

# Occurrence of Biogenic Amines in Soybean Food Products

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## 1. Introduction

Biogenic amines (BAs) are known as toxic substances and formed in foods as a result of microbial action during fermentation and storage (Shalaby, 1996; Santos, 1996). BAs could cause diseases with food poisoning symptoms such as stimulating the nerves and blood vessels in man and animals (Joosten, 1988). The most important BAs found in foods are putrescine, cadaverine,  $\beta$ -phenylethylamine, tyramine, spermine, histamine, spermidine, tryptamine and agmatine. BAs exist in fish, meat, egg, cheeses, vegetables, soybean, beer, wine, etc., and their products. BAs are also known as possible precursors of carcinogens, such as N-nitrosamines (Shalaby, 1996; Santos, 1996). They are frequently found in high concentrations in foods and can not be reduced by high-temperature treatment (Shalaby, 1996; Santos, 1996). BAs in food are extensively studied; a lot of information on formation and occurrence of the biogenic amines in foods is given in recent reviews (Davidek & Davidek, 1995; Halasz et al., 1994; Santos, 1996; Stratton et al., 1991; Suzzi & Gardini, 2003). There are various kinds of soy products such as soybean paste, soy sauce, soy milk and soy curd, in which biogenic amines can be analyzed. Major sources of biogenic amines in the soy foods include: fermented/non-fermented foods such as soy sauce, Miso and Tofu. Nutritionally, soybean milk, Tofu and Sufu have the same importance to people of Asia as they prefer the salt-coagulated bean curd, not only because it has the desired texture, but also because it serves as an important source of calcium (Wang & Hesseltine, 1970). BAs are formed in fermented soybean products by microorganisms during fermentation, and high levels of BAs have been reported for soy products (Chin & Koehler, 1983; Mower & Bhagavan, 1989; Nout et al., 1993; Stratton et al., 1991; Yen, 1986). As the microbial spoilage of food may be accompanied by the increased production of decarboxylases, the presence of biogenic amines might serve as a useful indicator of food spoilage. For these reasons, it is important to monitor biogenic amines levels in foods. Soy sauce, a Chinese traditional fermented condiment, is made from soybean and wheat flour. During the manufacturing process of soy sauce, soy sauce is traditionally prepared by growing the koji mold such as *Aspergillus oryzae* (*A. oryzae*) or *Aspergillus sojae* (*A. sojae*) on the raw material containing a mixture of steam-cooked defatted soybean and roasted wheat flour. Soy sauce mash obtained by mixing the finished koji with brine solution is then subjected to various periods of ageing (Whitaker, 1978). During the fermentation of soy sauce, proteins in the raw materials are hydrolyzed into small molecular weight peptides, amino acids and ammonia

by the proteases produced by *A. oryzae* or *A. sojae* (Whitaker, 1978). During the fermentation and ageing, the flavor may develop gradually. Meanwhile, soy sauce contains relatively high amount of free amino acids, which could be a potential sources of biogenic amine formation. There are many factors that affect the production of BAs, such as the ratio of soybean in the raw material, microbiological composition and duration of fermentation (Chin & Koehler, 1983; Nout et al., 1993).

There are various kinds of soy products such as soybean paste, soy sauce, soy milk and soy curd, in which biogenic amines can be analyzed. The most well known biogenic amines are the neurotransmitters such as serotonin, dopamine, noradrenaline and histamine, best known for their role in allergies. Others, which are less well known, include tyramine, tryptamine and  $\beta$ -phenylethylamine. These biogenic amines may act as neurotransmitters, be involved in local immune responses (such as the inflammation produced by histamine release), or regulate the functions of gut. The classic neurotransmitters serotonin dopamine, noradrenaline are all essential to proper brain functioning. Imbalances of these neurotransmitters can lead depression and anxiety. In relation to food intolerances however, we are more concerned with the biogenic amines contained in foods and beverages that can cause local symptoms in the gut including nausea, diarrhoea and irritable bowel syndrome, as well as triggering symptoms elsewhere in the body, such as migraines, asthma and hives. The chemical structure of biogenic amines can either be:

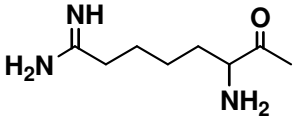
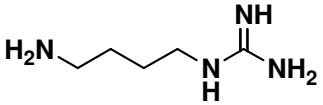
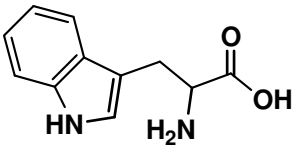
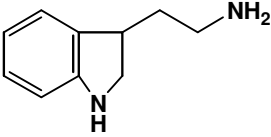
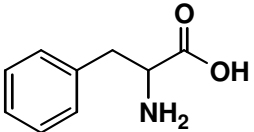
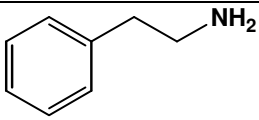
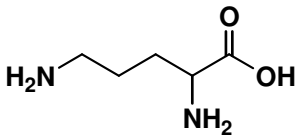
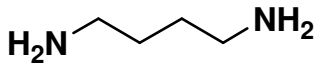
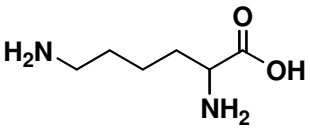
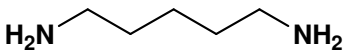
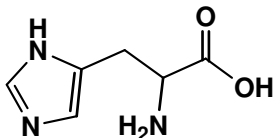
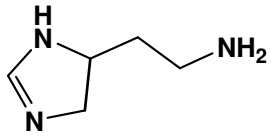
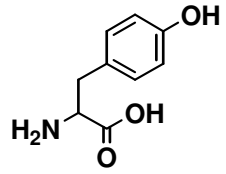
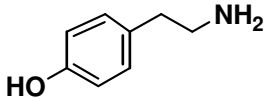
- aliphatic (putrescine, cadaverine, spermine, spermidine)
- aromatic (tyramine, phenylethylamine)
- heterocyclic (histamine, tryptamine) (Santos, 1996)

Amines such as putrescine, spermidine, spermine and also cadaverine are indispensable components of living cells and they are important in the regulation of nucleic acid fraction and protein synthesis and also in the stabilization of membranes (Bardocz, 1995; Majjala et al., 1993; Halasz et al., 1994; Santos, 1996).

## 2. Mechanism of biogenic amines formation

Amine build-up usually results from decarboxylation of free amino acids by enzymes of bacterial origin. Amino acid decarboxylation takes place by the removal of a carboxyl group to give the corresponding amine. Arginine is easily converted to agmatine, or as a result of bacterial activity can be degraded to ornithine from which putrescine is formed by decarboxylation. Lysine can be converted by bacterial action into cadaverine. Histidine can, under certain conditions, be decarboxylated to histamine. Tyramine, tryptamine and  $\beta$ -phenylethylamine come by the same manner from tyrosine, tryptophan and phenylalanine, respectively. Proteolysis, either autolytic or bacterial, may play a significant role in the release of free amino acids from tissue proteins which offer a substrate for decarboxylase reactions (Shalaby, 1996). The precursors of the main biogenic amines are described in Table 1. Prerequisites for biogenic amine formation by microorganisms are:

1. Availability of free amino acids (Joosten, 1988; Marklinder & Lonner, 1992; Soufleros et al., 1998).
2. Presence of decarboxylase-positive microorganisms (Tiecco et al., 1986; Brink et al., 1990; Huis in't Veld et al., 1990).
3. Conditions that allow bacterial growth, decarboxylase synthesis and decarboxylase activity (Brink et al., 1990; Santos, 1996).

Compound name	Precursor	Structure	Molecular weight
Agmatine	 Arginine		130.2
Tryptamine	 Tryptophan		160.2
2-Phenylethyl amine	 Phenylalanine		121.2
Putrescine	 Ornithine		88.2
Cadaverine	 Lysine		202.2
Histamine	 Histidine		111.0
Tyramine	 Tyrosine		137.3

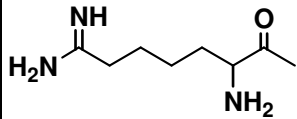
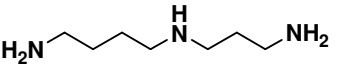
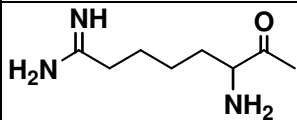
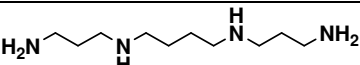
Spermidine	 Arginine		145.3
Spermine	 Arginine		348.1

Table 1. List of some important Biogenic amines and their precursors

### 3. Functions of biogenic amines

BAs are sources of nitrogen and precursors for the synthesis of hormones, alkaloids, nucleic acids and proteins (Santos, 1996). They can also influence the processes in the organism such as the regulation of body temperature, intake of nutrition and increase or decrease of blood pressure (Greif et al., 1999). In plants, polyamines such as spermidine and spermine are implicated in a number of physiological processes, such as cell division, flowering, fruit development, response to stress and senescence (Halasz et al., 1994). Polyamines are important for the growth, renovation and metabolism of every organ in the body and essential for maintaining the high metabolic activity of the normal functioning and immunological system of gut (Santos, 1996; Bardocz, 1995). Because of the diversity of the roles of polyamines in cellular metabolism and growth, the requirement for polyamines is particularly high in rapidly growing tissues. Indeed, the importance of putrescine, spermidine and spermine in tumour growth is widely recognized. Inhibition of polyamine biosynthesis in tumour-bearing individuals is one of the major targets of cancer therapy research.

BAs are potential precursors for the formation of carcinogenic N-nitroso compounds (Krizek & Kalac, 1998). The reaction of nitrosating agents with primary amines produces short-lived alkylating species that react with other components in the food matrix to generate products (mainly alcohols) devoid of toxic activity in the relevant contents. The nitrosable secondary amines (agmatine, spermine and spermidine, etc.) can form nitrosamines by reaction with nitrite, while tertiary amines produce a range of labile N-nitroso products (Halasz et al., 1994). In fatty foods, such as bacon, at high temperature and in the presence of water, the carcinogen N-nitrosopyrrolidine can be formed from putrescine or spermidine (Lovaas, 1991). Some BAs such as putrescine, cadaverine and spermidine can act as free radical scavengers. Tyramine has a remarkable antioxidative activity, which increases with its content. Thus, inhibiting effect depends on amino and hydroxy groups (Halasz et al., 1994). Spermine is able to regenerate tocopherol from the tocopheroxyl radical through hydrogenic donor from amino group. The spermine radical next binds lipid or peroxide radicals into a lipid complex (Greif et al., 1999).

Beginning in the early 1990s, a new era dawned in studies of Biogenic amines as neurotransmitter structure, function and regulation, illuminated by the cloning of transporter cDNAs and genes, the development of transporter-specific gene and protein probes, and the characterization of heterologous expression systems suitable for advanced biophysical analyses. It is believed that the brain contains several hundred different types of

chemical messengers (neurotransmitters) that act as communication agents between different brain cells. These chemical messengers are molecular substances that can affect mood, appetite, anxiety, sleep, heart rate, temperature, aggression, fear and many other psychological and physical occurrences. The biogenic amineneurotransmitters dopamine (DA), norepinephrine (NE) and serotonin (5-hydroxytryptamine, 5-HT) are very simple molecules with highly complex actions in the peripheral and central nervous systems ranging from the control of heart rate to the coloring of mood. Pharmacologists have been fascinated by the amines for decades, as the management of amine production, action or inactivation figures prominently in the treatment of autonomic, emotional and cognitive disturbances. The past decade began with an elucidation of the genes responsible for clearance of amines from the synaptic cleft (Povlock & Amara, 1997).

Scientists have identified three major categories of neurotransmitters in the human brain: Some common structures of biogenic amines as a neurotransmitters are shown in Fig.1 & Fig. 2.

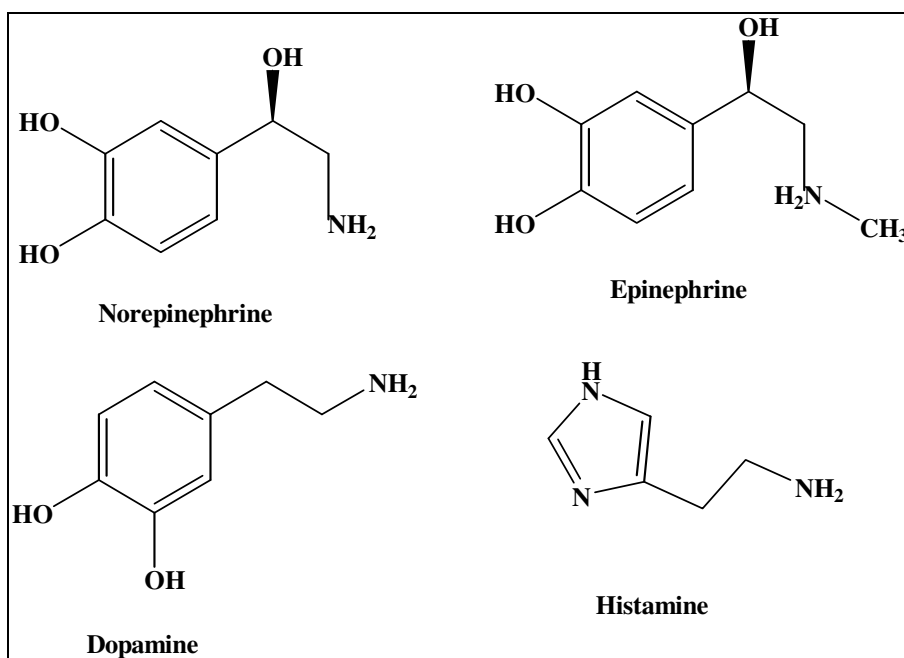


Fig 1. Common biogenic amines as neurotransmitters.

Biogenic amine neurotransmitters have been studied the longest and are probably the best understood in terms of their relationship to psychological disturbances. Some important biogenic amine neurotransmitters are:

Serotonin, is chemical messenger that a role in modulating anxiety, mood, sleep, appetite and sexuality. Serotonin reuptake inhibitors are generally considered first line medication to treat panic disorder.

Norepinephrine, which influences sleep and alertness, is believed to be correlated to fight or flight stress response.

Epinephrine, is usually thought of a stress hormone managed by the adrenal system, but it also acts as a neurotransmitter in the brain.

Dopamine, influences body movement and is also believed to be involved in motivation, reward, reinforcement and addictive behaviours. Many theories of psychosis suggest that dopamine plays a role in psychotic symptoms.

Histamine, is thought to influence arousal, attention and learning. It is also released in response to an allergic reaction. Antihistaine, which are commonly used to treat allergies, have common side effects of sedation, weight gain and low blood pressure.

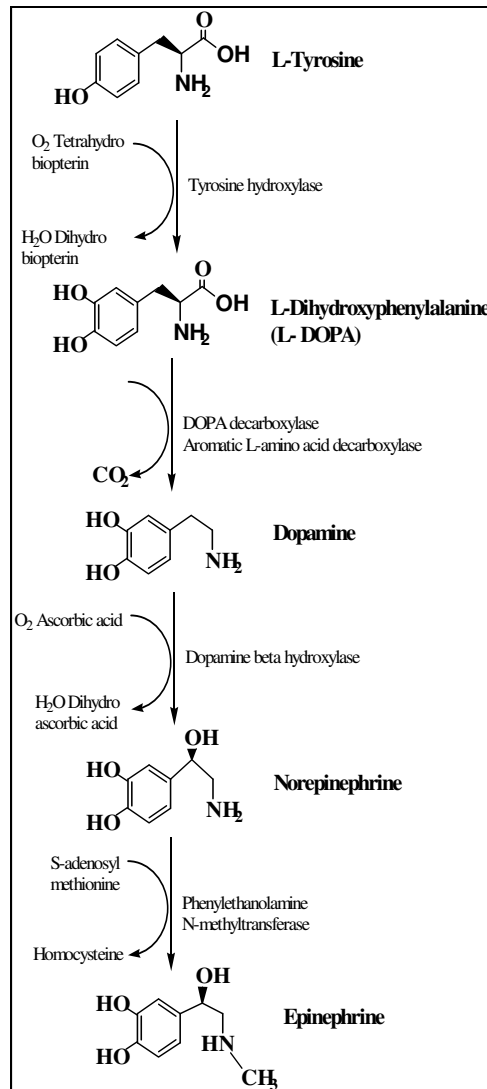


Fig. 2. A synthetic pathway for neurotransmitter biogenic amines

#### 4. Microorganisms producing biogenic amine in soybean food

Microorganisms have a different ability in synthesizing decarboxylases. Most soybean fermented and non-fermented foods are subjected to conditions that enable BAs synthesis. The amount of different amines formed is highly dependent on the nature of the food and the microorganisms present in the food (Brink et al., 1990). BAs are present in a wide range of fermented food products such as fish (Shalaby, 1996), meat (Maijala et al., 1993), dairy (Stratton et al., 1991), soybean products (Chin & Koehler, 1986), wine (Lehtonen et al., 1992) and beer (Dumont et al., 1992), as well as vegetables (Taylor et al., 1978). Soybean paste or Doenjang is a traditional Korean food produced through the fermentation of soybeans by naturally occurring bacteria and fungi, and has been consumed for centuries as a protein rich source and seasoning ingredient in Korea. This paste contains a relatively high concentration of amino acids degraded from soybeans and may be a source for BAs formation. Decarboxylase activity has been described in several microbial groups, including *Bacillus*, *Citrobacter*, *Clostridium*, *Klebsiella*, *Escherichia*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella*, *Photobacterium*, *Lactobacillus*, *Pediococcus* and *Streptococcus* (Rice et al., 1976; Brink et al., 1990; Huis in't Veld et al., 1990). In Miso (Japanese fermented soybean paste), tyrosine decarboxylase bacteria have been identified as *Enterococcus faecium*, *Lactobacillus bulgaricus* and histamine decarboxylase has been associated with *Lactobacillus* species and *Lactobacillus sanfrancisco* (Ibe et al., 1992). Amine-producing lactic bacteria such as *Lactobacillus brevis*, *Lactobacillus buchneri*, *Lactobacillus bulgaricus*, *Lactobacillus curvatus*, *Lactobacillus carnis*, *Lactobacillus dicergens* and *Lactobacillus hilgardii* have been isolated from meat products (Maijala et al., 1993). Moon et al. (2010) isolated two biogenic amine producing bacteria from traditional soybean pastes: one was a histamine producing *Clostridium* strain, and the other was a tyramine producing *Pseudomonas* strain. Moon et al. (2010) reported that *Clostridium* strain, isolated from traditional soybean pastes, was potent histamine producer among the tested cultures. *Clostridium perfringens* grows in protein rich media and can not survive in media that lacks essential amino acid supply (Shimizu et al., 2002). Accordingly, this bacterium is often detected in amino acid rich environment, including protein-fermented foods like Sufu, a traditional Chinese fermented soybean curd (Han et al., 2001). Tsai et al. (2007) identified some histamine producing bacteria belonging to *Lactobacillus* species in Natto products (traditional Japanese fermented soybean food) manufactured in Taiwan. In the case of fermented food and beverages, the introduction of starter cultures can affect the production of biogenic amines either directly or indirectly through interaction between different microbial populations, which are probably very important (Huis int Veld et al., 1990).

#### 5. Occurrence of biogenic amines in soybean food

Virtually, all foods that contain proteins or free amino acids are subjected to conditions, enabling microbial or biochemical activity; biogenic amines can be expected. The total amount of the different amines formed strongly depends on the nature of the food and the microorganisms present (Brink et al., 1990). Biogenic amines are present in a wide range of food products including fermented and non-fermented soybean products (Brink et al., 1990; Halasz et al., 1994; Santos, 1996; Shalaby, 1996; Soufleros et al., 1998). Since several

varieties of molds, yeasts and lactic acid bacteria are involved in the fermentation processes of soybean products where the raw material (soybean) contains considerable amounts of protein, the formation of various amines might be expected during the fermentation (Shalaby, 1996). Several studies have shown that biogenic amines in fermented soybean products are most likely formed by the lactic microflora that remains active during fermentation (Kirschbaum et al., 2000; Stratton et al., 1991). Tyramine and histamine have been found at various levels in fermented products (Stratton et al., 1991). The variability of biogenic amines levels in the commercial fermented soybean products samples had been attributed to the variations in manufacturing processes; variability in the ratio of soybean in the raw material, microbial composition, conditions and duration of fermentation (Shalaby, 1996). The data reported by several authors (Maijala et al., 1995a; Eerola et al., 1998) confirmed the key role played by the raw material quality. However, other variables such as pH, moisture content and NaCl can have an important effect on the production of BAs in soybean food and other food products. In non-fermented foods, the presence of BAs above a certain level is considered as indicative of undesired microbial activity, therefore, the amine level could be used as an indicator of microbial spoilage. However, the presence of biogenic amines in food does not necessarily correlate with the growth of spoilage organisms, because they are not all decarboxylase-positive (Santos, 1996). Shalaby (1996) reported that fermented soybean products (Miso) contained high levels of histamine (462 mg/100g), putrescine (1,234 mg/100g), cadaverine (634 mg/100 g) and tyramine (3,568 mg/100g). Cho et al. (2006) reported the presence of histamine and tyramine in traditional Korean paste Doenjang at a level of 952.0 mg/kg and 1,430.7 mg/kg. Tyramine was the most abundant BA in different types of soy sauces produced in China (Yongmei et al., 2009). Tsai et al. (2007) tested biogenic amine levels in seven soybean and eleven black bean douchi (traditional chinese fermented soybean product), among which four soybean douchi products had histamine levels greater than 5 mg/100 g while, among the black bean douchi samples, four samples contained histamine at 56.3, 62.1, 80.2 and 80.8 mg/100 g, levels greater than 50 mg/100 g, a hazard action level (Taylor, 1989). However, histamine is not the only compound responsible for scombrototoxicosis (acute onset of gastrointestinal symptoms such as headache, flushing and hypertension after ingesting spoiled fish), since ingestion of pure histamine does not automatically cause toxic symptoms (Bjeldanes et al., 1978). The differences in the contents of biogenic amines between black bean and soybean douchi products could be attributed to the variation of the substrate materials, the microbial composition, conditions and duration of fermentation (Yen, 1986). The toxic effects of histamine are increased in the presence of some other amines, such as putrescine and cadaverine, which inhibit histamine metabolizing enzymes in the small intestine (Arnold & Brown, 1978; Bjeldanes et al., 1978; Lehane & Olley, 2000).

Yen (1986) reported that the average amine contents in 15 samples of commercial Sufu from Taiwan and China were: cadaverine (0.039 mg/g), histamine (0.088 mg/g),  $\beta$  - phenylethylamine (0.063 mg/g), putrescine (0.473 mg/g), tryptamine (0.150 mg/g) and tyramine (0.485 mg/g). Tyramine and putrescine were the major amines found, and these might have a potential harmful effect on human beings if levels are very high. Biogenic amines in different varieties of soybean foods have been analyzed by several other authors as summarized in Table 2.



Food	Tyramine	Tryptamine	Histamine	Putrescine	Cadaverine	Phenylethylamine	Spermine	Spermidine	Reference
Fermented Soy	-	-	4,620	12,340	6,340	-	-	-	Shalaby, 1996
Tempe	4.3	15.6	4.1	116.9	-	-	-	11.6	Saaid et al., 2009
Soy bean sauce	1.0	ND	9.6	1.0	-	-	-	ND	
Salty soy sauce	ND	ND	2.0	ND	-	-	-	ND	
Taucu (salty bean)	ND	ND	0.8	59.0	-	-	-	ND	
Soya bean milk	1.7	20.2	17.5	ND	-	-	-	1.3	
Soy sauce (n mol/ g)	-	-	-	696	-	-	10	82	Nishibori et al., 2007
Miso (Japanese soybean paste) mol/g	-	-	-	296	-	-	5	12	
Korean Doenjang (traditional type)- mg/kg	669.5	105.5	596.4	462.6	23.5	244.7	3.8	15.6	Cho et al., 2006
Korean Doenjang (modern type)- mg/kg	133.0	22.4	83.6	46.4	3.2	6.5	2.4	7.4	
Miso (mg/kg)	48.6	22.6	0.9	19.8	3.0	4.4	2.2	15.7	
Chunngkukjang (mg/kg)	133.8	69.9	10.1	26.4	9.7	22.0	10.7	52.0	
Chunngkukjang powder (mg/kg)	68.1	35.0	1.0	10.2	12.1	17.0	15.5	54.6	
Chunjang (mg/kg)	44.3	16.6	16.8	10.7	3.3	7.0	1.1	6.1	
Soy sauce (traditional type) mg/kg	241.6	12.1	225.9	376.9	16.1	13.5	6.6	24.5	
Soy sauce (modern type) mg/kg	594.5	36.6	129.8	56.8	6.1	40.8	1.0	6.3	
Kochujang (mg/kg)	3.5	27.2	1.0	2.9	0.5	4.9	1.6	2.5	
Sufu (white) mg/100g	0.80	1.62	0.46	1.51	0.70	ND	1.64	ND	

Sufu (brown)mg/100 g	1.08	ND	2.72	0.24	5.00	ND	ND	ND	
Chinese soy sauce (mg/l)	0-673		0-592		0-550		0-145	0-486	Yongmei et al., 2009
Natto (Japanese) mg/100g	0.12	0.91	3.54	1.71	2.19	ND	0.86	4.50	Tsai et al., 2007
Natto (Taiwan) mg/100g	ND	ND	4.51	0.16	0.05	ND	ND	2.50	

ND: Not detected

Table 2. Biogenic amines in different soybean food products

## 6. Physiological role of biogenic amines

BAs play a number of crucial roles in the physiology and development of eukaryotic cells (Tabor & Tabor, 1985; Igarashi, 2001). A detail description of their physiological role has been summarized in Table 3. The most active BAs are histamine, putrescine and tyramine. Polyamines such as putrescine, spermine and spermidine also play essential roles in cell growth and differentiation via the regulation of gene expression and the modulation of signal transduction pathways. Histamine is present in many living tissues as a normal constituent of the body and has multiple effects in different mammalian and invertebrate organs (Maintz & Novak, 2007). In humans, it is found in different concentrations in the brain, lungs, stomach, small and large intestines, uterus and the ureter. It is produced and stored predominantly in mast cells, basophiles and neurons. Histamine modulates a variety of functions by interacting with specific receptors on target cells, namely H1, H2 and H3 receptors of the G-protein coupled receptor family. H1 receptors are found in the brain where they are involved in the control of the circadian rhythm, attention and cognition and in peripheral tissues where they mediate vascular and bronchial muscle responses to histamine in allergic processes (Jorgensen et al., 2007). H2 receptors, although widely distributed in body tissues, seem to have a central role only in the regulation of acid secretion. They respond to the presence of histamine, provoking gastric acid secretion and the contraction of intestinal smooth muscle (Rangachari, 1992). H3 receptors, originally described as presynaptic autoreceptors on brain histaminergic neurons that control histamine synthesis and release, were subsequently recharacterised as heteroreceptors on non-histaminergic neurons in the central and peripheral nervous systems. They have also been found in immune cells and in smooth muscle (Coruzzi et al., 2001; Passani et al., 2007) where they have been associated with immediate and allergic hypersensitivity. When histamine binds with these receptors, they affect the contraction of smooth muscle cells, the dilation of blood vessels and, therefore, an efflux of blood serum is established into the surrounding tissues (including the mucous membranes) and initiating the inflammatory process (Rangachari, 1992). Tyramine and  $\beta$ -phenylethylamine are included in the group of trace amines, a family of endogenous compounds with strong structural similarities to classical monoamine neurotransmitters, although the endogenous levels of these

compounds are at least two orders of magnitude below that of these neurotransmitters. The effects of these low physiological concentrations have been difficult to demonstrate, but it has been suggested that they serve to maintain the neuronal activity of monoamine neurotransmitters within defined physiological limits (Berry, 2007). Tyramine can be converted into octopamine when taken up in sympathetic nerve terminals, where it displaces norepinephrine (NE) from storage vesicles. A portion of this NE diffuses out of the nerve to react with receptors, causing hypertension and other sympathomimetic effects (Berry, 2007). The biological functions of amines are mainly the regulation of gene expression by altering DNA structure and by modulating signal transduction pathways. The optimal functioning of the cell therefore requires the intracellular polyamine content be strictly controlled at the levels of biosynthesis, catabolism, uptake and efflux (Linsalata & Russo, 2008). Small amounts of orally administered polyamines induce cell growth; larger quantities have no effect or may actually inhibit growth (Deloyer et al., 2001). Amines lies in their physiological functions related to cell membrane stabilization and cell proliferation, since they are involved in DNA, RNA and protein synthesis. Therefore, they are considered important food microcomponents during periods of intensive tissue growth (infant gut maturation, post-operational recovery, etc.), although in some pathological cases (individuals with tumours) the intake of amines should be minimized (Bardocz, 1995).

## 7. Toxicological effects of biogenic amines

BAs, such as tyramine and  $\beta$ - phenylethylamine, have been proposed as the starters of hypertensive crisis in certain patients and dietary-induced migraine. Another amine, histamine, has been implicated as the causitive agent in several outbreaks of food poisoning. Histamine intake ranged within 8 - 40 mg, 40 - 100 mg and higher than 100 mg may cause slight, intermediate and intensive poisoning, respectively (Parente et al., 2001). Nout (1994) pointed out that the maximum daily intake of histamine and tyramine should be in the range of 50 - 100 mg/kg and 100 - 800 mg/kg, respectively; over 1,080 mg/kg tyramine becomes toxic. Putrescine, spermine, spermidine and cadaverine have no adverse health effect, but they may react with nitrite to form carcinogenic nitrosoamines and also can be proposed as indicators of spoilage (Hernandez-Jover et al., 1997). Tryptamine can induce blood pressure increase, therefore causes hypertension, however there is no regulation on the maximum amount of tryptamine consumption in sausage in some countries (Shalaby, 1996). Food poisoning may occur especially in conjunction with potentiating factors such as monoamine oxidase inhibiting (MAOI) drugs, alcohol, gastrointestinal diseases and other food containing amines. Histaminic intoxication and hypertensive crisis due to interaction between food and MAOI anti-depressants as well as food-induced migraines are the most common reactions associated with the consumption of foods containing large amounts of biogenic amines (Marine-Font et al., 1995). The diamines (putrescine and cadaverine) and the polyamines (spermine and spermidine) favor the intestinal absorption and decrease the catabolism of the above amines, thus, potentiating their toxicity (Bardocz, 1995). Formation of nitrosoamines, which are potential carcinogens, constitutes an additional toxicological risk associated to biogenic amines, especially in meat products that contain nitrite and nitrate salts as curing agents (Scanlan, 1983). Determination of the exact toxicity threshold of biogenic amines in individuals is extremely difficult, since the toxic dose is strongly dependent on the efficiency of the detoxification mechanisms of each individual (Halasz et al., 1994). Normally, during the food intake process in the human gut, low amounts of

biogenic amines are metabolized to physiologically less active degradation products. This detoxification system includes specific enzymes such as diamine oxidase (DAO). However, upon intake of high loads of biogenic amines in foods, the detoxification system is unable to eliminate these biogenic amines sufficiently. Moreover, in case of insufficient DAO activity, caused for example by generic predisposition, gastrointestinal disease or inhibition of DAO activity due to secondary effects of medicines or alcohol, even low amounts of biogenic amines can not be metabolized efficiently (Bodmer et al., 1999).

Some biogenic amines, e.g., histamine and tyramine, are considered as anti-nutritional compounds. For sensitive individuals they represent a health risk, especially when their effects are potentiated by other substances. The intake of foods with high BA loads or the inadequate detoxification of BAs can lead to their entering the systemic circulation, inducing the release of adrenaline and noradrenaline and provoking gastric acid secretion, an increased cardiac output, migraine, tachycardia, increased blood sugar levels and higher blood pressure (Salabhy, 1996). The most serious and studied toxic effects of BA-rich foods have been investigated in patients treated with MAOIs (Stratton et al., 1991; Gardner et al., 1996; Rapaport, 2007). Indeed, the toxic effects of some BAs were first discovered in patients treated with MAOIs who suffered headaches after eating cheese (Blackwell, 1963; Hanington, 1967). Depending on the severity of the symptoms, the effects of BAs are described as a reaction, intolerance, or intoxication or poisoning. Reaction symptoms include nausea, sweating, rashes, slight variations in blood pressure and mild headache. If the amount ingested is too great for efficient detoxification to be performed, or the detoxification system is strongly inhibited, the symptoms become more severe (those of intolerance) with vomiting, diarrhoea, facial flushing, a bright red rash, bronchospasms, tachycardia, oral burning, hypo- or hypertension and migraine. In exceptional cases BA poisoning may occur, involving a hypertensive crisis (blood pressure >180/120 mmHg) that can lead to end-organ damage in the heart or the central nervous system (Blackwell, 1963).

Biogenic amine	Physiological effects	Toxicological effects
Histamine	Neurotransmitter, local hormone, gastric acid secretion, cell growth and differentiation, regulation of circadian rhythm, body temperature, food intake, learning and memory, immune response, allergic reactions	Headaches, sweating, burning nasal secretion, facial flushing, bright red rashes, dizziness, itching rashes, oedema (eyelids), urticaria, difficulty in swallowing, diarrhea, respiratory distress, bronchospasm, increased cardiac output, tachycardia, extrasystoles, blood pressure disorders
Tyramine	Neurotransmitter, peripheral vasoconstriction, increase respiration, elevate blood glucose, release of norepinephrine	Headaches, migraine, neurological disorders, nausea, vomiting, respiratory disorders, hypertension
Putrescine	Regulation of gene expression, maturation of intestine, cell growth and differentiation	Increased cardiac output, tachycardia, hypotension, carcinogenic effects

Table 3. Physiological and toxicological effects of biogenic amines

## 8. Recommended limits of biogenic amines in food

It is very difficult to establish a uniform maximum limit for ingested BAs since their toxic effects depend on the type of amine, the presence of modulating compounds and the efficiency of an individual's detoxification mechanism. Several studies have suggested that the absorption, metabolism and/or potency of one BA might be modified by the presence of another, which might explain why aged cheese is more toxic than its equivalent amount of histamine in aqueous solution (Taylor, 1986). Laboratory studies on the effects of BAs face a number of methodological problems. Most studies have focused on the effect of individual BAs administered intravenously to laboratory animals or healthy volunteers, but these results are difficult to transfer to food intake since the intravenous response is several times higher than that obtained with oral administrations (Simpson & White, 1984). The effects of trace amines are mainly based on clinical observations; no meta-analyses that might confirm their effects are therefore possible (Jansen et al., 2003). Ingestion limits based on case reports may be too high since, usually, only cases of BA poisoning are reported (Taylor, 1985; Rauscher-Gaberng et al., 2009). Although more in-depth studies on the toxic effects of BAs are necessary, some studies have reported minimum toxic levels for some BAs. Wohrl et al. (2004) reported that 75 mg of pure liquid oral histamine, a dose common in normal meals, can provoke immediate as well as delayed symptoms in 50% of healthy females with no history of food intolerance. A concentration of over 125 mg/kg of tyramine in food is considered to be toxic in normal individuals, almost 100 times the concentration considered potentially toxic when ingested in combination with MAOIs (McCabe-Sellers, 1986). Threshold values of 100 mg/kg for tyramine and 30 mg/kg for phenylethylamine have been suggested (Brink et al., 1990). However, since there is always more than one type of BA in food, a maximum total BA level of 750 - 900 mg/kg in food products has been proposed (Brink et al., 1990). Currently, the only BA for which maximum limits have been set in the European Union and the United States of America is histamine. The US Food and Drug Administration (FDA) consider a histamine level of  $\geq 500$  mg/kg in food to be a danger to health. This agrees with values cited in histamine intoxication reports in which over 500-1000 mg/kg of food had been ingested (Rauscher-Gaberng et al., 2009). Askar and Treptow (1996) have suggested histamine at a concentration of 500 mg/kg in food to be hazardous for human health. On the other hand, an upper limit of histamine for human consumption has to be 100 mg/kg, 100 - 800 mg/kg of tyramine and 30 mg/kg of phenylethylamine in food products have been reported to be toxic doses in foods (Brink et al., 1990). Total BA levels of 1,000 mg/kg in food are also considered hazardous for human health (Taylor, 1985). An intake of over 40 mg biogenic amines per meal has been considered potentially toxic (Nout, 1994).

## 9. Factors influencing biogenic amine production in soybean food

Since amines are formed by the enzymatic breakdown of food or by decarboxylase active bacteria, inhibition of such activity and prevention of bacterial growth would be very important for controlling the hazardous amine content of foods. Raw material and various manufacturing conditions influence the production of biogenic amines. Thus, tyramine, putrescine and cadaverine concentration in Tempe were low or high depending on the applied manufacturing process: soaked soybeans, kinds of fermentative microorganisms used and storage temperature (Nout et al., 1993). Biosynthesis of amino acids in fermented soybean paste is an enzymatic process which is catalyzed by synthetases (e.g. glutamine synthetase). Other amino acid metabolizing enzymes have been detected with higher levels,

e.g., aspartate amino transferase and especially histidine decarboxylase during the fermentation process in various food products (Picton et al., 1993). Thus, biogenic amine production in various fermented and non-fermented soybean foods has been related to factors such as variety of raw material, pH, salt concentration, and temperature.

### 9.1 Effect of pH

The pH is an important factor for fermentation and formation of biogenic amines because amino acid decarboxylase activity remains stronger in an acidic environment (Santos, 1996). Santos (1996) reported that the pH was an important factor influencing decarboxylase activity, and low pH about 3.0 - 6.0 was optimal for bacteria to produce decarboxylase. Teodorovi et al. (1994) also reported that amino acid decarboxylase activity was stronger in an acidic environment, being the optimum pH between 4.0 and 5.5. Furthermore, in such acidic environment, bacteria are more strongly encouraged to produce decarboxylase enzymes, as a part of their defence mechanisms against the acidity (Santos, 1996). In addition to this, Kim et al. (2003) reported that low pH of Doenjang samples, about 3.0 - 6.0, was effective for increasing the decarboxylase activity. Koessler et al. (1928) suggested that amine formation by bacteria was a physiological mechanism to counteract an acid environment. Bacterial amino acid decarboxylases usually have acid pH optimum (Gale, 1946). However, amine formation depends on the amount of growth of decarboxylating bacteria (Yoshinaga & Frank, 1982). High production of histamine can be related to inadequate pH decrease in the first day of ripening process (Buncic et al., 1993; Maijala et al., 1993). Also tyramine production by *Carnobacterium divergens* was lower at pH 4.9 than 5.3, associated with a reduced cell yield. This can explain the low tyramine amount found in nordic meat generally characterized by lower pH, which limits bacterial growth, and, consequently, tyrosine decarboxylase activity (Masson et al., 1999).

### 9.2 Effect of sodium chloride

The variation in the quantity of water and in the salt/water ratio during fermentation and storage of fermented soyproducts has an important role on microbial multiplication. The rate of amines production of a bacterial strain *L. bulgaricus* (now *L. delbrueckii* subsp. *bulgaricus*) was considerably reduced when salt concentration in the medium increased from 0 to 6% (Chander et al., 1989). Chin and Koehler (1986) demonstrated that NaCl concentration ranging from 3.5 to 5.5% could inhibit histamine production. This influence can be attributed to reduced cell yield obtained in the presence of high NaCl concentration and to a progressive disturb of the membrane located microbial decarboxylase enzymes (Sumner et al., 1990). A similar NaCl effect characterized cell yield and BA production in *Enterococcus faecalis* EF37 (Gardini et al., 2001). According to Santos (1996), the presence of sodium chloride activates the tyrosine decarboxylase activity and inhibits histidine decarboxylase activity. At 3.5% content of sodium chloride, the ability of *L. buchneri* to form histamine is partly inhibited, whereas its formation was stopped at the concentration of 5.0% NaCl (Maijala et al., 1995b). Hernandez-Herrero et al. (1999) reported that NaCl contents in the range of 0.5 - 10% had a stimulatory effect on histamine formation for *Staphylococcus capitis* and *Staphylococcus epidermidis*, whereas NaCl level in excess of 20% inhibited their growth and histamine formation.

### 9.3 Effect of temperature

It is well known that temperature has a marked effect on the formation of BAs in food products. Several authors reported that biogenic amine content depends on temperature

and time (Diaz-Cinco et al., 1992; Halasz et al., 1994). *Carnobacterium divergens* produced more tyramine at 25°C than at 15°C (Masson et al., 1999). Also the temperature has effects on the activity of proteolytic and decarboxylating enzymes and the relationship between the microbial population (Joosten & van Boeckel, 1988 and Maijala et al., 1995b). In addition, the processing temperature also has influence on the formation of biogenic amines in dry sausages as well as on the total amount of amines (Maijala et al., 1995b). Higher temperature can favor proteolytic and decarboxylating reactions, resulting in increased amine concentration after storage. At 15°C, microbial decarboxylases might remain active, even if during storage, most microbial populations have reached the stationary growth or death phase (Bover-Cid et al., 2000). In contrast, during a prolonged meat storage at 4°C before casing, putrescine can be produced due to the action of psychrotrophic pseudomonads (Paulsen & Bauer, 1997). However, lower BA amounts were detected in food products stored at 4°C with respect to those stored at 15°C (Bover-Cid et al., 2000). A better understanding of the mechanisms by which biogenic amines are produced is necessary to prevent their formation. Generally, biogenic amines in foods can be controlled by strict use of good hygiene in both raw material and manufacturing environments with corresponding inhibition of spoiling microorganisms. In case of fermented foods, the use of short fermentation with carefully selected active starter cultures instead of wild fermentations will help to prevent the formation of toxic amines.

## 10. Analytical methods for the detection of biogenic amines in food

There are two reasons for the determination of amines in foods: the first is their potential toxicity; the second is the possibility of using them as food quality markers. Various methods have been developed for the analysis of BAs in foods such as thin-layer chromatography (TLC), gas chromatography (GC), capillary electrophoretic method (CE) and high performance liquid chromatography (HPLC). Lapa-Guimaraes & Pickova (2004) introduced one dimensional, double development thin-layer chromatographic technique, using the solvent system Chloroform:diethylether:triethylamine (6:4:1) followed by chloroform:triethylamine (6:1) for separation and determination of the dansyl derivatives of BAs. One-dimensional TLC technique was used for the separation of eight biogenic amines. The quantitative determination of biogenic amines has been performed by densitometry at 254 nm (Shalaby, 1996).

Few reports have been published on simultaneous detection of multiple amines. Gradient HPLC with pre- or post-column derivatization is a reproducible and accurate method for the determination of histamine, putrescine, cadaverine and tyramine in fish (Luten et al., 1992). Continuous flow analysis and isocratic HPLC with precolumn derivatization is suitable for the analysis of histamine alone. Good repeatability and reproducibility have been reported with extraction into trichloroacetic acid clean-up by cation exchange and HPLC separation using UV and fluorescence separation for determining putrescine, cadaverine, histamine and tyramine in fish and fish products Feier & Goetsch (1993). A convenient method was described for the analysis of biogenic amines by means of reversed-phase HPLC (Lehtonen et al., 1992).

Various chemical derivatization reagents have also been used for the BAs analysis, for example ninhydrine and o-phthalaldehyde as a postcolumn derivatization reagent, dansyl and benzoyl chloride, fluoresceine and 9-fluorenylmethyl chloroformate with precolumn derivatization (Wei, 1990; Seiler, 1986; Beljaars, 1998). Simplest method for determination of biogenic amines in foods is by chromatography in an amino acid analyser, including the ion-

exchange chromatographic method (Simon-Sarkadi & Holzapfel, 1994). Zhang and Sun (2004) described sensitive capillary zone electrophoresis (CZE) with lamp-induced fluorescence detection method for the simultaneous analysis of histamine and histidine. Kim et al. (2005) developed a method for the determination of biogenic amine in low salt fermented soybean paste by using benzoylchloride as a derivatization agent and amounts of amine were quantified by HPLC analysis. Previously other researchers also reported a similar method for the determination of biogenic amines in Miso and Natto products (Kung et al., 2007; Tsai et al., 2007). Saaid et al. (2009) determined biogenic amines in some Malaysian soybean products such as soybean sauce, tempe, salty soy sauce, taucu and soybean milk. These samples were extracted with 0.1 M HCl and then derivatized with dansyl chloride and finally analyzed by using HPLC. The BAs are determined in derivatized forms as trifluoroacetyl, trimethylsilyl or 2, 4-dinitrophenyl derivatives (Ascar & Treptow, 1986).

Fluorometric methods are used owing to fluorescence of BAs at some pH and reaction of BAs with suitable agents to the fluorescence derivatives. Using these methods, histamine can be determined by *o*-phthalaldehyde and tyramine by  $\beta$ -naphthol (Ascar & Treptow, 1986). At suitable conditions amino acid analyzer can be used not only for the determination of BAs as well their representative precursor amino acids (Halasz et al., 1999). Recently due to the commercial availability of enzymes like MAO and putrescine oxidase, several research groups tried to couple the enzymatic reactions with electrochemical sensors in order to obtain simple and reproducible biosensors. In some cases, the BAs have been coupled with oxygen sensors or hydrogen peroxide sensors. The biosensor procedure has advantages, such as low cost, short analysis time and simplicity of use and it can be used outside an organized laboratory. The biosensors show a low detection limit with life-time estimated at one month with a 10 - 30% loss of sensitivity (Casella et al., 2001).

Enzymatic methods including radioimmuno assay and enzyme linked immunosorbent assay system (ELISA) have been applied for the detection of histamine (Guesdon et al., 1986), with the advantages of rapidity and not requiring expensive instrumentation like HPLC (Stratton et al., 1991). Lange and Wittman (2002) developed an enzyme sensor array methods for the simultaneous detection of biogenic amines (histamine, tyramine and putrescine) in different food samples within the duration of 20 min. Aygun et al. (1999) compared ELISA and HPLC method for the detection of histamine in cheese and found that the ELISA was suitable for the determination of histamine in cheese. Many other authors also reported various analytical detection methods for the determination of biogenic amines in different food samples as summarized in Table 4.

Amines	Food samples	Sample pretreatment	Derivatization	Detection / wavelength	References
Histamine	Natto	Extraction with 6% trichloroacetic acid	Derivatization with dansyl chloride	HPLC, UV-Vis detector/ 254 nm	Tsai et al., 2007
Various amines	Chinese soy sauce	Extraction with 0.4 M-perchloric acid	Derivatization with dansyl chloride	HPLC, Diode-array detector/ 254 nm	Yongmei et al., 2009
Histamine	Sufu	Extraction with 6% trichloroacetic acid	Derivatization with benzoyl chloride	HPLC, UV-Vis detector 254 nm	Kung et al., 2007



Putrescine, Cadaverine, Histamine, Spermidine and Spermine	Fish	Extraction with 5% trichloroacetic acid	Precolumn derivatization with dansylchloride	HPLC, UV detector/ 254 nm	Rosier & Peteghem, 1988
Various amines	Fermented sausages	Extraction with 0.6 M-perchloric acid	Postcolumn derivatization with o-phthaldialdehyde and 3-mercaptopropionic acid	HPLC, Fluorimetric (excitation 390 nm and emission 475 nm)	Straub et al., 1993
Various amines	Doenjang, Miso, Chungkukjang, Soy sauce, Kochujang	Extraction with 0.1 N HCl, 0.4 M perchloric acid and 5% trichloroacetic acid	Derivatization with dansyl chloride	HPLC, UV detector 254 nm	Cho et al., 2006
Various amines	Korean traditional fermented soybean paste	Extraction with 0.4 M-perchloric acid	Derivatization with dansyl chloride	HPLC UV-Vis detector/ 254 nm	Shukla et al., 2010
Various amines	Low salt fermented soybean paste	Extraction with trichloroacetic acid	Derivatization with benzoyl chloride	HPLC, Photodiode array detector/ 225 nm	Kim et al., 2005
Various amines	Leafy vegetables	Extracted with 10% trichloroacetic acid	-	Amino acid analyzer, Colorimetric detection/ 570 nm	Simon-Sarkadi & Holzapfel, 1994
Various amines	Meat products	Extraction with 0.6 M perchloric acid	Derivatization with dansylchloride	UPLC, UV detector/ 225 nm,	Dadakova et al., 2009
Various amines	Alcoholic beverages	-	-	Integrated pulsed amperometric detection (25 UV-Vis detector at 276 nm by Ion exchange chromatography)	De Borba & Rohrer, 2007
Various amines	Variety of Food samples	-	-	Enzyme sensor array detection method	Lange & Wittmann, 2002
Various amines	Milk and cheese	Extraction with 0.6 M	Derivatization with o-phthaldialdehyde	HPLC, Fluorimetric (excitation 340 nm	Masson et al., 1996

		trichloroacetic acid		and emission 445 nm)	
Various amines	Alcoholic beverages in Nigeria	-	Derivatization with dansylchloride	HPLC, UV detector 254 nm	Lasekan & Lasekan, 2000
Various amines	Fish products	Extraction with 0.1 M hydrochloric acid	Derivatization with dansylchloride	HPLC, Photodiode array detector/ 254 nm	Park et al., 2010
Various amines	Turkish red wines	-	Derivatization with o-phthalaldehyde	HPLC, diode array detector/ 200-550 nm	Anli et al., 2004
Various amines	Sucuk (Turkish dry fermented sausage)	Extraction with 0.4 M perchloric acid	Derivatization with dansylchloride	HPLC, diode array detector	Gencelep et al., 2008
Various amines	Dressed fried fish meat product	Extraction with 20% trichloroacetic acid	-	Enzyme based colorimetric method, UV-VIS Spectrophotometer at 505 nm.	Yeh et al., 2006
Histamine	Dressed fried fish meat product	Extraction with deionized water	-	Competitive direct enzyme- linked immunosorbent assay (as described by Neogen Corp.), detected at 650 nm.	Yeh et al., 2006
Various amines	Jeotkals, Korean salted and fermented fish products	Extraction with 0.4 M perchloric acid	Derivatization with dansylchloride	HPLC, Photodiode array detector/ 254 nm	Mah et al., 2002

Table 4. Various methods for the detection of biogenic amines in different food samples

## 11. Conclusions

The biogenic amines represent a group of low molecular mass organic bases occurring in all organisms. Enzymatic decarboxylation of free amino acids and other metabolic processes can lead to the presence of BAs in soybean products. These BAs can also be produced by bacterial decarboxylation of amino acids. Therefore, any fermented soybean foodstuffs produced by fermentation or exposed to microbial contamination during processing or storage may contain BAs. Therefore the concentration of BAs like histamine, tyramine, cadaverine, putrescine and spermidine gives therefore a good indication of the freshness of foods. The determination of biogenic amines in non-fermented or fresh and processed foods is of great interest not only due to their toxicity but also because they can be a useful index of spoilage or ripening. For these reasons, it is important to monitor the levels of BAs in foodstuffs. On the other hand, the same raw material can lead to very different amine levels in final products depending on the presence of decarboxylating microorganisms, either derived from environmental contamination or from starter cultures, and the conditions

supporting the growth activity of amine - producing bacteria. However the quality of raw materials seems to be only one of the many factors affecting amine formation in fermented soybean products. In this perspective, the control of hygiene and storage conditions is essential for the reduction of biogenic amine accumulation.

Analytical determination of biogenic amines (BAs) is not simple because of the complexity of the real matrix to be analyzed. The extraction of amines from real matrices is the most critical in terms of obtaining adequate recoveries for all amines. The most of the analyses include derivatization step. Therefore, estimation of BAs is important not only from the point of view of their toxicity, but also because they can be used as indicators of the degree of freshness or spoilage of food.

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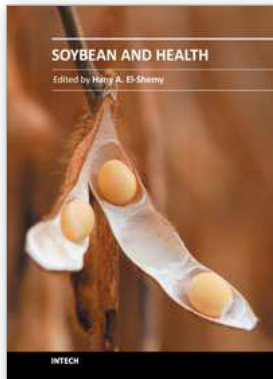
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## **Soybean and Health**

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Worldwide, soybean seed proteins represent a major source of amino acids for human and animal nutrition. Soybean seeds are an important and economical source of protein in the diet of many developed and developing countries. Soy is a complete protein, and soy-foods are rich in vitamins and minerals. Soybean protein provides all the essential amino acids in the amounts needed for human health. Recent research suggests that soy may also lower risk of prostate, colon and breast cancers as well as osteoporosis and other bone health problems, and alleviate hot flashes associated with menopause. This volume is expected to be useful for student, researchers and public who are interested in soybean.

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