concentration can be varied by cultivar difference. For example, the amounts of sinigrin in two cultivars of white cabbage which are Heckla and Predikant are 463 μmol/g dry mass and 588 μmol/g dry mass, respectively (Wennberg et al., 2006).

Table 2.5 Types and mean concentration (μmol/g dry mass) of glucosinolates in different *Brassica* vegetables (edible parts)

Glucosinolates	Broccoli	Brussels sprouts	White Cabbage	Cauliflower	Kale
Sinigrin	0.1 ± 0.4	8.9 ± 0.2	7.8 ± 0.1	9.3 ± 0.1	10.4 ± 0.0
Gluconapin	1.0 ± 1.5	6.9 ± 0.7	0.7 ± 0.4	0.3 ± 0.2	1.0 ± 0.1
Glucobrassicanapin	0.3 ± 0.1	0.5 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Progoitrin	1.0 ± 0.8	2.4 ± 0.4	0.2 ± 0.2	0.3 ± 0.1	0.6 ± 0.1
Epiprogoitrin	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Glucoiberin	0.1 ± 0.2	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Glucoraphanin	7.1 ± 2.5	1.0 ± 1.3	0.1 ± 0.6	0.4 ± 0.0	1.0 ± 0.2
Glucobrassicin	1.1 ± 0.4	3.2 ± 0.2	0.9 ± 0.1	1.3 ± 0.1	1.2 ± 0.0

Source: Kushad et al. (1999)

Generally, glucosinolates are stable compound and located in vacuole. Myrosinases are soluble proteins and located in myrosin cells. Intact glucosinolates are inactive until plant cells are damaged resulting in releasing glucosinolates from the vacuole and then are hydrolyzed by myrosinase (Barbieri et al., 2008). The hydrolysis generates glucose and an unstable aglycone intermediate, thiohydroxamate-*O*-sulfonate, which is spontaneously converted to different breakdown products, i.e., isothiocyanates, nitriles, thiocyanates, epithionitriles and oxazolidine-2-thione (Figure 2.6). The optimum condition of myrosinase activity is temperature ranging from 50-60 °C (Yen and Wei, 1993; Rouzaud et al., 2004). Myrosinase structures are denatured resulting in a lower conversion of substates hydrolyzing to breakdown products at the temperature above

60 °C (Wennberg et al., 2006). Myrosinase are also present in many bacteria commonly associated with human intestinal microflora. This implied that glucosinolates can be converted to bioactive compound in human cell after ingestion (Fahey et al., 2001; Rouzaud et al., 2004).

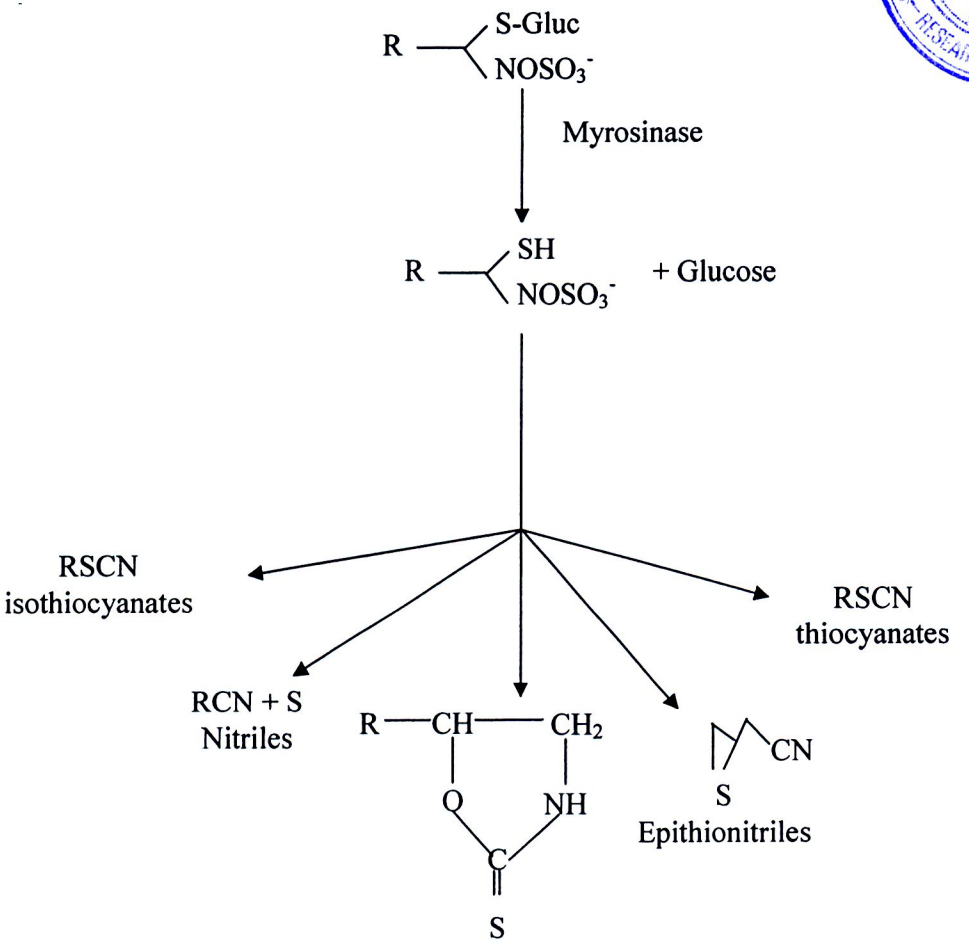


Figure 2.6 Breakdown products from glucosinolate (Jones et al., 2006)

Types of hydrolysis product depend on many factors such as substrate, pH condition, ferrous ions and the level and activity of epithiospecifier protein (ESP) (Vaughn et al., 2005). One of important hydrolyzed products of glucosinolates is isothiocyanate, which largely considered for bioactive compounds (Rouzaud et al., 2004; Jones et al., 2006; Moreno et al., 2006). Isothiocyanates appear to act at a number of points in the tumor development process (Jiao et al, 1998). These compounds act as blocking agents by modifying the metabolism of carcinogenic compounds through their influence on biotransformation enzymes. The action of isothiocyanates is generally to enhance the activity of phase II enzymes, which are detoxicification enzymes and inhibit phase I enzymes, which are responsible for bioactivation of carcinogen (Keck and Finley, 2004). These enzymes are reported to help inactivate cancer by blocking normal cells from DNA damage (Verkerk et al., 2001). Isothiocyanates also act as suppressing agents during the promotion phase of the neoplastic process (Rouzaud et al., 2004).

Among the isothiocyanates, sulforaphane (methylsulfinylbutyl-isothiocyanate), which is hydrolyzed from glucoraphanin, has the most powerful chemoprotection (Cartea and Velasco, 2008). Sulforaphane is heat sensitive and its thermal susceptibility is much dependent on an experimental system (Shen et al., 2010). Heating at certain temperatures and time has been reported to increase the rate of sulforaphane formation. Matusheski et al. (2004), for example, studied the effect of heating on sulforaphane formation in broccoli florets. Their results showed that heating at 60 °C for either 5 or 10 min led to significant increase of sulforaphane content. Rungapamestry et al. (2006) also reported that heating at temperatures in the range of 50-60 °C helped accelerate the rate of sulforaphane formation in cabbage. However, in other studies (Jin et al., 1999;

Van Eylen et al., 2007), heating at 50-60 °C was noted to cause sulforaphane degradation.

2.3 Dietary Fiber

Dietary fiber (DF) makes up the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine, with complete or partial fermentation in the large intestine (Anderson et al., 2009). DF plays important roles in the prevention of several diseases. Consumption of DF helps reduce the risk of colorectal cancer by increasing the volume of fecal bulk and shortening intestinal transit times. It has also been reported that DF helps diabetes patients to control blood glucose level by retarding the digestion and absorption of carbohydrates. In addition, the effects of DF in decreasing the level of cholesterol and preventing coronary heart disease have been reported in some epidemiological studies (Anderson et al., 2009).

2.3.1 Dietary Fiber Composition

Dietary fiber (DF) can be classified into 2 categories according to their water solubility, which are insoluble dietary fiber (IDF) and soluble dietary fiber (SDF).

2.3.1.1 Insoluble Dietary Fiber

Insoluble dietary fiber (IDF) can be defined as plant fibers that do not dissolve in water. Insoluble dietary fiber consists of plant cell wall material including cellulose, hemicelluloses and lignin (Anderson et al., 2009). They are usually found in wheat and vegetables. These fibers increase fecal bulk and cause feces to move more rapidly through the intestines (Meyer, 2004).

Cellulose is the most abundant molecule in nature. It consists of the beta isomer of starch, which is a long (up to 10,000 sugar residues) linear polymer of 1,4β-linked glucose units. Hydrogen bonding between sugar residues in adjacent chains imparts a crystalline microfibrill structure (Kay, 1982).

Hemicelluloses are those cell wall polysaccharides solubilized by aqueous alkali after removal of water soluble and pectic polysaccharides. They contain backbone of β-1,4 linked pyranoside sugars, but differ from cellulose in that they are smaller in size and are usually branched (Kay, 1982).

Lignin is an aromatic polymer containing about 40 oxygenated phenylpropane units including coniferyl, sinapyl, and p-coumaryl alcohols that have undergone a complex dehydrogenative polymerization process. Lignin has greater resistance to digestion than any other naturally occurring polymer (Kay, 1982).

2.3.1.2 Soluble Dietary Fiber

Soluble dietary fiber (SDF) is plant fibers that can be dissolved in water. SDF consists of noncelluosic polysaccharides including pectin, gums and mucilage. Plant foods, especially fruits and vegetables have been found to be good sources of soluble DF. These fibers are known to slow the emptying time of the stomach, delay the absorption of glucose into the bloodstream and lower serum cholesterol (Lorenzani, 1998).

Pectin is a complex group of polysaccharides in which D-galacturonic acid is a principal constituent. They are structural components of plant cell walls and also act as a cementing material in the cell walls of all plant tissues (Kay, 1982). Pectin has

beneficial health effect in response to postprandial glycemia and serum insulin levels. This is because addition of natural or chemically processed fiber has been shown to decrease both the postprandial and fasting blood glucose by delaying carbohydrate absorption. It has been reported to be associated with absorption of zinc and reported to significantly reduce liver cholesterol concentrations (Chawla and Patil, 2010).

Gums are water-soluble or dispensable vegetable exudates containing long-chain polysaccharides. They may be unbranched, significantly branched, or even covalently cross-linked, but they must interact significantly with water. Supplementation of diet with gums helps to reduce evaluated LDL-cholesterol without affecting the high-density lipoprotein (HDL)-cholesterol fraction (Kay, 1982; Chawla and Patil, 2010).

Mucilages are polysaccharides that usually contain galactose, galaturonic acid residues, and often xylose and arabinose. They are found in the seed portion of the plant, within grains, nuts, seeds and legumes; psyllium seed husk; slip bark; marshmallow root. Mucilages help chelate heavy metals and lower cholesterol levels (Kay, 1982; Chawla and Patil, 2010).

2.3.2 Dietary Fiber in Agricultural By-products

Fruits and vegetables are good sources of DF. In addition to fresh consumption, fruits and vegetables can be processed into many forms such as juices, canned or dried products. During processing, fruit and vegetable residues are usually considered as waste in spite of the fact that these residues still contain a high content of DF and various phytochemicals. By-products from fruits and vegetables processing can be

transformed into commercial products. These materials are of interest since they are inexpensive and in abundant supply.

Many agricultural by-products can be potentially used as starting raw materials for commercial production into many products. Citrus residues after juice extraction, for example, contains a high amount of organic acid, DF, especially pectin and many other bioactive compounds including vitamin C, polyphenols and flavonoids. These residues have been reported to be raw materials for production of fiber concentration, commercial pectin and citrus essential oil (Figuerola et al., 2005; Silva and Viotto, 2010). Carrot peels and pomace, by-products from the food industry, are reported to be a good source of pigment, DF and antioxidants such as β-carotene and phenolic compounds. Therefore, many attempts were made to utilize carrot residues in food such as cake, cookies and for production of colorant (Schieber et al., 2001; Singh et al., 2006). Chantaro et al. (2008) reported that carrot peel could be used as raw materials for production of antioxidant DF powder. Apple pomace, a good source of pectin substances, has been reported to be important material for production of pectin (Canteri-Schemin et al., 2005). This residue has also been utilized for the production of cider by fermenting with *Acedobacter* bacteria (Paverkar and Joshi, 2000).

Brassica vegetable by-products such as cabbage outer leaves, broccoli leaves and stems are rich in antioxidant and anticarcinogenic compounds, such as vitamin C, phenolic compounds and glucosinolates (Kaur et al., 2006; Wennberg et al., 2006). Recently, it has been reported that there is a potential to transform cabbage outer leaves into DF powder (Jongaroontaprangsee et al., 2007). Nilnakara et al. (2009) reported that DF powder produced from cabbage outer leaves contained high fiber (43%) and

antioxidants such as phenolic compounds (78.44 mg/100 g dry weight) and vitamin C (108.13 mg/100 g dry weight).

2.4 Production of Dietary Fiber Powder

DF powder can be produced by using fruits, vegetables and by-products from agricultural industries as starting raw materials. The steps for DF powder production are shown in Figure 2.7. The typical process for production of DF powder starts from washing raw material and then cutting into small pieces. Treatments such as blanching and dipping in chemicals to inhibit various enzymes may be applied. After that, the materials are dried until the final moisture content and ground to obtain fine powder. The particle size in range of DF powder (150-430 μm) is recommended for commercial DF powder (Larrauri, 1999). The main characteristics of the commercialized fiber product consist of total DF content above 50%, moisture content lower than 9% wet basis, a low content of lipids, a low caloric value and neutral flavor and taste (Larrauri, 1999).

2.5 Drying

Drying is a process for food preservation by supplying heat to the materials and then removing the moisture from food to a final concentration, which assures microbial stability and expected shelf-life of the product (Harison and Andress, 1994). Drying can be performed at atmospheric pressure or under vacuum. This section describes brief information of drying kinetics. Details of drying methods are also given.

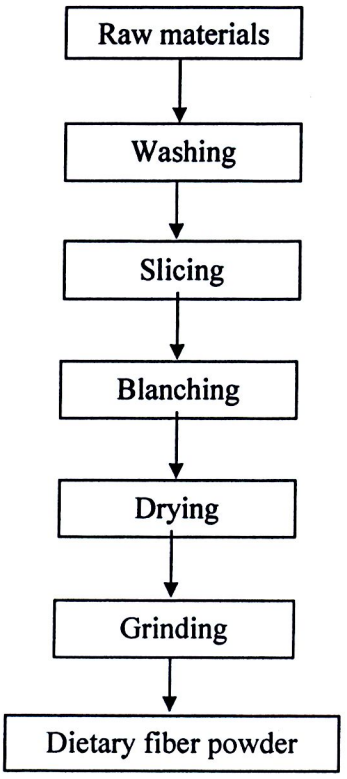


Figure 2.7 Production of DF (Larrauri, 1999)

2.5.1 Drying Kinetics

Drying kinetic is the description of the change of moisture content of material during drying. It can be expressed as a drying curve as shown in Figure 2.8 (a). Drying curve is obtained experimentally by plotting the free moisture content versus drying time (Okos et al, 1992). This plot can be converted into a drying rate curve (Figure 2.8 (b)).

Drying rates curve can be characterized into four stages; the heating period (AB), the constant rate period (BC), the first falling rate period (CD) and the second falling rate period (DE). The initial removal of moisture (AB) occurs as temperature of the product and the water within the product experience a slight increase. Following the initial stage

of drying, significant reduction in moisture content will occur at a constant rate (BC) and at a constant product temperature. In this stage of drying the rate-controlling step is the transport of the water vapor across the air-moisture interface. This period continues until water from the interior is no longer available at the surface of food material. Point C distinguishes the constant rate period from the subsequent falling rate period and is called the critical moisture content. The surface of the solid is no longer wet. The falling rate period has two sections as shown in the figure. From C to D the wet areas on the surface of drying material become completely dry. When the surface is dry (point D) the evaporation front continues moving toward the center of the solid. (Sing and Heldman, 2009).

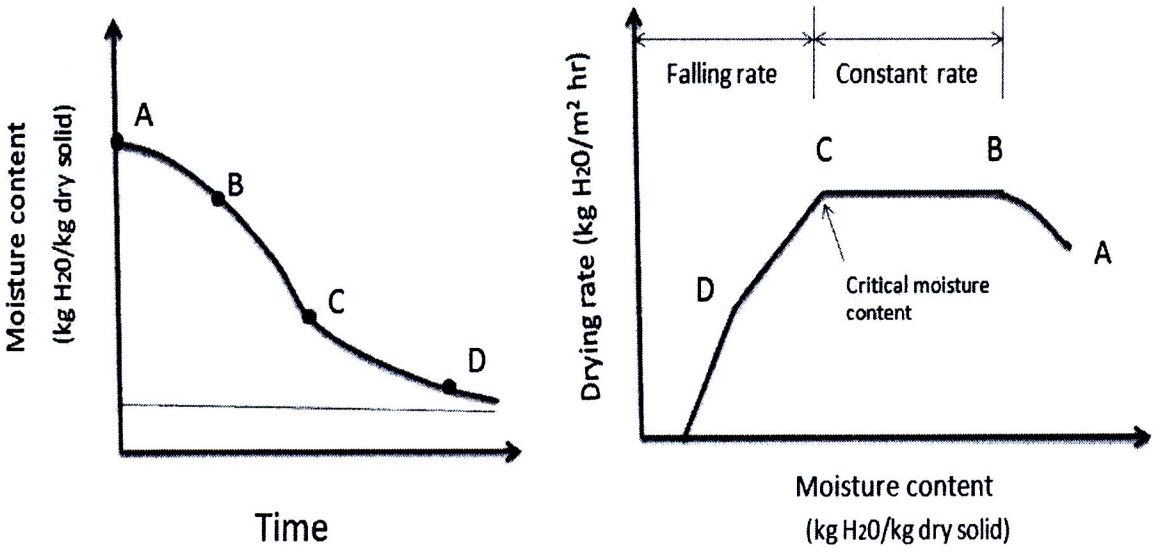


Figure 2.8 Illustration of (a) drying curve and (b) drying rate curve (Singh and Heldman, 2009)

2.5.2 Drying method

Drying method is one of the factors affecting the drying kinetics and quality of food products. There are many drying methods used in food industry. However, hot air and vacuum drying are widely used for drying process. The characteristics of these drying techniques are reviewed briefly as follows.

2.5.2.1 Hot Air Drying

Hot air drying is commonly used for food drying. In drying, warm temperatures cause the moisture to evaporate. Low humidity allows moisture to move quickly from the food to the air. Air current speeds up drying by moving the surrounding moist air away from the food. This method is not complicatedly to operate and low operating cost. The use of hot air drying for producing dried fruits and vegetables and pre-drying raw materials before further processing has been reported (Pedreschi and Moyano, 2005; Mrkìc et al., 2006). Pedreschi and Moyano (2005) reported that hot air drying of potato before frying resulted in increase of crispness dramatically and significant reduction of oil absorption of potato chips.

However, hot air drying is a time-consuming process. Because of the oxygen-rich drying, this method results in degradation of product quality especially in term of nutrition values (Negi et al., 2000; Gong et al., 2007). Larrauri et al. (1997) reported that hot air drying caused 32.6% reduction of polyphenols in red grape pomace peel.

2.5.2.2 Vacuum Drying

Vacuum drying is a method that removal of moisture from food products takes place under low pressure (Chan et al., 2009). The lower pressure allow drying temperature to

be reduced and higher quality to be obtained than with air conventional process at atmospheric exposed to high temperature, faster drying and more efficient heat recovery. Thus, this method is suitable for products, which are heat-sensitive compared with hot air drying. Vashisth et al. (2007) reported that vacuum drying resulted in better retention of phenolic in muscadine pomace and shorter drying time than hot air drying. Gong et al. (2007) determined vitamin C content and color in cabbage after vacuum drying and hot air drying at 70 °C. Their results revealed that vacuum drying gave higher retention of vitamin C and better color than hot air drying.

However, vacuum drying requires higher operating cost than hot air drying. Therefore, it is often used as a secondary drying method. The moisture content of high moisture food is reduced to 20-25% by a conventional method, such as hot air drying and then vacuum is applied to bring the moisture down to 1-3% (Sokhansanj and Jayas, 1995).

2.6 Pretreatment

Pretreatment is required to improve the final quality product in terms of appearance and retention of bioactive compounds. This process is operated for the purpose of inactivating enzymes, modifying texture; preserving color, flavor, and nutritional value; (Podsędek, 2007; Volden et al., 2008). Different pretreatment methods have been developed for food industry, including blanching and use of chemicals (Wiriya et al., 2009; Abano and Samp-Amoah, 2011).

2.6.1 Chemical Treatment

The use of chemicals is a pretreatment used to prevent fruits and vegetables such as apples, bananas, peaches, and potatoes from turning brown (Baiano et al., 2003). The

chemical treatments of materials before drying include sulfiting, immersion in sodium chloride, calcium chloride or acid solution and use of surfactants (Lewicki, 2006). Sulfiting can retard nonenzymatic browning of fruits and vegetables (Ozkan and Cameroglu, 2002). Immersion in solutions containing sulfites reduces oxidation of β-carotene (Negi and Roy, 2000). It facilitates drying by reacting with proteins and breaking disulfide bond leading to reduce firmness of the material (Lewicki, 2006). Treatment with calcium salts also retards nonenzymatic browning. It is reported to improve texture of dried product (Lewicki, 2006). Calcium bides to the plan cell walls and cross-link, especially with pectin of the middle lamella hence affects texture and shrinkage of the material during drying. Abano and Samp-Amoah (2011) showed that dipping sliced banana into ascorbic acid and lemon juice before drying helped increase moisture diffusivity. Dipping in surfactants such as ethyloleate containing potassium carbonate is reported to facilitate drying process by alerting the structure of waxy layer then leading to reduce resistance of moisture diffusion (Hui et al., 2006).

2.6.2 Blanching

The frequent treatment processing drying is blanching. Blanching has many beneficial effects on the quality of the dried product. Blanching helps prevent product from darkening because this method inactivate enzymes, which cause enzymatic browning reaction such as polyphenoloxidase, peroxidase and phenolase (Lewicki, 2006). By Comparing to other enzymes, peroxidase has been found to be the most stable to moderate heat treatments. Thus, peroxidase activity is used as an overall indicator of the adequacy of blanching of fruits and vegetables (Negi and Roy, 2000). Blanching can modify the plant structure. Loosening of the cellular network and separation along the middle lamella is observed during blanching and this results in softening of the tissues

and then reduce cohesiveness of the plant matrix (Akissoe et al., 2003). Reduction of cohesiveness of plant matrix improves absorption of water and provides better rehydration properties of a dried product (Kaymak-Ertekin, 2002). This process has been reported to shorten drying time and increase drying rates (Lewicki, 2006). Heating during blanching causes loss of tugor and affects permeability of the cellular membranes which results in increase drying rate (Akissoe et al., 2003). Blanching also reduces microbial load on the surface of material (Lewicki, 2006).

Hot water and steam blanching are the commonly pretreatments for blanching in food industry. Water blanching is performed in hot water at temperatures ranging typically from 70 °C to 100 °C (Johnson, 2011). Water blanching usually results in a more uniform treatment, allowing processing at lower temperatures. However, during blanching soluble compounds and minerals can leak to the surrounding water. This process requires longer processing times, resulting in increased losses of heat sensitive compounds (Podsędek, 2007; Volden et al., 2008).

In steam blanching, the raw material are suspended above the boiling water and heated only by the steam. This blanching method requires less time than water blanching because the heat transfer coefficient of condensing steam is greater than that of hot water (Roy et al., 2009). Because steam blanching minimizes the leaching of water soluble phytochemicals such as phenolic compounds and vitamin C, it provides higher retention of the compounds in final products (Volden et al., 2008). Nevertheless, operating cost of steam blanching is higher than water blanching because a boiler used for steam generation is one of the most expensive pieces of equipment to operate in a food processing plant, given the high cost of energy (Johnson, 2011).

2.7 Effects of Processing on Phytochemicals

The processing such as blanching, canning, sterilization, freezing and drying may affect content and activity of phytochemical compounds. As retention of phytochemicals in foods are of great importance to consumers, many works have reported the changes of phytochemicals during processing (Wennberg et al., 2006; Kongsoontornkijkul et al., 2006; Podsędek, 2007; Mrkic et al. 2010). In this section, the details on the effects of processing on antioxidants and glucosinolates are given.

2.7.1 Effect of Processing on Antioxidants

As many antioxidants can be degraded when expose to heat, light, oxygen, any processing step involved thermal and mechanical damage can cause changes of antioxidants. Blanching can have significant effect on the antioxidants in fruits and vegetables. For example, Chu et al. (2000) evaluated flavonoid contents and antioxidant activity in green leaves of sweet potatoes after blanching in boiling water for 30, 60 and 120 s. They found that flavonoid contents and antioxidant activity significantly decreased when blanching time over 60 s. Ismail et al. (2004) determined the total antioxidant activity and phenolic contents of fresh and thermally treated vegetables (i.e., kale, spinach, cabbage, shallot and swamp). Their results showed that the means of total antioxidant activity of all fresh vegetables were higher than thermally treated vegetables by blanching in boiling water for 1 min. Ismail and Lee (2005) evaluated the effect of different blanching times (5 min, 10 min and 15 min) at 98 °C on the antioxidant activity and phenolic content of white cabbage. Their study revealed that longer blanching time resulted in a significant reduction of phenolic content and antioxidant activity. Volden et al. (2008) determined the effects of blanching (95 °C, 3 min), boiling (10 min) and steaming (10 min) on antioxidants in red cabbage. Their results showed

that blanching and boiling caused significant losses of total phenol and total monomeric anthocyanin acid whereas steaming caused no losses of total phenol.

Drying methods and condition also have significant effect on the antioxidants of dried fruit and vegetable products. Mrkìc et al. (2006) studied the effect of drying temperature (50-100 °C) on phenolic compounds and ascorbic acid in broccoli. It was observed that higher drying temperature resulted in higher losses of pheonolic compounds and ascorbic acid. Kongsoontornkijkul et al. (2006) showed that low pressure super-heated steam drying provided the better retention of vitamin C in Indian gooseberry than vacuum drying and hot air drying. Kerkhofs et al. (2008) studied the changes of various antioxidants and their activities in dried tomatoes after force-air drying at 42 °C. They found that dried tomatoes showed significant decrease of ascorbic acid, total phenolics contents and total antioxidant activity and increase of lycopene contents.

2.7.2 Effect of Processing on Glucosinolates

Processing such as chopping, blanching, boiling and drying that cause of cell damage may result in loss of glucosinolates. Chopping or shredding is a common step for food preparation before further processing. This process causes a disruption of plant cells, hence resulting in a loss of glucosinolates due to converting of glucosinolate to isothiocyanate by myrosinase. Song and Thornalley (2007) showed that total glucosinolate in shredded cabbage decreased up to 75% after storing at ambient temperature (12-22 °C) for 6 h.

Many previous works have reported about the effect of blanching, boiling and cooking on glulcosinolates. Wennberg et al. (2006) has been reported that blanching in boiling

water for 5 min caused approximately 50-74% reduction in glucosinolate content in white cabbage due to leaching into boiling water and thermal degradation. Jones et al. (2006) found that blanching broccoli in hot water at 95 °C for 2 min resulted in 99% reduction of sulforaphane. Boiling red cabbage for 30 min resulted in significant losses of glucosinates due to leaching into cooking water whereas steaming (0-20 min), stirred frying (0-5 min), microwaving (0-3 min, 900W) had not significant effect on loss of total glucosinolate content (Song and Thormally, 2007). Volden et al. (2008) studied the effects of processing (blanching, boiling and steaming) on the content of glucosinolates in cauliflower. Thiers results showed that total glucosinolates decreased about 42%, 55% and 19% after hot water blanching (96-98 °C) for 3 min, boiling for 10 min and steaming for 10 min, respectively.

There are not many works reported about the effect of drying on glucosinolates. Jones et al. (2006) reported that drying broccoli at 50-55 °C did not give significant effect on glucosinolates. Mrkic et al. (2010) studied the effect of temperature (50-100 °C) of the air that was used to dry broccoli and reported that glucosinolates content decreased upon drying, especially at higher drying temperatures. The degradation of glucosinolates was found when temperature was 75 °C. There is no report about the effect of drying on sulforaphane and isothiocyanates.

2.8 Microwave-assisted Extraction

Microwave-assisted extraction (MAE) is a process of using microwave energy to heat solvent in contact with a sample in order to partition analytes from the sample matrix into the solvent (Chan et al., 2011). MAE is of interest for extraction of constituents from plant matrix because it requires shorter extraction time, consume less solvent and

increase yield of extracted compounds. This section gave details of principle of MAE, parameters influencing MAE and review on MAE application for phytochemicals.

2.8.1 Principle of MAE

Microwave is an electromagnetic wave. It consists of electric field and magnetic field, which oscillates perpendicularly to each others in frequency ranged from 0.3 to 3 GHz (Chan et al., 2011). Schematic diagram of MAE is shown in Figure 2.8. Microwave device consists of four major components. Microwave generator is equipment used for generating microwave energy. Wave guide is used to propagate the microwave from the source to the microwave cavity. Applicator is used for placing sample and circulator is a device that allows the microwave to move only in the forward direction (Chan et al, 2011). The extraction is carried out in a vessel which is directly exposed to the propagation of microwave radiation. The upper part of the vessel is connected to a reflux unit to condense any vaporized solvent.

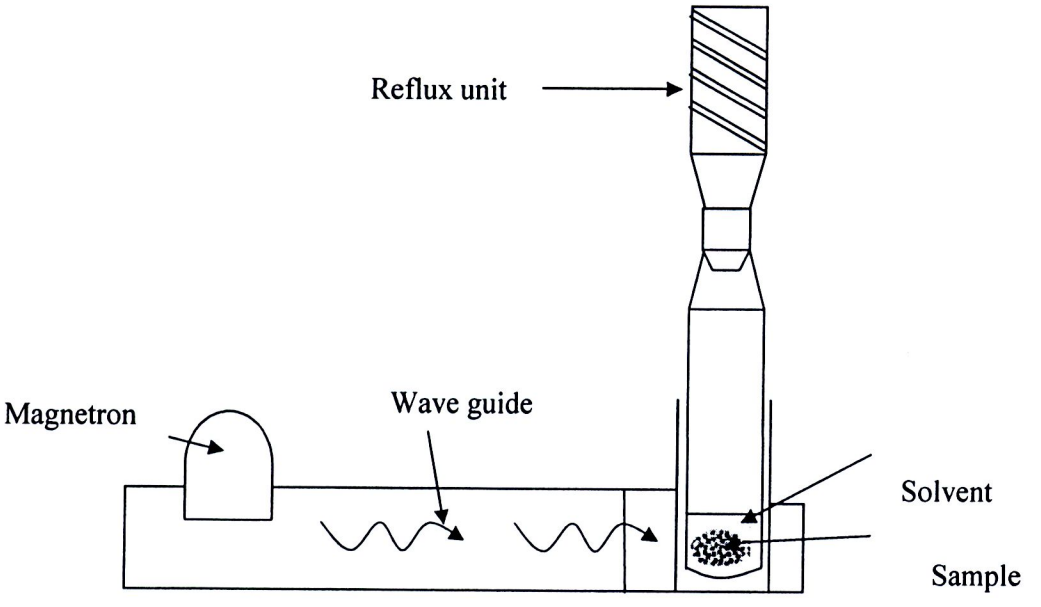


Figure 2.9 Schematic diagram of MAE

Microwave can penetrate into materials and interacts with the polar components to generate heat. The principle of heating using microwave energy is based on the direct effect of microwaves on molecules by ionic conduction and dipole rotation. In most cases, these 2 mechanisms occur simultaneously (Eskilsson and Björklund, 2000). Ionic conduction is the electrophoretic migration of ions when an electromagnetic field is applied. The resistance of the solution to the migration of ions will generate friction, which eventually heats up the solution. Dipole rotation means realignment of dipoles with the applied field. The frequency at 2450 MHz which is used in commercial systems, the dipoles will align and randomize 4.9×10^9 times per second (Eskilsson and Björklund, 2000). The alignment of the solvent molecules generates heat through the friction force (Chan et al., 2011). The ability of a solvent to absorb microwave energy and transfer the energy in form of heat to the surrounding molecules will partly depend on the dissipation factor (tan δ). The dissipation factor, the dielectric loss tangent is given by the equation:

$$\tan \delta = \varepsilon'' / \varepsilon'$$

where ε'' is the dielectric loss which refers the efficiency of converting microwave energy into heat. ε' is the dielectric constant which is the measure of the polarizibility of a molecule in an electric field. Table 2.6 shows the dielectric constants and dissipation factors for some solvents commonly used in MAE. Water has the higher dielectric constant but lower the dissipation factor than other solvents. This means that the rate at which water absorbs microwave energy is higher than the rate at which the system can dissipate the heat. This phenomenon is called superheating effects. The superheating effects have positive or negative effects depending on the matrix. In some cases, the diffusivity of analyze in the matrix is increased. However, in other cases decomposition

Table 2.6 Dissipation factor and dielectric constants for some solvent commonly used in MAE

Solvent	Dielectric constant, ε'	Dissipation factor, tan δ ($\times 10^{-4}$)	Boiling point (°C)
Acetone	20.7	5555	56
Dichloromethane	8.93	4117	39.8
Ethanol	24.3	2500	78
Ethyl acetate	6.02	5316	71.1
Hexane	1.89	0.1	68.7
Methanol	32.6	6400	65
2-Propanol	19.9	6700	82
Water	78.3	1570	100

Source: (Chan et al., 2011; Verma et al., 2011)

of analyze and/or explosion of solvent can occurs due to the intense heating. Therefore, the best solvent should have a high dielectric constant and a high dissipation factor.

2.8.2 Parameters Influencing MAE Process

To determine the optimum condition for MAE, parameters affected on the extraction yield will be studied. The most commonly studied parameters are solvent composition, solvent volume, extraction temperature, extraction time and matrix characteristics including water content.

2.8.2.1 Choice of Solvent

A correct choice of solvent is fundamental for obtaining an optimal extraction process. When selecting solvent, consideration should be given to the dissipation factor and dielectric constants of the solvent, the interaction of the solvent with the matrix, and the analyte solubility in the solvent (Mandal, et al. 2007). Preferably the solvent should have a high selectivity towards the analyte of interest excluding unwanted matrix components. Another important aspect is the compatibility of the extraction solvent with the analytical method used for the final analysis step. Optimal extraction solvents cannot be deduced directly from those used in conventional procedures. If the solvent molecule is not able to absorb microwave energy there will be no heating (Eskilsson and Björklund, 2000).

2.8.2.2 Solvent Volume

The amount of solvent needed for a single sample is often in the range of 10–30 mL (Eskilsson and Björklund, 2000). In some cases solvent volume may be an important parameter for efficient extraction. The solvent volume must be sufficient to ensure that the entire sample is immersed, especially when having a matrix that will swell during the extraction process. Hydrocarbons have been extracted from sediment samples in the range of 1-15 g with solvent volumes between 10 and 30 mL (Lopez-Avila et al., 1995). This investigation led to the conclusion that the proportion of sample in the extraction solution should not exceed 30-34% (w/v). Generally in conventional extraction techniques a higher volume of solvent will increase the recovery, but in MAE a higher solvent volume may give lower recovery. This is probably due to inadequate stirring of solvent during MAE (Eskilsson and Björklund, 2000).

2.8.2.3 Temperature

The most investigated parameter in MAE is the extraction temperature, which is not surprising since the temperature is an important factor contributing to increased recovery, not only for MAE but for all extraction techniques. When MAE is conducted in a closed vessel, the temperature may reach well above the boiling point of the solvent. These elevated temperatures result in improved extraction efficiencies, since desorption of analytes from active sites in the matrix will increase. Additionally, solvents have higher capacity to solubilize analytes at higher temperatures, while surface tension and solvent viscosity decrease with temperature, which will improve sample wetting and matrix penetration, respectively (Eskilsson and Björklund, 2000).

2.8.2.4 Extraction Time

Extraction times in MAE are very short compared to conventional techniques. Generally, longer extraction time results in increase of extracting yield (Mandal et al., 2007). However, in the case of thermolabile compounds, long extraction times may result in degradation (Eskilsson and Björklund, 2000). Dai et al. (2010) reported that the degradation of phenolic compounds in peppermint leaves occurred when the extraction time was longer than 30 min and this was due to increasing temperature in product mixture. The effect of this parameter depends on the extraction solvent used in the method due to difference in dielectric properties of solvent (Mandal et al, 2007). Solvent such as water, ethanol and methanol microwave power has the higher dielectric constant but lower the dissipation factor. These solvents may heat up largely on longer exposure leading to degradation of heat sensitive compounds (Mandal et al, 2007). Extraction time is also influenced by microwave power. Extraction time is decreased

when microwave power is increased due to microwave can be absorbed more intensively at higher microwave power (Charalampos and Komaitis, 2008).

2.8.2.5 Food Matrix Characteristics

The nature of the matrix in which the analytes of interest are bound can have a profound effect on the yield of the compounds (Chan et al., 2007). Some works reported that the matrix moisture improves the extraction yield. Higher water volume in plant cells resulted in larger internal pressure upon microwave irradiation leading to more rupture of plant cells and leaching extracted compound into solvent (Eskilsson and Björklund, 2000). Ng and Hupé (2011) studied the effect of moisture content in tobacco on yield of nicotin during microwave extraction. Their results showed that nicotin yield increased from 3 to 70% as moisture level in tobacco raised from 3-13% wet basis.

2.8.3. Review on MAE Application for Phytochemicals

MAE has been successfully used to extract phytochemicals from plants. For example, Liazid et al. (2001) developed MAE to extract anthocyanin from grape seeds. The result showed that anthocyanins could be microwave extracted from grape seed for 5 min using 100 °C as extraction temperature and 40% methanol in water as the extraction solvent. Ng and Hupé (2003) compared the efficiency of MAE and Soxhlet methods. Their results revealed that MAE required 6 min of extaction time and 25 mL solvent volume whereas Soxhlet method consumed 2 h of extraction time and 75 mL solvent volume for extraction of nicotine from tobacco. MAE can be considered as an effective technique for rapid and selective extraction of piperine. In comparison to conventional solvent extraction techniques, MAE provides higher extraction efficiency, more rapid than conventional solvent extraction (Raman and Gaikar, 2002). Quan et al. (2006)

reported that MAE for 6 min exhibited higher yield of polyphenols from fresh tea than conventional extraction at room temperature (25 °C) for 24 h, conventional heating extraction at reflux temperature (25 °C) for 60 min and ultrasound-assisted extraction for 60 min. Victório et al. (2009) reported that MAE was the best method for extraction of flavonoids from *Alpinia zerumbet* when compared with shaking maceration, ultrasonic and stirring. Liszard et al. (2011) also reported that extraction time was reduced from 5 h to 5 min when using MAE instead of maceration method.