Chapter 2
Literature Review

2.1 *Thua Nao* and similar soy-fermented products

Thua Nao, a traditional fermented soybean of Thailand, is an alkaline-fermented food with unique desirable umami taste and ammonical or fishy smell. It is mainly consumed in northern region of Thailand such as Chiang Mai, Chiang Rai, Mae Hong Son, Lam Phun and Lam Pang. Similar products include *Chungkukjang*, *Daddawa*, *Kinema* and *Natto*. In general, the overall production process of these soy-fermented foods is quite similar; cooked soybeans are spontaneously fermented by natural microorganisms (Sarkar and Tamang, 1995; Omafuvbe *et al*., 2002; Lee *et al.,* 2005a; Chukeatirote *et al*., 2006). It should be noted, however that this is except for the *Natto* process which is a commercial fermentation with pure selected *Bacillus subtilis* (natto) strain as starter inoculum (Wei *et al*., 2001). The traditional *Bacillus*-fermented soybeans usually have strong unpleasant ammoniacal smell, dark grayish or brownish colour and slightly slime matter (Sarkar and Tamang, 1995; Omafuvbe *et al*., 2002; Lee *et al.,* 2005a; Chukeatirote *et al*., 2006), whereas the typical characteristics of *Natto* product are white mucous substance, unique flavour, palatably soft texture, light yellow, and able to generate silky and sticky mass (Wei *et al*., 2001). The features of traditional *Bacillus*-fermented soybeans are summarised in Table 2.1.

Table 2.1 Traditional *Bacillus*-fermented soybeans

Product	**Country**	**Uses**	**Reference**
Thua Nao	Thailand	- Flavour enhancer in local dishes - Main dish: mashed fresh *Thua Nao* combination with salt, chili, lemongrass, onion and garlic after that packed in banana leave into small pocket and cooked by atmosphere steaming or roasting, eaten with sticky rice.	Sundhagul *et al.*, (1972)
Natto	Japan	- Eaten directly with rice - Flavour enhancer in local dishes i.e. Miso soup and sushi - Accompaniments to dinks i.e. sake and tea - Main dish: mixed with Miso, salt, sugar, garlic, and other ingredients, mashed together and stored in chinaware for few days to produce a pickled dish	Kiuchi and Watanabe (2004)
Kinema	India	Fried in vegetable oil for 3-5 min and added with tomato, onion, spices, salt and a little amount of water to produce a thick curry.	Tamang *et al.* (1988); Nout *et al.* (1998)
Dawadawa	Nigeria and Ghana	Flavour enhancer in local dishes	Omafuvbe *et al.* (2002)
Chungkukjang	Korea	Flavour enhancer in local dishes	Kwak *et al.* (2007)

2.2 Fermentation process

Traditional *Bacillus*-fermented soybeans are produced by a diversity of artisanal practices and are different depending on area, local culture, raw materials and equipment. The details of production processes of *Thua Nao* and other related products are explained as follows:

Thua Nao: the conventional process of the product commonly involves the soaking and boiling of soybean seeds for 3 - 4 h until soft. The cooked soybeans are then packed in bamboo basket lined and covered with banana leaves and allowed to undergo spontaneous fermentation at ambient temperature for 2 - 3 days. However, there are differences in the procedures and equipment employed in different areas and communities. For example, in the production of *Thua Nao* at Fang District in Chiang Mai, dry soybeans are cooked in boiling water for 7 h after being washed twice with clean water, then fermented in woven bamboo baskets lined with fern leaves, then covered with the plastic bags and placed outside the building exposed to sunlight for 3 days (Leejeerajumnean *et al*., 2001). According to the production process of Muang Chiang Mai District, soybean seeds are soaked overnight (12 - 14 h) in water before being boiled for a shorter time (4 - 6 h). Besides, the fermentation conditions are also different, as cooked seeds are packed in bamboo basket lined and covered with banana leaves and left to stand at ambient temperature in the household for 2 - 3 days. The production of *Thua Nao* in Lam Phun and Lam Pang follows closely the method of Fang District except for omitting the soaking step of the seeds, instead dry soybean seeds are immediately cooked by boiling in water for 3 - 4 h, the banana leaves are used to enclose the cooked soybeans in bamboo baskets and allowed to spontaneous ferment at ambient temperature in the household for 3 - 4 days (Sundhagul *et al*., 1972). The diversity of soaking and cooking steps including the variation of equipment and fermentation conditions leads to the inconsistency and safety qualities of the product. The complete fermented soybean is indicated by grayish brown colour of soybean seeds, the appearance of a slight mucilaginous substance and is dominated by a strong ammonia-like odour. *Thua Nao* is regarded as spoiled when they liberate putrid or rancid smell or appeared contaminated with mould or yellow-pigmented slimy material on the beans (Sundhagul *et al*., 1972). A major problem of cooked *Thua Nao* is a short shelf-life, about 2 days, if stored under ambient conditions. Hence, in order to extend the shelf-life of this product, sun-dried processed of *Thua Nao* is employed and it has another local name of *Thua Nao Kab* which could be kept for several months.

Natto* or Itohiki-*Natto is a traditional Japanese non-salt whole-soy food fermented by *Bacillus subtilis* (natto). It is the most popular *Bacillus*-fermented soybean with unique characteristics such as beans covered with a white coloured mucous substance, palatably soft texture, light yellow colour, have fruity or nutty aroma and able to generate silky and sticky mass when mixed/stirred with a pair of chopsticks (Steinkraus, 1983). The traditional production of *Natto* involves wrapping cooked soybeans (usually prepared by boiling method) in rice straw, which donates several strains of *Bacillus* as starter cultures, and fermenting at ambient temperature. However, nowadays the modern techniques employ the use of a monoculture of *Bacillus subtilis* selected strain inoculated in pressure cooked soybean including the use of controlled conditions for incubation of inoculated soybeans, which as a consequence leads to consistent good quality of the product. The quality of *Natto* product varies greatly with the conditions of soaking, steaming, fermentation, and cultivars of soybean (Wei *et al.*, 2001; Wei and Chang, 2004). The major differences between traditional *Thua Nao* and commercial *Natto* productions are summarised as in Figure 2.1.

(A)

Soybeans
↓
Wash in water
↓
Soak in water for overnight at ambient temperature
↓
Cook by boiling for 3 – 4 h
↓
Pack in bamboo basket
↓
Cover with banana leaves
↓
Ferment at ambient temperature (25-37°C) for 3 days
↓
Thua Nao

(B)

Soybeans
↓
Separate small soybeans by sieves and remove contaminants and foreign substances
↓
Wash in water
↓
Soak in water for 20 h at 10°C
↓
Steam at 1.5 kg/cm^2 pressure for 20 min
↓
Inoculate with spore suspension of *Bacillus subtilis* (natto) approximately 10^3 CFU/g soybean
↓
Pack 25 to 150 g in paper cups or polystyrene paper trays
↓
Ferment at 40°C for 16 – 18 h in controlled room
↓
Cool and age for 24 h at <10°C
↓
Natto

Figure 2.1 Flow chart of *Thua Nao* (A) (Chukeatirote *et al.*, 2006) and commercial *Natto* (B) productions (Hosoi and Kiuchi, 2003).

Kinema is a soy-fermented food consumed mainly in Nepalis, Lepchas and Bhutias as a low-cost protein diet. Production processes of *Kinema* are not uniform depending on locally available materials, fuel and tradition (Nout *et al*., 1998). According to Sarkar and Tamang (1995), the traditional method of *Kinema* preparation is as follows: soybean seeds are cleaned, washed, soaked overnight (12 – 20 h) at ambient temperature (10 – 25°C), cooked by boiling in water (about 90 min) until they can be crushed easily, wrapped in fern or banana leaves and sackcloth, and fermented naturally for 1 – 3 days at ambient temperature. The completed fermented *Kinema* indicated by formation of a typical *Kinema* flavour, dominated by ammonia and a white viscous fluid (Tamang *et al.,* 1988; Sarkar and Tamang, 1995).

Soy-*Daddawa* or soy-*Dawadawa* is a traditional Nigerian fermented soybean as a taste enhancer in traditional dishes and low-cost meat substitute (Omafuvbe *et al*., 2002). The typical characteristic of this product is sticky with a strong ammonical smell (Ogbadu and Okagbue, 1988). In the traditional method of soy-*Daddawa* preparation, soybeans are cleaned, soaked overnight in tap water, dehulled, cooked by boiling for 2 h, placed in calabashes lined with plantain of banana leaves, covered with more of leaves and left to ferment naturally for up to 3 days in warm place (28 - 35°C) (Popoola and Akueshi, 1985; Omafuvbe *et al*., 2000; 2002).

Chungkukjang is a traditional Korean fermented soybean similar to *Natto* as produced from fermenting steamed soybeans with *Bacillus* species. *Chungkukjang* is prepared simply by fermenting steamed soybeans in a closed and humid space maintained at about 40°C for 2 to 3 days with airborne microbes or microflora present in rice straw (Kwak *et al*., 2007).

2.3 Microorganisms related to the fermentation

The diversities of microorganisms principally involved in alkaline soy-fermented foods are shown in Table 2.2. However, *B. subtilis* and related strains such as *B. licheniformis* and *B. pumilus* are the most predominant in these products. *B. subtilis* is a Gram-positive bacteria, aerobic spore-forming rod of less than 1 μm in diameter and presents mostly single rods, seldom in chains. The width of a rod is 0.7-0.8 μm and the length is 2 - 3 μm and its spores are very heat-resistant. The optimum temperature for germination is about 40°C. The optimum temperature for growth is

39 - 43°C and growth will stop and lysis will occur at 55°C (Yamamoto *et al.,* 1978; Muramatsu *et al.,* 2001). The optimum pH for growth is neutral and germination is inhibited at pH 4.5 or lower. The colonies of *B. subtilis* on agar medium appear round or irregular in shape and the surface is dull. It may be wrinkled and may become cream or brown coloured. The *Natto* bacterium is *B. subtilis* (natto), formerly classified as *B. subtilis.* However, *B. subtilis* (natto) requires biotin to grow and develop properly so vegetative cells cannot grow on media without biotin and their spores do not germinate (Watanabe *et al.,* 1975), whereas *B. subtilis* does not have this requirement, even though the other characteristics are very similar.

Table 2.2 Predominant bacteria involved in alkaline fermented soybeans

Product	**Microorganisms involved**	**References**
Thua Nao	*B. subtilis*, *B. licheniformis*, *B. pumilus*, *B. megaterium, B. cereus, Lactobacillus* sp. Gram-positive cocci	Sundhagul *et al*. (1972); Leejeerajumnean (2003); Chukeatirote *et al*. (2006)
Kinema	*B. subtilis*, *B. licheniformis*, *B. cereus*, *B. circulans*, *B. thuringensis*, *B. sphaericus*, *Enterococcus faecum*, *Candida parapsilosis*, *Geotrichum candidum*, *Escherichia coli*, coliform and Enterobaceriaceae	Tamang (1993); Sarkar *et al*. (1994); Nout *et al.* (1998); Sarkar *et al*. (2002)
Soy-*Daddawa* or *Dawadawa*	*B. subtilis*, *B. licheniformis*, *B. pumilus, B. cereus, B. firmus, B. circulans, B. metaterium, Staphylococcus saprophysicus, S. epidermidis, Micrococcus luteus, Pseudomonas aeroginosa*	Jideani and Okeke (1991); Omafuvbe *et al.* (2000); Dike and Odunfa (2003); Dakwa *et al*. (2005)
Chungkukjang	*B. subtilis*	Kwak *et al*. (2007)
Natto	*B. subtilis* (natto)	Kiuchi and Watanabe (2004)

2.4 Nutritional values of fermented soybean

2.4.1 Proximate composition

Enzymatic reactions particularly proteolytic, amylolytic and lipolytic reactions play an important role in the changes in biochemical quality that occur during *Bacillus*-fermentation of soybeans. For example, Campbell-Platt (1980 reported that 9 of *Bacilllus* species, isolated from *Dawadawa* samples produced proteolytic and amylolytic, and 76 produced lipolytic activity. The main role of enzymatic reactions involved in the fermentation of *Natto* (Sakurai, 1960 , *Kinema* (Sarkar *et al.,* 1997 and *Thua Nao* (Chantawannakul *et al*., 2002; Visessanguan *et al.,* 200 ; Chukeatirote *et al*., 2006; Chunhachart *et al*., 2006; Inatsu *et al.* 2006 have also been reported. Of these enzymatic reactions, hydrolysis of proteins is the most predominant occurring during fermentation of soybean and released small peptides and free amino acids contribute to a typical desirable umami taste of the product (Yoshida, 1998 . Subsequently, bacteria utilise amino acids as carbon and energy source resulting in the accumulation of ammonia and consequently increased pH value (Ohta, 1986; Steinkraus, 1991; Sarkar *et al*., 1993 . Ammonia becomes volatile and causes unpleasant strong ammonical smell whilst pH reaches 8.0 - 8.3 (Campbell-Platt, 1980; Odunfa, 1986; Sarkar *et al*., 1993 . Ammonogenesis can be suppressed by addition of a humectant, such as glycerol and sodium chloride, part way through the fermentation and conducting the fermentation in a container that limits the oxygen supply (Allagheny *et al*., 1996 . In the case of *Natto*, limiting ammonia formation is usually achieved by employing low-temperature storage to restrict bacteria growth while the proteolytic reactions continue to work (Ohta, 1986 . The variable proximate compositions of *Bacillus*-fermented soybeans are summarised in Table 2.3.

Table 2.3 Proximate composition of *Bacillus*-fermented soybeans

Chemical composition	*Kinema*[1]	*Thua Nao*[2]	*Dawadawa*[3,4]	*Chungkukjang*[5]	*Natto*[6]
Protein (%)	48	38.76	31.25	15.70	42.51
Fat (%)	17	16.97	19.90	9.0	21.11
Ash (%)	7	5.73	1.72	5.2	4.38
Carbohydrate (%)	28	26.38	22.86	16.1	30.80
Fiber (%)	-	11.93	3.47	-	-
Reducing sugar (%)	-	-	-	-	8.20
Ammonia (mg/100g)	-	-	-	-	240.49
Moisture (%)	62	-	59.40	54.00	59.30
pH	7.89	8.0-8.6	8.2-8.9	8.49	6.83

Source: [1]Sarkar *et al*. (1994). [2]Sundhagul *et al*. (1972).[3]Jeff-Agboola and Oguntuase (2006). [4]Omafuvbe *et al*. (2000). [5]Lee *et al*. (2002). [6]Wei and Chang (2004). (-), data not available.

2.4.2 Amino acid profiles

Soybean is considered as a good source of protein with average content being 40% of total components in soybean. Proteins in soybean comprise of a complex mixture of different types; however the storage proteins, β-conglycinin and glycinin are the major components of soy proteins with the proportion of 65 to 80% of total seed proteins (Nielsen, 1985). Amino acids such as Glu, Asp, Leu, Lys, Arg, Pro and Ser are abundant in soybean while there is a deficiency in sulphur-containing Met and Cys (Gupta, 1983). For enhanced bioactive amino acids, microbial fermentation of soybean is indicated with the important role of proteolytic enzymes that are produced from starter organisms to hydrolyse initial soy proteins during the fermentation process to increase the free amino acids content in the product (Sarkar *et al*., 1997; Dakwa *et al*., 2005). The diverse profiles of amino acids in alkaline fermented soybeans are summarised in Table 2.4 with the effect of type of soybean, cooking method, strains of fermented organisms, fermentation period, and incubated condition in the production processes (Sarkar *et al*., 1997; Dakwa *et al*., 2005; Lee *et al*., 2005a). Besides nutritive values, free amino acids have direct influence on the typical taste, e.g. the umami or MSG-like taste of the product related to Glu and Asp, the bitter taste affected by Leu and other hydrophobic amino acids, and sweet taste responsible from Ala, Gly, Ser and Thr (Tseng *et al*., 2005). Yanfang and Wenyi

(2009) revealed that the large contents of Glu, Asp, Arg, Leu and Ala are important contributors to the taste of soy sauce. Flavour volatiles of fermented soybeans are also affected by free amino acids because these compounds act as important precursor substances in the formation reactions of volatile compounds. For example pyrazines, which are typical nutty aroma compounds, derive from the precursor of amino acids like Thr, Gly, Ala, Val, Ile and Leu through Strecker degradation and Maillard reaction (Shu, 1998). Furthermore, the role to promote health benefits such as antihypertensive effect (Gibbs *et al*., 2004), reduce blood cholesterol concentration (Potter *et al.,* 1995), reduction in coronary heart disease (Anderson *et al.,* 1995) and antioxidant activity (Saito *et al.,* 2003) have also been investigated. Gibbs *et al.* (2004) indicated several biologically active peptides in fermented soybeans (*Natto* and *Tempeh*) mostly derived from glycinin soy protein.

Table 2.4 Content of free amino acids (g/kg dry sample) in alkaline fermented soybeans

Free amino acid	***Kinema***[1]	***Dawadawa***[2]	***Chungkukjang***[3]	***Natto***[4]	**Red *Sufu***[5]
Ala	4.73	0.12	0.40	0.66	6.6
Arg	0.22	0.15	1.02	0.17	1.2
Asp	6.25	0.15	0.50	0.84	7.0
Cys	<0.01	0.03	0.51	0	-
Glu	21.06	0.12	4.58	4.12	21.3
Gly	5.08	0.11	0.51	0.72	3.1
His	4.25	0.07	1.07	1.70	1.4
Ile	6.22	0.12	3.62	2.10	5.5
Leu	9.50	0.22	7.73	4.56	8.8
Lys	8.23	0.18	1.30	3.24	5.6
Met	3.15	0.03	1.43	1.24	1.5
Phe	11.82	0.14	5.74	5.26	6.3
Pro	3.54	0.15	0.12	0.30	4.1
Ser	3.24	0.15	1.49	0.44	3.1
Thr	2.79	0.11	0.06	0.62	3.4
Tyr	4.86	0.09	1.88	3.77	4.5
Trp	<0.01	0.03	-	-	-
Val	7.05	0.13	-	2.19	4.9

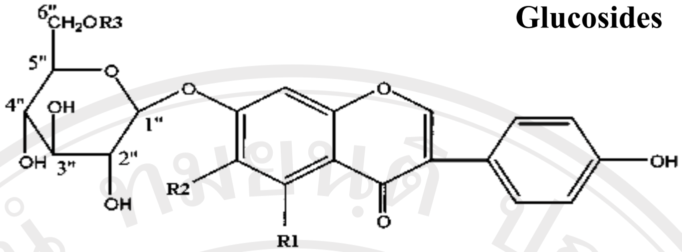
Source: [1]Sarkar *et al*. (1997). [2] Dakwa *et al*. (2005). [3] Lee *et al*. (2005a). [4]Nikkuni *et al*. (1995). [5]Han *et al*. (2004). (-), data not available.

2.4.3 Isoflavone compounds

Isoflavones, a type of phenolic compounds, are formed through condensation of a phenylpropane compound by means of participation of three molecules of malonyl coenzyme A. They are found in soybeans and soybean based food products in various forms comprising aglycone (daidzein, genistein, and glycitein), β-glucoside (daidzin, genistin, and glycitin), malonylglucoside (malonyldaidzin, malonylgenistin, and malonylglycitin), and acetylglucoside (acetyldaidzin, acetylgenistin, and acetylglycitin) forms (Figure 2.2). These compounds are most interesting because of their biological functionality such as anticarcinogenic activity (Gotoh *et al*., 1998; Peterson *et al*., 1998; Jung *et al*., 2006), inhibition of oxidative damage to low-density lipoprotein (Okamoto *et al*., 1995; Potter *et al*., 1998; Park *et al*., 2003), improved bone health (Ishimi *et al.,* 2002), elimination of menopause symptoms (Eden, 1998; Kim *et al*., 2006), antimutagenic effects (Park *et al*., 2003), antidiabetic activity (Liu *et al*., 2006), antiestrogenic effects (Makela *et al.*, 1995), and antioxidant activity (Lee *et al.*, 2005b; Kwak *et al.*, 2007; Lee *et al.*, 2007a; Moktan *et al.*, 2008; Georgetti *et al*., 2009).

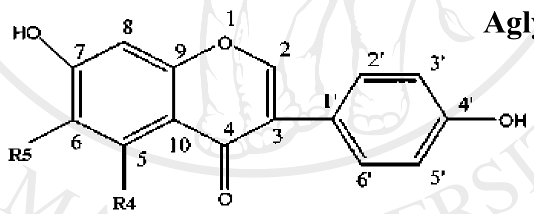
The bioactivity and metabolic fate of dietary soybean isoflavones differ depending on their chemical structure form. Day *et al*. (1998) revealed that after ingestion, isoflavone glucosides can be converted through hydrolysis of intestinal mucosal and bacterial β-glucosidase into aglycone form prior to when they are absorbed directly or further metabolised by microflora in human large intestine. Also Izumi *et al*. (2000) reported that aglycone isoflavones are faster absorbed in humans than their glucosides. Murota *et al*. (2002) suggested that higher efficiency absorption of aglycone derivatives in human intestinal was because of their moderated lipophilicity. Hence, ingestion of isoflavones in available form from dietary sources is expected to give a superior advantage.

Glucosides



R1	R1	R3	Compounds
H	H	H	daidzin
OH	H	H	genistin
H	OCH_3	H	glycitin
H	H	$COCH_3$	acetyldaidzin
OH	H	$COCH_3$	acetylgenistin
H	OCH_3	$COCH_3$	acetylglycitin
H	H	$COCH_2COOH$	malonyldaidzin
OH	H	$COCH_2COOH$	malonylgenistin
H	OCH_3	$COCH_2COOH$	malonylglycitin

Aglycones



R4	R5		Compounds
H	H		daidzein
OH	H		genistein
H	OCH_3		glycitein

Figure 2.2 Structures of soy isoflavone compounds (Liu, 2004).

In soybean seeds, variable content and composition of isoflavones are observed due to growth stages, variety, genotype, crop year, location, and storage time (Wang and Murphy, 1994; Hoeck *et al.,* 2000; Lee *et al*., 2003; Kim and Chung, 2007), however malonylglucoside isoflavones are often reported as the most predominant form in soybean, being followed by β-glucosides, aglycones, and acetylglucosides (Lee *et al*., 2004; Kim and Chung, 2007) (Table 2.5).

Table 2.5 Isoflavone compositions (mg/g dry basis) of non-fermented and fermented soybeans

Isoflavone	**Non-fermented soybean**		**Fermented soybean**		
	Soybean[1]	**Steamed bean**[1]	***Chungkukjang***[1]	***Tofu***[2]	***Natto***[3]
Malonylglucosides					
Malonyldaidzin	330	-	2.02	100	-
Malonylgenistin	680	-	2.36	200	-
Malonylglycitin	20	-	3.06	6.5	-
Acetylglucosides					
Acetyldaidzin	-	-	0.66	1.3	-
Acetylgenistin	4.7	-	-	2.9	-
Acetylglycitin	-	2.14	4.31	-	-
Glucosides					
Daidzin	130	6.04	0.35	57	375
Genistin	150	3.90	0.23	63	765
Glycitin	11	1.76	-	4.5	138
Aglycones					
Daidzein	3.4	0.77	21.59	7.8	786
Genistein	2.6	0.56	19.00	12	349
Glycitein	-	1.97	3.99	0.5	11

Source: [1]Kwak *et al*. (2007). [2]Jackson *et al*. (2002). [3]Wei *et al*. (2008). (-), data not available.

Transformation of glucosides into aglycone isoflavones in soybean is also caused by food processes and enzymatic reactions. In the production process of fermented soybean, soaking and moist heating steps are important for conversion to isoflavone derivatives. During the soaking process, the conversions of glucosides into aglycones are suggested by the hydrolysis of β-glucosidase (Toda *et al*., 2001; Kao *et al*., 2004) depending on soybean cultivars, soaking temperature and time. Whereas moist heating step, both of malonyl and acetyl ester groups of conjugated isoflavones or glucose group of β-glucoside form are cleaved easily by the affect of moist heating bringing about the β-glucoside form or via another pathway, the aglycone form (Toda *et al*., 2000; Chein *et al.*, 2005). Apart from this, microbial fermentation is also considered to play an important role for producing aglycones with the deglycosylation reaction of β-glucosidase that is produced from microorganisms during fermented of soybean (Kao *et al*., 2004; Chien *et al*., 2006). The diverse profiles of isoflavones distributed in fermented soybeans including cooked stage are summarised in Table 2.5.

2.4.4 Antioxidant property

Phenolic compounds are distributed in the embryo, cotyledon and seed coat of soybeans and are indicated as mainly antioxidant compounds in soybean because of their redox properties, which allow them to act as reducing agents, hydrogen donators, single oxygen quenchers, and metal chelating potential (Rice-Evans *et al*., 1995). The OH at the C-4′ position is a key to the antioxidant activity which is further enhanced by an OH at the C-5 location (Wei *et al.,* 1995; Hu *et al.,* 1995). Kim *et al*. (2006) showed supporting evidence antioxidants potential of phenolic substances in soybean with a strong correlation between levels of phenolic components and antioxidant capacity in nine Korean soybean varieties. Various contents of total phenolic compounds, range 730 - 1812 μmol/g were investigated in nine soybean varieties over storage for 4 years (Kim *et al*., 2004a). The quantity and structural conformation of phenolic compounds also affect the degree of antioxidant activity. Among isoflavone components, glucoside genistin had antiradical power greater than other glucosides and aglycones derivatives and based on low-density lipoprotein (LDL) oxidation efficiency, aglycone genistein possessed the strongest inhibition activity as well as its glucoside. There have been reports that stronger antioxidant activity is due to aglycone derivatives of phenolic compounds which are markedly increased in soybeans after microbial fermentation than that presented in original non-fermented soybeans (Kwak *et al*., 2007; Lee *et al*., 2007a; Moktan *et al*., 2008; Georgetti *et al*., 2009). These studies explained that the activity of β-glucosidases, which are released from starter organisms, were the major cause of hydrolysis of original glucoside phenolic substances into aglycone derivatives in fermented soybeans. Antioxidants derived from Korean fermented soybean (Kim *et al*., 2008) and free phenolic compounds of soybean flour fermented with different β-glucosidase-producing fungi (Georgetti *et al*., 2009) could decrease oxidative stress and inhibit free radicals according to *in vitro* and *in vivo* studies. Also *Chungkukjang*, *Kinema*, *Natto* and *Tempeh* have been recommended as a good source of antioxidant components superior to their non-fermented stage (Hattori *et al*., 1995; Shon *et al*., 2007; Moktan *et al*., 2008; Chang *et al*., 2009).

Phenolic acids have also been found to partially possess antioxidant activity in soybean products. Pratt and Birac (1979) and Hammerschmidt and Pratt (1978) found several derivatives of phenolic acids like chlorogenic, isochlorogenic, caffeic, ferulic, syringic, vanillic, feruric, *p*-coumaric and *p*-hydroxybenzoic acids, possessing antioxidant activity in soybean extracts with the potential activity increasing in order as *p*-coumaric, ferulic, chlorogenic and caffeic acids, respectively. While, Chen *et al* (2005) identified 4 phenolic acids included β-hydroxybenzoic, vanillic, syringic, and feruric acid in methanol extracts of *Douchi* with the highest antiradical power of syringic acid follow by feruric, vanillic and β-hydroxybenzoic acid, respectively. In addition, the other antioxidant components such as tocopherols, phospholipids, peptides, free amino acids, and melanoidins (a by-product of the Maillard reaction) have also been found in microbially fermented soybeans as potentially antioxidant components (Pratt and Birac, 1979; Chen *et al.*, 1998; Rufian-Henares and Morales, 2007a; Wang *et al*., 2008). Peptides containing His and Tyr have been reported as possessing antioxidant activity in soy protein hydrolysis (Chen *et al*., 1998). Supporting evidence also revealed by Wang *et al*. (2008) for the excellent antioxidant activity of aqueous extracts of *Douchi* due to peptides and free amino acids which were formed after fungal fermentation. The diversity concentrations of phenolics and potentially antioxidants distributed in microbial fermented soybeans are summarised in Table 2.6.

Table 2.6 Antioxidant qualities of alkaline fermented soybeans

Product	**Fermented microbial**	**Extracted solvent**	**Antioxidant components**	**Antioxidant activity**
Chungkukjang[1] (yellow soybean)	*Bacillus* species	Ethanol	Isoflavones and other polyphenolic compounds	anti-DPPH radicals, LDL oxidation
Chungkukjang[2] (black soybean)	*Bacillus subtilis*	Methanol	Anthocyanin, total phenolics, daidzein, genistein	anti-DPPH radicals, anti-hydroxy radicals, total antioxidant
Kinema[3]	*B. subtilis* DK-W1	Methanol	Total phenolics	- anti-DPPH radicals, metal chelator, LPIA
Douchi[4]	*Aspergillus oryzae*	Aqueous	Peptides, isoflavones	50% inhibition of DPPH

Abbreviations: LDL, low density lipoprotein; LPIA, lipid peroxidation inhibition activity.
Source: [1]Kim *et al*., 2008; [2]Shon *et al*., 2007; [3]Moktan *et al*., 2008; [4]Wang *et al*., 2008.

2.4.5 Aroma and flavouring agents

In raw soybean, the typical green and grassy aromas are produced by hexanal and hexanol (Sugawara *et al*., 1985). The different profiles of volatile compounds in cooked soybean have been reported with the temperature and time of heating (Table 2.7). The predominant components in 1.5 - 5 h boiled soybean have been reported to be 1-octen-3-ol, furfuryl alcohol, 2-butyl-octenal, hexanal, 2,6-di-tert-butyl-p-cresol and pentanol (Sugawara *et al*., 1985; Chairote, 1987; Dakwa *et al.,* 2005). Whereas the most volatiles involved in longer boiling time (7 h) of soybeans are 2-pentylfuran, 2-ethylfuran and 1-octen-3-ol (Leejeerajumnean *et al*., 2001). Of autoclaved soybean, 1-octen-3-ol, benzaldehyde, 2,5-dimethylpyrazine, hexanal and hexanol are the major substances that contribute to soybean cooked flavour (Owens *et al*., 1997). Apart from cooking process, differences of soybean cultivar, exaction of volatiles and analysis method are another main effect leading to various volatiles profile of soybean. For example, Owens *et al.* (1997) and Leejeerajumnean *et al.* (2001) used a Tenax trap technique for extracting and collecting volatile compounds of cooked soybeans whilst Chairote (1987) and Dakwa *et al.* (2005) employed simultaneous steam distillation method.

Table 2.7 Flavour volatile compounds identified in cooked non-fermented soybeans

Volatile compounds	Autoclaved[1]	Boiled			
	121°C/20min	1.5 h[2]	3 h[3]	4-5 h[4]	7 h[5]
Alcohol					
1-Hexanol	+	-	-	-	-
1-Octen-3-ol	+	-	+	-	+
3-Octenol	-	-	-	-	+
Hexanol	-	-	+	-	-
n-Hexanol	-	-	-	+	-
Furfuryl alcohol	-	-	+	-	-
Ethanol	-	-	-	+	-
3-Octanol	-	-	-	+	-
1-Amyl alcohol	-	-	-	+	-
n-Amyl alcohol	-	-	-	+	-
β-Phenyl ethanol	-	-	-	+	-
Aldehydes					
Hexanal	+	+	-	-	+
Nonanal	+	-	-	-	-
Decanal	+	-	-	-	-
2-Butyl-octenal	-	+	-	-	-
Pentanal	-	-	-	-	+
4-Pentanal	-	-	-	-	+
n-Hexanal	-	-	-	+	-
Allyl butylaldehyde	-	-	-	+	-
Ketones					
2-Heptanone	+	-	-	+	-
3-Octanone	+	-	-	+	-
Acetophenone	-	-	+	-	-
Acids and esters					
Docyl butanoate	+	-	-	-	-
Hexadecanoic acid	-	+	-	-	-
Ethyl acetate	-	-	-	+	-
Ethyl formate	-	-	-	+	-
Vinyl stearate	-	-	-	+	-
Pyrazines					
Methylpyrazine	+	-	-	-	-
2,5-Dimethylpyrazine	+	-	-	-	-
2,6-Dimethylpyrazine	+	-	-	-	-
2-Ethyl-5-methylpyrazine	+	-	-	-	-
2-Ethyl-6-methylpyrazine	+	-	-	-	-
Trimethylpyrazine	+	-	-	-	-
3,5-Dimethyl-2-ethylpyrazine	+	-	-	-	-

Table 2.7 (*continued*)

Volatile compounds	Autoclaved[1]	Boiled			
	121°C/20min	1.5 h[2]	3 h[3]	4-5 h[4]	7 h[5]
3,6-Dimethyl-2-ethylpyrazine	+	-	-	-	-
5,6-Dimethyl-2-ethylpyrazine	+	-	-	-	-
3,5-Dimethyl-2-methylpyrazine	+	-	-	-	-
Aromatic compounds					
Styrene (vinyl benzene)	+	-	-	-	-
Benzaldehyde	+	-	-	+	+
2-Methoxyphenol (guaiacol)	+	-	-	-	-
4-Vinyl-2-methoxyphenol	+	-	-	-	-
P-Xylene	-	+	-	-	-
Chrorobenzene	-	+	-	-	-
1,3-dimethylbenzene	-	+	-	-	-
Furans					
2-Pentylfuran	+	-	+	-	+
2-Ethylfuran	-	-	-	-	+
2-n-pentylfuran	-	-	-	+	-
Miscellaneous					
Indole	+	-	-	-	-
2,4-bis(1,1-dimethyl ethyl) phenol	-	+	-	-	-
Docosane	-	+	-	-	-
2-Chloropropane	-	-	-	-	+
4-Ethyl-2-methylthiazole	-	-	+	-	-
(E)-3,5-Dimethyl-1,2,4-trithiolane	-	-	+	-	-
(Z)- 3,5-Dimethyl-1,2,4-trithiolane	-	-	+	-	-
2,6-Dimethyl-1,3-dithiin	-	-	+	-	-
Thialdine	-	-	+	-	-
Diethyl ether	-	-	-	+	-
3-Methyl heptane	-	-	-	+	-
Thiofenchone	-	-	-	+	-
Myrcene	-	-	-	+	-
2-Methyl-1-pentene	-	-	-	+	-
Linalool	-	-	-	+	-
2-Methyl fecalin	-	-	-	+	-
2,6-Di-tert-butyl-p-cresol	-	-	-	+	-
Diethyl phthalate	-	-	-	+	-

Source: [1]Owens *et al.* (1997). [2]Dakwa *et al.* (2005). [3]Sugawara *et al.* (1985). [4]Chairote (1987). [5]Leejeerajumnean *et al*. (2001). (-), data not available.

Microbial fermentation is also shown as one of the key factors in producing flavour volatiles of soy-fermented foods. During microbial fermentation of soybeans, metabolic activities of the microbes generate various precursors of aroma volatile compounds (i.e. acetolactate, acetoin, free amino acids, ammonia, alcohols, aldehydes and ketones) that are converted to various kinds of aroma compounds (i.e. pyrazines, aldehydes, alcohols, acids, esters, and other compounds) by Strecker degradation and Maillard reactions. High concentrations of flavour volatile compounds after fermentation of soybeans by *Bacillus* sp. have been reported in *Dawadawa* (Owens *et al.*, 1997), *Natto* (Sugawara *et al.*, 1985), *Chungkukjang* (Tanaka *et al.,* 1998), and *Thua Nao* (Leejeerajumnean *et al.,* 2001). In addition, the diversity of volatiles is also presented in these products as summarised in Table 2.8. The unique aroma is the character of the fermented soybeans relating to quality of the product. Odour like *Natto* or fruity/nutty is indicative of good quality of the product, but ammonia-like and fishy odour resulting nasty smell indicates spoilage of food.

Table 2.8 Flavour volatile compounds of *Bacillus*-fermented soybeans

Volatile compounds	***Thua Nao***[1]	***Chung-kukjang***[2]	**Commercial *Natto***		***Dawadawa***	
			Mito[1]	***Natto***[2]	**Pure culture**[3]	**Traditional**[4]
Alcohols						
Ethanol	-	-	-	-	-	+
Methanol	-	-	-	+	-	-
1-Butanol	-	+	-	+	-	-
2-Butanol	+	-	-	+	-	-
2-Buten-1-ol	-	+	-	-	-	-
2-Methyl-1-propanol	+	+	-	+	-	+
1-Mehtyl cyclohexanol	+	-	-	-	-	-
3-Methyl-3-buten-1-ol	-	+	-	+	-	-
1-Hexanol	+	+	-	+	-	-
2-Ethyl-1-hexanol	-	+	-	+	-	-
1-Octen-3-ol	+	+	+	-	+	-
2,3-Butadiol	-	-	-	-	-	+
Phenyl ethanol	-	-	-	-	-	+
3-Methyl-1-butanol	-	+	-	+	-	-
2-Methyl-2-butanol	-	-	-	+	-	-
1-Propanol	-	+	-	+	-	-
1-Penten-3-ol	-	+	-	+	-	-

Table 2.8 (*continued*)

Volatile compounds	***Thua Nao***[1]	***Chung-kukjang***[2]	**Commercial *Natto***		***Dawadawa***	
			Mito[1]	***Natto***[2]	**Pure culture**[3]	**Traditional**[4]
3-Propen-1-ol	-	+	-	-	-	-
Furfuryl alcohol	-	+	-	+	-	-
2-Butoxyethanol	-	+	-	-	-	-
Aldehydes						
3-Methyl-1-butanal	+	+	-	-	-	+
2-Methyl butanal	+	+	-	-	-	-
2-Methyl-2-butenal	-	+	-	-	-	-
2-Methyl propanal	-	+	-	-	-	-
(E,E)-2,4-Decadienal	-	-	-	-	-	+
5-Methyl-2-phenyl-2-hexanal	-	-	-	-	-	+
Acetaldehyde	-	+	-	+	-	-
2-Butenal	-	+	-	-	-	-
Pentanal	-	-	-	-	-	-
Hexanal	-	+	-	-	+	-
Nonanal	-	-	-	-	+	-
Decanal	-	-	-	-	+	-
Tetradecanal	-	-	-	-	+	-
Ketones						
Acetone	-	+	-	+	-	-
Acetophenone	-	+	-	-	-	-
Butanone	+	-	-	-	-	-
2-Butanone	-	+	-	+	+	-
2-Heptanone	+	+	-	+	+	-
3-Methylbutanone	+	-	-	-	-	-
3-Methylpentanone	+	-	-	-	-	-
3-Hydroxy-2-butanone (acetoin)	+	+	+	+	+	-
3-Methyl-2-butanone	-		-	+	+	-
3-Methyl-2-pentanone	-	+	-	+	-	-
2-Methyl-3-pentanone	-	+	-	-	-	-
4-Methyl-2-pentanone	-	+	-	+	-	-
3-Hydoxy-3-methyl-2-butanone	-	+	-	+	-	-
2,3-Butanedione (diacetyl)	-	-	-	+	+	-
2,3-Pentaindione	-	+	-	+	-	-
2-Pentanone	-	-	-	+	+	-
Methyl-2-pentanone	-	-	-	-	+	-
5-Methyl hexanone	-	+	-	-	-	-
5-Methyl-2-hexanone	-	-	-	+	+	-
6-Methyl-2-heptanone	-	-	+	+	+	-

Table 2.8 (*continued*)

Volatile compounds	***Thua Nao***[1]	***Chung-kukjang***[2]	**Commercial *Natto***		***Dawadawa***	
			Mito[1]	***Natto***[2]	**Pure culture**[3]	**Traditional**[4]
5-Methyl-2-heptanone	-	-	-	+	+	-
3-Octanone	-	-	-	-	+	-
4-(acethyloxy)-2-butanone	-	+	-	+	-	-
3-Hydroxy-3,5-dimethyl-2-hexanone	-	+	-	-	-	-
2-Decanone	-	-	-	-	+	-
2-Tridecanone	-	-	-	-	+	-
Pentadecanone	-	-	-	-	+	-
Acids and esters						
Ethyl acetate	-	+	-	+	-	+
Methyl acetate	-	-	-	+	-	-
Methyl phenyl acetate	-	-	-	-	+	-
Butyric acid	-	+	-	+	-	-
Ethyl butyrate	-	+	-	-	-	-
Isobutyric acid	-	+	-	+	-	-
Methyl isobutyrate	-	-	-	+	-	-
Ethyl isobutyrate	-	-	-	+	-	-
2-Methylbutanoic acid	+	+	-	-	-	-
Ethyl-2-metylbutanoate	+	-	-	-	-	-
Ethyl pentanoate	+	-	-	-	-	-
Methylpentanoate	+	-	-	-	-	-
Methyl propionate	-	-	-	+	-	-
Ethyl propionate	-	+	-	-	-	-
Butyl propionate	-	+	-	-	-	-
Ethyl-2-nitropropionate	-	+	-	-	-	-
Ethyl hexanoate	-	+	-	-	-	-
Ethyl heptanoate	-	+	-	-	-	-
3-Methylbutyl pentanoate	-	-	-	-	-	+
3-Methyl-2-butenoic acid	-	+	-	-	-	-
Hexadecanoic acid	-	-	-	-	-	+
Acetic acid	-	+	-	+	+	-
3-Methyl-butanoic acid	-	+	-	-	-	-
Ethyl isovalerate	-	+	-	-	-	-
3-Methyl butanoate	-	-	-	-	+	-
Decyl butanoate	-	-	-	-	+	-
Methyl -2-methylbutanoate	-	+	-	+	+	-
Ethyl -2-methylbutanoate	-	+	-	-	-	-

Table 2.8 (*continued*)

Volatile compounds	***Thua Nao***[1]	***Chung-kukjang***[2]	**Commercial *Natto***		***Dawadawa***	
			Mito[1]	***Natto***[2]	**Pure culture**[3]	**Traditional**[4]
Pyrazines						
2,5-Dimethylpyrazine	+	+	+	+	+	-
Trimethylpyrazine	+	+	+	+	+	+
2,6-Dimethylpyrazine	-	+	-	-	+	-
3-ethyl-2,5-dimethylpyrazine	-	-	+	-	-	+
3,5-Dimethyl-2-methylpyrazine	-	-	+	-	+	-
2,3,5-Trimethyl-6-ethylpyrazine	-	-	+	-	-	-
Tetramethylpyrazine	+	-	+	-	+	+
Pyrazine	-	+	-	+	-	-
2-Methylpyrazine	-	+	-	+	+	-
2,3-Dimethylpyrazine	-	-	-	-	+	-
3,5-Dimethyl-2-methylpyrazine	-	-	-	-	+	-
3,5-Dimethyl-2-ethylpyrazine	-	-	-	-	+	-
3,6-Dimethyl-2-ethylpyrazine	-	-	-	-	+	-
5,6-Dimethyl-2-ethylpyrazine	-	-	-	-	+	-
2-Ethyl-5-methylpyrazine	-	-	-	-	+	-
2-Ethyl-6-methylpyrazine	-	-	-	-	+	-
Aromatic compounds						
Benzene methanol	-	-	-	+	-	-
Benzaldehyde	+	+	+	-	+	+
Ethyl benzene	-	+	-	+	-	-
Dichlorobenzene	-	+	-	+	-	-
Trimethyl benzene	-	-	-	+	-	-
Tetramethyl benzene	-	-	-	+	-	-
Vinylbenzene (styrene)	-	+	-	+	+	-
Benzyl alcohol	+	-	-	-	-	-
Chlorobenzene	-	-	-	-	-	+
Furans						
3-Methylfuran	-	-	-	+	-	-
2-Pentylfuran	+	+	-		+	
Sulphur-containing compounds						
Dimethyl disulfide	+	-	-	-	+	+
Diallyl disulfide	-	+	-	-	-	-
Dimethyl trisulfide	-	-	-	-	-	+
Miscellaneous						
Chloroform	-	-	-	+	-	-
Xylene	-	+	-	+	-	-
p-Xylene	-	-	-	-	-	+

Table 2.8 (*continued*)

Volatile compounds	***Thua Nao*[1]**	***Chung-kukjang*[2]**	**Commercial *Natto***		***Dawadawa***	
			Mito[1]	***Natto*[2]**	**Pure culture[3]**	**Traditional[4]**
Toluene	-	+	-	+	-	-
Ethyl toluene	-	+	-	-	-	-
Thiophene	-	-	-	+	-	-
Indole	+	-	-	-	+	+
Limonene	-	-	-	+	-	-
Phenol	-	+	-	-	-	-
2-Methoxy phenol (guaiacol)	-	+	-	-	+	-
4-Vinyl-2-methoxyphenol	-	-	-	-	+	-
3-Methyl-3-propyl-cis-oxirane	+	-	-	-	-	-
2-Propylbutane	+	-	-	-	-	-
Pyridine	-	-	-	+	-	-
Trimethyloxazole	-	-	-	-	+	-

Source: [1]Leejeerajumnean *et al.* (2001). [2]Tanaka *et al.* (1998). [3]Owens *et al.* (1997). [4]Dakwa *et al.* (2005). (-), data not available.

Pyrazines are important flavour and aroma compounds in heated foods especially those with specific roasted nutty, corny bready and cooked flavour (Reineccius, 2006). Besides, it has also been reported that formation of pyrazine compounds could be induced by *B. subtilis* IFO 3013 during soybean fermentation (Besson *et al*., 1997; Larroche *et al*., 1999). Tetramethylpyrazine was first identified in *Natto* and cultures of *B. subtilis* (natto) with aroma very similar to *Natto* (Kosuge and Kamiya, 1962). Subsequently, other derivatives such as 2,5-dimethylpyrazine, trimethylpyrazine, methylpyrazine, 2,3-dimethylpyrazine, 2,6-dimethylpyrazine, ethylpyrazine, 2-ethyl-6-methylpyrazine, 3-ethyl-2,5-dimethylpyrazine, 2,6-diethyl-3-methylpyrazine and 2-methyl-5-propenylpyrazine have been verified and suggested to be the key compounds of fruity/nutty aroma in *B. subtilis* fermented *Natto* (Sugawara *et al*., 1985; Yamaguchi *et al.,* 1993; Tanaka *et al*., 1998; Leejeerajumnean *et al.*, 2001). A mechanism for the production of 2,5-dimethylpyrazine and tetramethylpyrazine by *B. subtilis* in solid substrate fermentation on ground soybeans was explained by Larroche *et al*. (1999) as shown in Figure 2.3.

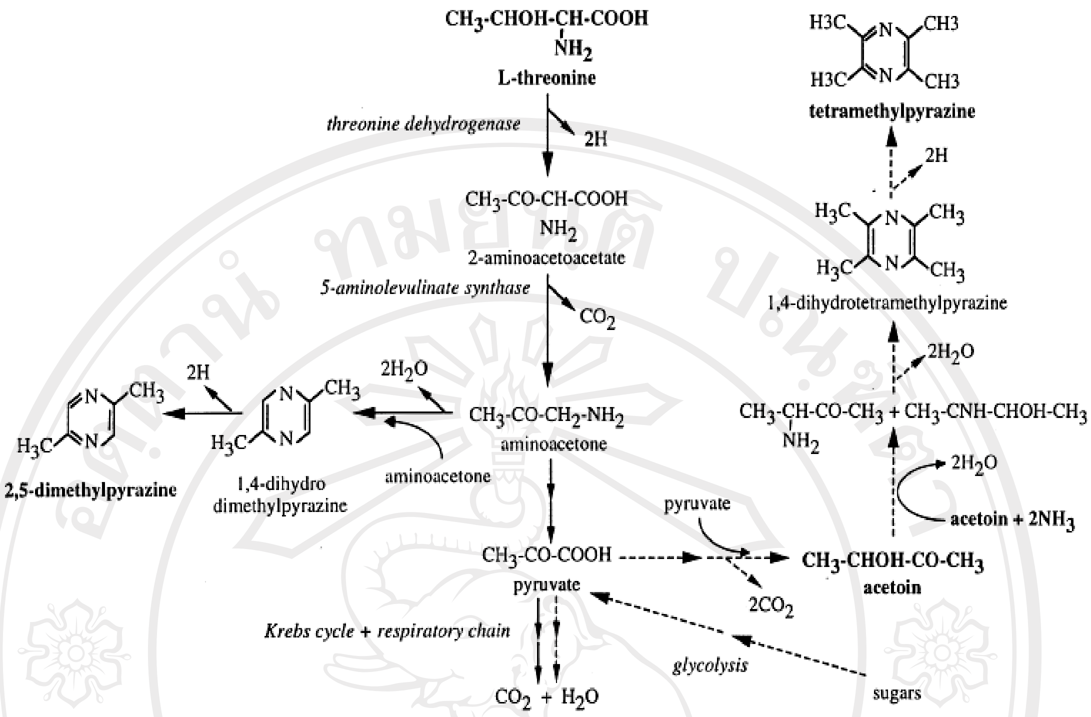


Figure 2.3 Metabolic pathways for 2,5-dimethylpyrazine and tetramethylpyrazine synthesis by *B. subtilis* in solid substrate fermentation on ground soybeans (Larroche *et al*., 1999).

After microbial fermentation, distinct quantities of some groups of volatiles were observed in soybeans. During *Thua Nao* fermentation, ketones, acids and esters, pyrazines, aromatic components and sulphur-containing compounds were generated from fermentation process and responsible for a unique flavour and odour of the product (Leejeerajumnean *et al*., 2001). Similar results have been also reported in *Daddawa* for intense increasing of ketones, pyrazines and aromatic compounds (Owens *et al*., 1997) (Figure 2.4).

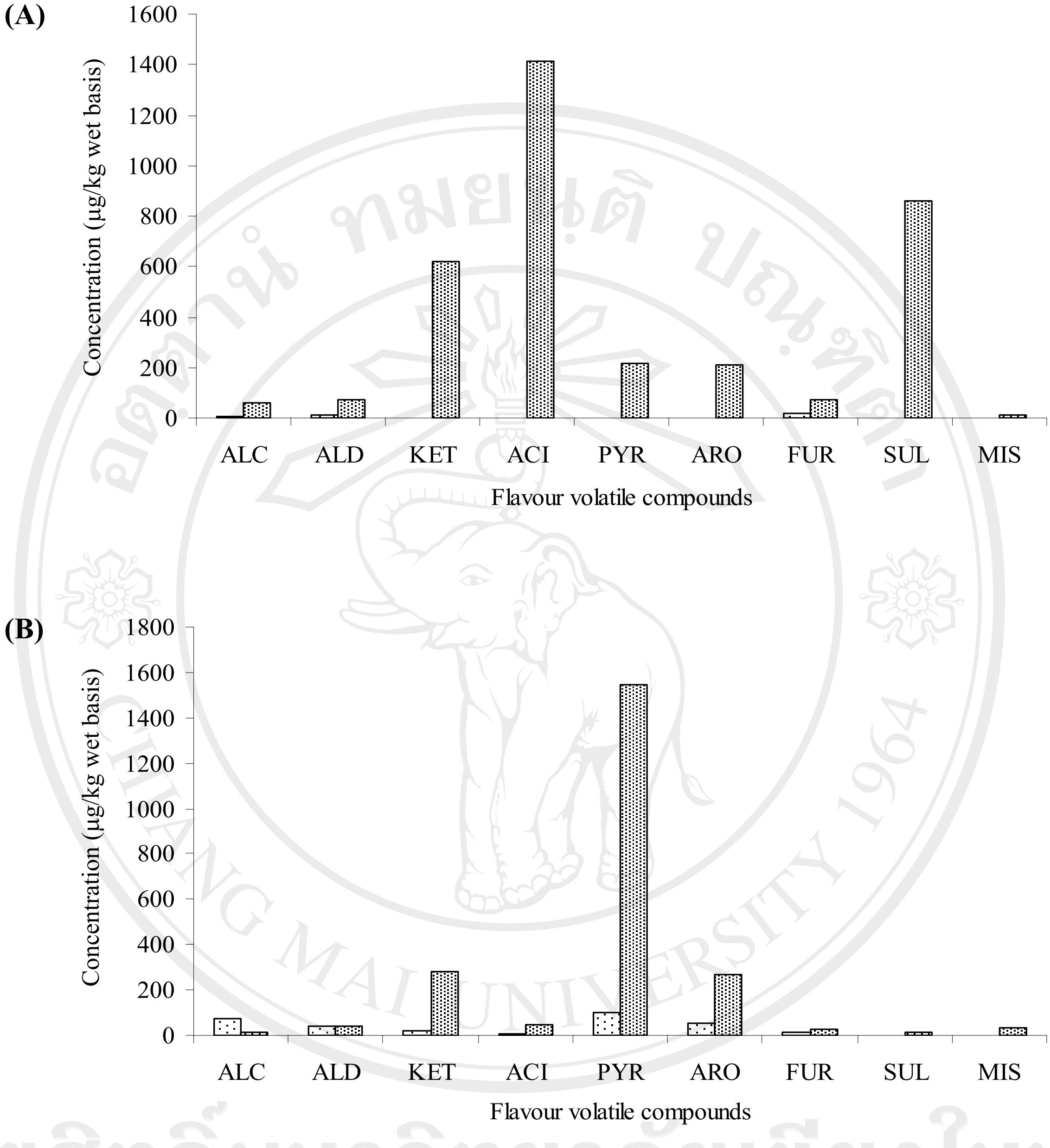


Figure 2.4 Flavour volatile compounds in before (□) and after (▩) fermentation of *Thua Nao* (A) (Leejeerajumnean *et al*., 2001) and *Daddawa* (B) (Owens *et al*., 1997). ALC, alcohols; ALD, aldehydes; KET, ketones; ACI, acids and esters; PYR, pyrazines; ARO, aromatic compounds; FUR, furans; SUL, sulphur-containing compounds; MIS, miscellaneous.

2.4.6 Antimicrobial activity

To date there have been a few investigations of antimicrobial activity in crude extracts of *Bacillus*-fermented soybeans (Zheng and Slavik, 1999; Kim *et al*., 2004b; Yun, 2005) but those of *Bacillus* sp., a key organisms involved in soy fermented foods, have been studied extensively. Several antibiotic substances and bacteriocins against other organisms produced from *B. subtilis* and related strains are verified as summarised in Table 2.9. Bacteriocins have a concentration-dependent mode of action which is also affected by physiological condition such as ionic strength, temperature, pH and by the growth phase of the target organisms. Besides antibiotic substances, phytochemical isoflavones, peptides, amino acids and melanoidins are expected to synergistically inhibit activity in crude extracts of soybean (Naim *et al*., 1974), Chinese fermented soybean seasoning (Zheng and Slavik, 1999), *Tempeh* (Kiers *et al*., 2002), *Chungkukjang* (Kim *et al*., 2004b), and *Doenjang* (Yun, 2005).

Table 2.9 Antibiotic substances produced by *Bacillus subtilis* and related strains

Microorganisms	**Antimicrobial components**	**Target microorganisms**	**Reference**
B. subtilis	Bacillomycin F	Fungi and *Micrococcus luteus*	Mhammedi *et al.* (1982)
	Subtilocin A	Gram-positive bacteria	Babasaki *et al*. (1985)
	Botrycidin AJ1316	Fungi	Zuber *et al*. (1993)
	Subtilin	Gram-positive bacteria	Klein *et al*. (1993)
	Subtilosin	Gram-positive bacteria	Paik *et al*. (1998)
	Alirin B-1	Fungi	Shenin *et al*. (1995)
	Peptide antibiotics	*Botrytis cinerca* and *Alternaria brassicicola*	Leifert *et al*. (1995)
	Phenylacetic acid	*Barsaphelenchus xylophilusi*	Kawazu *et al.* (1996)
	Bacillocin22	*Bacillus cereus* and *Listeria monocytogenes*	Zheng and Slavik (1999)
	Bacilysocin	*Staphylococcus aureus, Saccharomyces cerevisiae, Candida pseudotropicalis, Cryptococcus neoformans*	Tamehiro *et al*. (2002)
	Bacteriocin-like substance	*Bacillus cereus* and *Listeria monocytogenes*	Motta *et al*. (2004)
	Sublancin	Gram-positive bacteria	Stein *et al*. (2004)

Table 2.9 (*continued*)

Microorganisms	**Antimicrobial components**	**Target microorganisms**	**Reference**
B. licheniformis	Phenylacetic acid	*Staphylococcus, Escherichia coli, Candida albicans*	Kim *et al*. (2004b)
B. pumilus	Supernatant	*Micrococcus luteus, Staphylococcus aureus, Bacillus cereus, Enterococcus facium, Listeria monocytogenes, Escherichia coli, Samonella typhimurium, Shigella dysenteriae, Yersinia enterocolitica, Aspergillus ochraceus, Penicillium rogueforti*	Ouoba *et al*. (2007)

2.4.7 Other health benefits

The potential role in reducing cancer risk played by soybeans and soybean products are confirmed by many researchers. Reduction both of hormone-dependent and hormone-independent cancer cells including breast (Peterson and Barnes, 1991; Lee *et al*., 1991), prostate (Peterson and Barnes, 1993; Naik *et al*., 1994), leukemia (Makishima *et al*., 1993), gastric (Matsukawa *et al*., 1993), melanoma (Rauth *et al*., 1997) and colonic cancer (Messina and Bennink, 1998) have been found to be associated with soy intake. Park *et al*. (2003) showed a strong inhibitory effect against various carcinogens or mutagens and alflatoxin B1 antimutagenic activity of *Doenjang* and *Chungkukjang* extracts. Besides these, Kim *et al*. (2008) also reported the antigenotoxic potential of *Chungkukjang* in animal studies; oral intake of *Chungkukjang* extracts for 2 weeks was able to reduce malondialdehyde formation (DNA damage and micronucleated reticulocytes) in mice. The improvement post-menopausal symptoms and its related diseases such as osteoporosis and cardiovascular diseases which usually affect women in the post-menopausal stage have also been reported with respect to soy diet intake (Thorneycroft, 1989; Alekel *et al*., 2000; Peluso *et al*., 2000; Park *et al*., 2005). Yokota *et al*. (1996) and Iwai *et al*. (2002) showed the potential of *Natto* fractions to prevent atherosclerosis development together with a significant reduction in serum cholesterol and triacylglycerol levels in animal studies by the effective of inhibitory oxidation of low-density lipoprotein activity. In addition, decreasing of serum total cholesterol concentration in Japanese men and women who have a high intake of soy products has been confirmed by Nagata *et al*. (1998). Nattokinase, antithrombotic and fibrinolytic enzyme extracts from *B. subtilis* fermented *Natto* (Sumi *et al*., 1987) and *Chungkukjang* (Kim *et al*., 1996; Lee *et al*., 1998) strains might be used to prevent cardiovascular disease. The mechanisms for breakdown of fibrin by nattokinase have been consequently discovered by Milner and Makise (2002). Also, derived protein from fermented *Natto* by *B. subtilis* strain 168 is reported to have fibrinolytic function (Omura *et al*., 2005). Osteoporosis disease is also expected to improve by dietary intake of *Natto* with the partial role of vitamin K (menaquinone-7). Significantly increased serum vitamin K has found in men with dietary intake of *Natto* (Tsukamoto *et al*., 2000). In addition, antidiabetic actions of soy-fermented *Chungkukjang* have been demonstrated by Kwon *et al*. (2006) and Kwon *et al*. (2007).

2.5 Improvement of soy-fermented food

Traditional soy-fermented foods are usually produced based on small-scale household practices, which bring about inconsistent product quality, liberates stronger ammonical smell and greater risk of contamination with pathogenic microorganisms. Several strains of foodborne and spoilage organisms such as *Bacillus cereus*, *Staphylococcus saprophysicus*, *S. epidermidis*, *Micrococcus luteus*, *Pseudomonas aeroginosa*, Enterobacteriaceae, coliforms and *Escherichia coli* are identified in traditional productions of soy-*Dawadawa* (Jideani and Okeke, 1991; Dike and Odunfa, 2003), *Thua Nao* (Leejeerajumnean, 2003), and *Kinema* (Nout *et al*., 1998).

In order to eliminate these problems and to improve upon traditional soybean fermentation technology, inoculation of soybean cultivars with selected starter organisms in combination with fermentation under controlled conditions similar to *Natto*, which has employed a pure culture of *B. subtilis* since 1928 (Hosoi and Kiuchi, 2003), are mostly used. Improvement of *Kinema* by fermenting autoclaved soybeans with starter culture of *B. subtilis* isolated from traditional *Kinema* fermentations have been reported (Tamang *et al*., 1988; Sarkar and Tamang, 1995; Tamang and Nikkuni, 1996). Omafuvbe *et al*. (2002) also reported for the satisfactory organoleptical qualities of soy-*Daddawa* the use of pure starter of *B. subtilis* isolated strains; subsequently the optimum temperature of incubation starter *Daddawa* was published

(Omafuvbe, 2008). For Korean *Chungkukjang*, Lee *et al*. (2005a) reported that high quality product was produced with starter culture of *Bacillus* sp. isolated from traditional *Chungkukjang*. However, in spite of the effect of starter culture strain, knowledge about the effects on quality of soybean cultivar, seed size, production process (e.g. inoculum size, temperature and time of soaking and sterilising steps, and incubation condition) is scarce and restricted to *Natto* (Ohta, 1986; Taira *et al.*, 1987; Maruo and Yoshkawa, 1989; Taira, 1990; Matsumoto *et al*., 1993; 1995; Wei *et al*., 2001; Wei and Chang, 2004).

Concerning *Thua Nao* production, Chantawannakul *et al*. (2002) and Visessanguan *et al*. (2005) have demonstrated faster rates of soybean fermentations by using pure starter culture of *B. subtilis* isolated strains with their potent highly active proteolytic activities. Also Tangjitjaroenkun *et al*. (2004) reported that vitamin B12 could improve in *Thua Nao* produced by mixture cultures of *Bacillus* sp. B4 and *Klebsiella* sp. KB2 isolated from local product. However, reports of the improvement of organoleptical and other nutritional qualities of the product remain limited. This study hence proposes to utilize another *B. subtilis* TN51 which has been previously isolated from commercial *Thua Nao* and reported to produce the highest proteolytic and amylolytic activities (Dajanta *et al*., 2009) to yield improved biochemical and microbiological qualities, including sensory attributes of *Thua Nao* product.