



Biologically active amines in fermented and non-fermented commercial soybean products from the Spanish market



N. Toro-Funes, J. Bosch-Fuste, M.L. Latorre-Moratalla, M.T. Veciana-Nogués, M.C. Vidal-Carou*

Department of Nutrition and Food Science-XaRTA, INSA, Faculty of Pharmacy, Campus de l'Alimentació Torribera, University of Barcelona, Avda. Prat de la Riba 171, 08921 Santa Coloma de Gramenet, Barcelona, Spain

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ABSTRACT

Biologically active amines were determined in commercial soybean products. The antioxidant polyamines were found in both non-fermented and fermented soybean products. Natto and tempeh showed the highest content of polyamines (75–124 and 11–24 mg/kg of spermidine and spermine, respectively). On the other hand, the bacterial-related biogenic amines, tyramine, histamine, tryptamine and β -phenylethylamine, were detected in practically all fermented products with a high variability. The highest contents were found in sufu, tamari and soybean paste. Extremely high tyramine and histamine contents, 1700 and 700 mg/kg, respectively, found in some sufu samples could be unhealthy. However, biogenic amines observed in the other soybean products should not be a risk for healthy consumers. However, individuals who take monoamine and diamine oxidase inhibitors drugs should be strongly recommended to avoid this kind of products in order to suffer no adverse health effects. These biogenic amines were not detected in non-fermented soybean products.

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1. Introduction

Soybean has been used as one of the main protein sources in Eastern countries since ancient times to produce a variety of traditional non-fermented and fermented products. The consumption of soybean foods could have potential health benefits, particularly to prevent different types of cancers, osteoporosis and cardiovascular diseases (Omoni & Aluko, 2005). Due to these potential benefits, soybean products have been progressively introduced in the European market in the last 20 years, being first only distributed in specific dietetic shops, but now available in most food stores and supermarkets.

Biologically active amines are organic bases with an aliphatic, aromatic, or heterocyclic structure which take part of normal metabolism of animals, plants and microorganisms. They can be classified in two groups depending on their origin. The physiological polyamines spermidine (SPD) and spermine (SPM) are present in all animal and vegetal tissues and their origin is not related to microbial activity. On the contrary, the called biogenic amines, mainly including tyramine (TY), histamine (HI), tryptamine (TR), β -phenylethylamine (PHE), putrescine (PU) and cadaverine (CA), are present in food as a consequence of bacterial enzymatic

decarboxylation of their precursor amino acids. In spite of their recognised bacterial origin, PU and CA could be also considered as naturally occurring amines in some foods since they can be synthesized at low amounts in both vegetal and animal organisms.

Polyamines are naturally occurring amines involved in growth and differentiation of cells and in the stabilization of membranes and nucleic acids. They have a recognised role as antioxidant compounds against the cellular oxidative stress (Das & Misra, 2004; Kalač & Krausová, 2005).

Usually, biogenic amines are known to be found in many foods, such as fish and meat products, cheese, wine, beer (Spano et al., 2010; Vidal-Carou, Veciana-Nogués, Latorre-Moratalla, & Bover-Cid, 2007). Biogenic amines have been proposed as chemical markers of the hygienic conditions of raw material and/or manufacturing practices since their accumulation is associated with the activity of contaminant bacteria (Latorre-Moratalla et al., 2008; Linares, Martín, Ladero, Alvarez, & Fernández, 2011). However, only legal limits for HI have been established in fish and fish products by Food and Drug Administration (FDA) and by European Union (EU).

The presence of high amounts of biogenic amines in food constitutes a potential public health concern. In healthy individuals, biogenic amines ingested from foods are detoxified by intestinal and hepatic enzymatic systems. Thus, dietary TY is mainly metabolized by monoamine oxidase enzyme (MAO) and HI by diamino

* Corresponding author. Tel.: +34 934033785; fax: +34 934035931.

E-mail address: mcvidal@ub.edu (M.C. Vidal-Carou).

oxidase (DAO) and histamine-N-methyltransferase. The ingestion of high amounts of TY and HI might saturate these enzymatic systems and cause clinical symptoms of intoxication, including hypertensive crisis and allergic effects on blood vessels and smooth muscles (Jarisch, 2004; Maintz & Novak, 2007). Besides, impairment of DAO activity due to genetic predisposition, gastrointestinal diseases, or medication with DAO inhibitors (DAOIs) could lead to the disorder called histamine intolerance causing migraine, skin and gastrointestinal problems, even after the ingestion of small amounts of HI tolerated by healthy individuals (Maintz & Novak, 2007). PU, CA, SPM, and SPD do not seem to have adverse health effects by themselves, but, they may reduce the catabolism of TY and HI, blocking the enzymatic systems and, thus, enhancing their toxicity (Vidal-Carou et al., 2007).

The European Food Safety Authority (EFSA) published, in 2011, a scientific opinion on the risk of biogenic amine formation in fermented foods, which reports it is not possible to make a quantitative risk assessment for HI and TY due to insufficient available information. However, the EFSA proposed safety HI and TY levels per person and per meal that are not associated with adverse health effects, which are: (a) 50 mg HI for healthy individuals, but below detectable limits for those with histamine intolerance; (b) 600 mg TY for healthy individuals, 50 mg for those taking third generation monoamine oxidase inhibitors (MAOIs) drugs or 6 mg for those taking classical MAOIs drugs (EFSA, 2011). In this scientific opinion, soybean products were not included due to limited data and the small number of available samples (EFSA, 2011).

Several authors have reported the occurrence of biogenic amines in non-fermented and fermented soybean products with a wide variability, such as soymilk, tofu, soybean sprouts, sufu, tempeh, miso, natto and soy sauce (Table 1). However, the information about their content in soybean products of the European countries is scarce. The lack of tradition in the soybean products consumption and the lack of consumer's information about the use of this type of products in terms of storage conditions and size of servings, may increase the risk arising from the presence of biogenic amines in them. For this reason, the following study was

addressed to provide data of the presence of biogenic amines in soybean products from the Spanish market. Moreover, from this data, a first approach to risk assessment of HI and TY of these soybean products has been carried out. In addition, the content of the health-related polyamines was also studied to contribute to the knowledge of the potential health benefits of soybean.

2. Materials and methods

2.1. Samples

Different soybean products, classified as non-fermented products (fresh tofu, hard tofu, soymilk and soybean sprouts) and fermented products (miso, tamari, soy sauce, natto, tempeh, soybean paste and sufu), were purchased from Spanish local stores. Three batches from one brand for each type of product were studied and examined by duplicate for biogenic amine and polyamine contents.

Although several brands of fresh tofu, hard tofu, soymilk and soybean sprouts are nowadays available in the Spanish market, for this work the most popular and representative brand of each product are chosen. These products were produced in Spain and they are subjected to quality standards required by the European regulation.

On the contrary, miso, tamari, soy sauce, natto, tempeh, soybean paste and sufu are fermented products, most of them imported from Asian countries. So, the specific conditions used for their fermentation and manufacturing processes are unknown. With the exception of soy sauce, the other products are scarcely distributed in the Spanish market, mainly found in specific shops.

Samples were kept at $-30\text{ }^{\circ}\text{C}$ until their analyses.

2.2. Biogenic amine and polyamine determination

An aliquot of 5–10 g of sample were extracted two times with 10 mL of perchloric acid 0.6 M in a magnetic stirring plate for

Table 1
Biogenic amine and polyamine contents (mg/kg) in non-fermented and fermented soybean products.

Soybean product		TY	HI	TR	PHE	CA	PU	SPD	SPM	Reference
Tofu	<i>n</i> = 2	–	–	–	–	18.4	1.8	15.5	6.7	Nishimura et al. (2006)
	<i>n</i> = 19	nd	3.52–5.76	1.06–9.46	nd-5.36	nd-3.35	nd-4.73	14.97–35.30	4.05–19.15	Byun, Bai, and Mah (2013)
Soybean sprouts	<i>n</i> = 2	–	–	–	–	134.6	3.8	18.2	3.3	Glória, Tavares-Neto, Labanca, and Carvalho (2005)
Soymilk	<i>n</i> = 2	–	–	–	–	13.9	2.2	16.3	2.8	Nishimura et al. (2006)
	<i>n</i> = 1	1.7	17.5	20.2	–	–	nd	1.3	–	Saad, Saad, Hashim, Mohamed Ali, and Saleh (2009)
Natto	<i>n</i> = 2	–	–	–	–	203.3	11.4	87.1–154.0	17.8	Nishimura et al. (2006)
	<i>n</i> = 39	nd-45.0	nd-457.0	nd-301.0	nd	nd-42.0	nd-27.0	nd-124.0	nd-71.0	Tsai et al. (2007)
Tempeh	<i>n</i> = 21	nd-300.2	nd-34.4	nd-45.8	nd-51.5	nd-36.8	nd-43.1	246.5–478.1	18.8–80.1	Kim et al. (2012)
	<i>n</i> = 2	–	–	–	–	35.2	45.3	85.5	13.5	Nishimura et al. (2006)
Miso	<i>n</i> = 1	4.3	4.1	15.6	–	–	116.9	11.6	–	Saad et al. (2009)
	<i>n</i> = 11	nd-49.8	nd-26.4	nd-78.4	nd-55.8	nd-30.0	nd-143.1	–	–	Yen (1986)
Soy sauce	<i>n</i> = 40	nd-49	nd-221	nd-762	nd	nd-201	nd-12	nd	nd-216	Kung, Tsai, and Wei (2007)
	<i>n</i> = 30	nd-3568.0	nd-461.6	nd-351.5	nd-1020.6	nd-633.7	nd-1233.7	–	–	Yen (1986)
(mg/kg dw) [*]	<i>n</i> = 40	nd-673	nd-592	–	–	nd-550	–	nd-486	nd-109	Yongmei et al. (2009)
	<i>n</i> = 2	nd-1.0	2.0–9.6	nd	–	–	nd-1.0	nd	–	Saad et al. (2009)
Sufu	<i>n</i> = 42	3.0–659.9	nd-395.0	–	nd-121.9	nd-68.6	nd-58.1	–	–	Guidi and Gloria (2012)
	<i>n</i> = 23	nd-5250	0.2–126	–	0–190	0–170	0–775	–	–	Stute, Petridis, Steinhart, and Biernoth (2002)
Sufu	<i>n</i> = 15	nd-1125.4	nd-373.7	nd-401.3	18.8–131.3	5.4–99.4	20.3–1351.7	–	–	Yen (1986)
	<i>n</i> = 22	nd-40	nd-158	nd-81	nd	nd-371	nd-45	nd	nd-82	Kung, Lee, et al. (2007)
	<i>n</i> = 11	–	–	–	–	nd-883.7	1.1–1776.3	nd-154.8	nd-17.9	Liu et al. (2011)
	<i>n</i> = 10	nd-48.0	nd-40.2	26.0–57.0	nd-4.2	–	21.2–47.3	nd-27.1	nd-22.9	Tang et al. (2011)
	<i>n</i> = 38	nd-446.6	nd-196.9	nd-104.1	nd-33.6	0.6–85.8	0.5–316.9	nd-4.0	nd-6.9	Guan et al. (2013)

^{*} Biogenic amine and polyamine contents in mg/kg of dry weight of soybean product.

20 min. Thereafter, the two phases were separated by centrifugation at 5600 g at 4 °C for 20 min. The supernatants collected were combined and the final volume was adjusted to 25 mL with 0.6 M perchloric acid. Before chromatographic analysis, perchloric extracts were passed through a 0.22 µm filter (GHP, Waters Corp, Milford, MA).

Twelve biogenic amines and polyamines, namely octopamine, dopamine, TY, PU, CA, HI, agmatine (AG), serotonin, PHE, SPD, TR and SPM, were determined following the method described by Latorre-Moratalla et al. (2009). Amine separation was performed through a Acquity UPLC BEH C-18 reverse phase column (Waters corp., Milford, MA, USA), followed by a post-column derivatization with *o*-phthalaldehyde and spectrofluorimetric detection (ex: 340 nm and em: 445 nm). The detection and determination limits were different for each amine.

2.3. Statistical analysis

The content of biogenic amines and polyamines were compared among batches by using the Kruskal–Wallis test. Statistical tests were performed by means of the Statistical Software Package for Windows SPSS Statistic 20.0 (SPSS, Chicago, IL, USA).

3. Results and discussion

3.1. Polyamines in soybean products

Polyamine contents in all batches of non-fermented and fermented soybean products are shown in Table 2. SPD levels were

higher than SPM, as it was expected since SPD level is commonly higher than SPM in vegetable foods (Kalač & Krausová, 2005). Polyamines were found in all samples, with the exception of Sufu samples, in which SPD was not detected. Liu et al. (2011) and Tang et al. (2011) reported an important decrease in SPD levels during fermentation of sufu due to microorganisms consuming the SPD as an alternative source of nitrogen. This fact suggests that not only raw material but also microbiota could also affect the polyamine content. Statistically significant differences in SPD and SPM contents between batches in non-fermented and fermented soybean products were observed, except in soymilk, soy sprouts and tamari. Moreover, the content of both polyamines also varied in a wide range between non-fermented and fermented soybean products.

The sum of both SPD and SPM levels were lower than 50 mg/kg in non-fermented and fermented products, except in tempeh and natto. Tempeh showed the highest contents of SPD and SPM with 124 and 21 mg/kg, respectively, followed by natto samples which contained 75 and 11 mg/kg of SPD and SPM, respectively. These results are consistent with the ones reported by Nishimura, Shiina, Kashiwagi, and Igarashi (2006), who found similar concentrations of these polyamines in tempeh and natto samples, but lower than the levels of 250–475 mg/kg of polyamines reported by Kim, Byun, and Mah (2012) in natto (Table 1). Thus, tamari, which is a special long fermented soy sauce, also showed high contents of polyamines (38 and 7 mg/kg of SPD and SPM, respectively) probably because it contains other ingredients in its formulation, such as wheat which is known to be a source of polyamines (Ali, Poortvliet, Strömberg, & Yngve, 2011). The relationship between

Table 2
Biogenic amine and polyamine contents (mean ± SD) in commercial non-fermented and fermented soybean products (mg/kg fresh weight).

	Batch No	Biogenic amine content (mg / kg fresh weight)							
		TY	HI	TR	PHE	CA	PU	SPD	SPM
<i>Non-Fermented products</i>									
Fresh tofu	1	nd	nd	nd	nd	0.85 ± 0.01 ^a	0.74 ± 0.05 ^a	18.91 ± 0.19 ^a	4.47 ± 0.04 ^a
	2	nd	nd	nd	nd	1.16 ± 0.02 ^b	1.12 ± 0.02 ^b	22.72 ± 0.36 ^b	5.91 ± 0.16 ^b
	3	nd	nd	nd	nd	1.43 ± 0.04 ^c	1.28 ± 0.03 ^b	24.75 ± 0.01 ^b	7.16 ± 0.04 ^b
Hard tofu	1	nd	nd	nd	nd	0.81 ± 0.06 ^a	1.49 ± 0.05 ^a	30.16 ± 0.67 ^a	9.27 ± 0.22 ^a
	2	nd	nd	nd	nd	0.62 ± 0.01 ^a	0.66 ± 0.01 ^b	17.16 ± 0.31 ^b	5.14 ± 0.18 ^b
	3	nd	nd	nd	nd	nd	0.77 ± 0.02 ^b	26.37 ± 0.17 ^c	8.09 ± 0.20 ^a
Soymilk	1	nd	nd	nd	nd	0.41 ± 0.01 ^a	0.84 ± 0.06 ^a	10.39 ± 0.18 ^a	2.11 ± 0.20 ^a
	2	nd	nd	nd	nd	0.42 ± 0.02 ^a	0.41 ± 0.02 ^a	9.76 ± 0.07 ^a	1.79 ± 0.20 ^a
	3	nd	nd	nd	nd	nd	1.82 ± 0.12 ^a	10.07 ± 0.08 ^a	1.49 ± 0.43 ^a
Soy sprouts	1	nd	nd	nd	nd	0.33 ± 0.01 ^a	41.13 ± 0.07 ^a	10.51 ± 0.13 ^a	0.94 ± 0.01 ^a
	2	nd	nd	nd	nd	0.31 ± 0.01 ^a	45.67 ± 0.51 ^a	11.37 ± 0.15 ^a	0.28 ± 0.01 ^a
	3	nd	nd	nd	nd	nd	47.33 ± 0.22 ^a	11.67 ± 0.21 ^a	0.32 ± 0.01 ^a
<i>Fermented products</i>									
Natto	1	nd	nd	nd	nd	5.43 ± 0.04 ^a	9.49 ± 0.05 ^a	75.21 ± 1.57 ^a	11.15 ± 0.06 ^a
	2	nd	nd	nd	nd	2.79 ± 0.04 ^b	5.81 ± 0.04 ^b	56.91 ± 0.01 ^b	9.17 ± 0.03 ^b
	3	nd	nd	nd	nd	3.81 ± 0.02 ^c	7.01 ± 0.05 ^c	66.97 ± 0.54 ^c	10.08 ± 0.13 ^c
Tempeh	1	nd	nd	nd	nd	1.52 ± 0.02 ^a	31.06 ± 0.15 ^a	105.5 ± 0.9 ^a	21.89 ± 0.31 ^a
	2	nd	nd	nd	nd	1.99 ± 0.02 ^b	20.91 ± 0.82 ^b	97.3 ± 1.5 ^b	6.06 ± 0.11 ^b
	3	10.68 ± 0.03	nd	nd	nd	7.21 ± 0.07 ^c	17.53 ± 0.11 ^c	124.0 ± 4.0 ^c	9.74 ± 0.07 ^c
Miso	1	nd	3.99 ± 0.07 ^a	nd	nd	3.83 ± 0.12 ^a	17.81 ± 0.23 ^a	9.91 ± 0.01 ^a	2.64 ± 0.07 ^a
	2	nd	1.49 ± 0.01 ^b	nd	nd	1.99 ± 0.01 ^b	2.73 ± 0.03 ^b	7.65 ± 0.18 ^b	3.54 ± 0.01 ^b
	3	nd	4.62 ± 0.06 ^c	nd	nd	nd	14.54 ± 0.11 ^a	7.54 ± 0.03 ^b	2.54 ± 0.05 ^a
Tamari	1	76.66 ± 0.85 ^a	50.19 ± 0.81 ^a	11.49 ± 0.17 ^a	11.26 ± 0.09 ^a	1.23 ± 0.02 ^a	13.07 ± 0.10 ^a	36.78 ± 0.33 ^a	3.61 ± 0.10 ^a
	2	73.11 ± 0.53 ^a	57.65 ± 0.28 ^a	12.57 ± 0.21 ^a	10.69 ± 0.01 ^a	1.81 ± 0.02 ^b	17.08 ± 0.18 ^b	38.02 ± 0.36 ^a	6.77 ± 0.22 ^b
	3	40.19 ± 0.61 ^b	38.11 ± 0.49 ^b	10.35 ± 0.27 ^a	9.91 ± 0.48 ^a	1.69 ± 0.03 ^b	15.60 ± 0.64 ^b	29.45 ± 0.88 ^b	2.78 ± 0.05 ^c
Soy sauce	1	0.93 ± 0.03 ^a	0.99 ± 0.03 ^a	2.57 ± 0.14 ^a	5.51 ± 0.01 ^a	0.18 ± 0.01 ^a	7.01 ± 0.18 ^a	22.89 ± 0.21 ^a	2.06 ± 0.02 ^a
	2	1.45 ± 0.01 ^b	nd	nd	5.33 ± 0.06 ^a	2.01 ± 0.01 ^b	7.61 ± 0.03 ^a	19.95 ± 0.13 ^b	1.54 ± 0.12 ^b
	3	1.53 ± 0.05 ^b	0.81 ± 0.01 ^b	2.05 ± 0.01 ^b	7.07 ± 0.10 ^b	2.21 ± 0.03 ^b	6.98 ± 0.09 ^a	22.71 ± 0.46 ^a	2.14 ± 0.02 ^a
Soybean paste	1	nd	nd	5.38 ± 0.10 ^a	nd	nd	10.29 ± 0.07 ^a	4.25 ± 0.08 ^a	1.11 ± 0.04 ^a
	2	156.8 ± 1.5	51.21 ± 0.70	6.36 ± 0.13 ^b	6.88 ± 0.36	1.62 ± 0.03	22.21 ± 0.30 ^b	0.25 ± 0.01 ^b	0.12 ± 0.01 ^b
	3	nd	nd	6.04 ± 0.05 ^b	nd	nd	14.81 ± 0.34 ^c	4.98 ± 0.06 ^a	1.23 ± 0.06 ^a
Sufu	1	930.0 ± 0.6 ^a	730.0 ± 8.0 ^a	nd	4.27 ± 0.08 ^a	33.39 ± 0.05 ^a	14.46 ± 0.17 ^a	1.36 ± 0.03	2.21 ± 0.09 ^a
	2	910.6 ± 2.7 ^a	486.3 ± 5.5 ^b	nd	2.51 ± 0.09 ^b	36.96 ± 0.61 ^b	18.76 ± 0.06 ^b	nd	1.21 ± 0.03 ^b
	3	1730 ± 11 ^b	127.6 ± 1.4 ^c	nd	1.69 ± 0.01 ^c	35.09 ± 0.16 ^b	9.33 ± 0.04 ^c	nd	0.88 ± 0.01 ^b

Abbreviations: TY: tyramine, HI: histamine, TR: tryptamine, PHE: β-phenylethylamine, CA: cadaverine, PU: putrescine, SPD: spermidine, SPM: spermine.
a–c Values in the same product with different letters were significant different ($p < 0.05$).

polyamines and age-associated conditions should be more investigated. However, some authors recommended an ingestion of polyamine-rich foods, such as fermented soybean as natto and tempeh, for maintenance health especially in seniors, because it is known their synthesis decreases with age (Nishimura et al., 2006; Hunter & Burritt, 2012). Likewise, a diet rich in polyamines has been also recommended for the prevention of age-associated chronic diseases, especially cardiovascular diseases, an effect in part mediated by the anti-inflammatory properties of polyamines (Soda, 2010; Minois, Carmona-Gutierrez, & Madeo, 2011). However, in carcinogenic processes, a polyamine-reduced diet is recommended due to polyamines are involved in growth and differentiation of cells (Wallace & Caslake, 2001; Cipolla, Guillí, & Moulinoux, 2003; Gerner & Meyskens, 2004). In addition, it should be considered that some authors have recently linked for the first time, the high polyamines intake with the development of tumors in the gastrointestinal tract. The same authors recognised the limitation of their study and indicate the need of future studies to confirm their findings (Vargas et al., 2012).

Sometimes, PU is considered as a polyamine and reported together with SPD and SPM since PU is the precursor of the formation of SPD and SPM in both plants and animals (Ali et al., 2011; Kalač & Krausová, 2005). In the current work, PU were found in most of the soybean products, both non-fermented and fermented products, and for its low concentration, it was considered as naturally occurring amine. Although wide variations of PU contents in non-fermented vegetable foods have been reported, <80–100 mg/kg of PU has been usually attributed to naturally occurring amine in vegetables (Ali et al., 2011; Kalač & Krausová, 2005; Nishibori, Fujihara, & Akatuki, 2007). PU content, in general, was higher in fermented soybean products than in non-fermented products. However, the highest amount of PU were found in soybean sprouts, but it cannot be attributed to bacterial amino acid decarboxylating activity since soybean sprouts results from the germination of the bean and PU is present at high levels because acts as a plant growth factor (Kusano, Berberic, Tateda, & Takahashi, 2008; Takahashi & Kakehi, 2010).

3.2. Biogenic amines in soybean products

Biogenic amine contents from all batches of non-fermented and fermented soybean products are shown in Table 2, which did not include octopamine, agmatine, dopamine and serotonin since they were found in very low levels or not detected in any sample. As it can be seen, biogenic amine contents differed quite markedly between fermented and non-fermented soybean products.

In non-fermented products, the bacterial-related biogenic amines (TY, HI, TR and PHE) were not detected in any sample. The little amounts of PU and CA found in these products could be considered as naturally occurring amines. These results are in agreement with those reported previously in soymilk, tofu and soybean sprouts, who also found low amounts of these compounds (Table 1).

In fermented soybean products, biogenic amines were detected in many samples with a high variability, although only high contents (>50 mg/kg) were found in sufu, tamari and soybean paste (Table 2). The variability of the biogenic amine contents found in these products could be attributed to the different processes and hygienic conditions used for their manufacture and distribution. Thus, the differences of the biogenic amine contents could be explained by: (1) the different proportion of soybean used as the raw material, which contains considerable amounts of proteins, and, the availability of free amino acids, (2) technological processing conditions, such as the starter cultures and the duration of fermentation specific of each soybean product, (3) the proteolytic

activity from bacteria and (4) the occurrence of potential contaminant microorganisms with decarboxylase activity.

In fermented soybean products, TY was the main amine, followed by HI. Then, in lower amounts, the following amine was PU, and finally, CA, PHE and TR. However, the amounts of PU and CA observed in practically all fermented soybean samples were in the range of those found in the non-fermented products suggesting that they could have a physiological origin coming from the raw materials, soybean and other ingredients. Certain varieties of lactic acid bacteria involved in the fermentation processes of soybean could be the responsible for the formation of the biogenic amines (Shalaby, 1996). However, their presence could not be only related to these technological bacteria but also could be formed by contaminant bacteria, especially by enterobacteriaceae (Ibe, Nishima, & Kasai, 1992; Kung, Lee, Chang, & Wei, 2007).

Sufu, tamari and soybean paste were the fermented soybean products with the highest amounts of TY and HI (Table 2). Concretely, sufu showed extremely high TY and HI levels achieving more than 1700 and 700 mg/kg in one sample, respectively. It should be noticed that although there is a wide range of contents of TY and HI reported in the literature, some of the samples of this work showed higher amounts of them. Thus, the contents previously reported in sufu ranged from not detected to 1125 mg/kg for TY, and not detected to 373 mg/kg for HI (Table 1). Sufu is a fermented soybean curd that is produced in an open-type environment and stored at room temperature, where there could be some uncontrolled factors that explain the high contents of the biogenic amines (Guan et al., 2013). Moreover, all batches of sufu showed the highest amounts of CA (>30 mg/kg). Other studies also reported high amounts of PU and CA related to fermentation of sufu when high amounts of TY and HI were formed (Yen, 1986; Kung, Lee, et al., 2007; Tang et al., 2011; Guan et al., 2013) (Table 1). However, Liu et al. (2011) detected very high contents of PU (1776 mg/kg) and CA (883 mg/kg) although did not find TY and HI formation in stinky tofu, a product similar to sufu.

Among batches, statistically significant differences were also found in TY and HI contents in sufu, tamari and soybean paste ($p < 0.05$). In soybean paste, for example, TY and HI, that achieved 157 mg/kg and 51 mg/kg, respectively in one batch, were not found in the others. Likewise, among batches, the relative standard deviations (RSD) of HI contents achieved 16% and 55% in tamari and sufu, respectively, whereas for TY was 25% and 32%. A high variability in HI and TY levels in soy sauces was also reported by the most of authors recompiled in Table 1. The variability among batches of these products could be explained by the uncontrolled fermentation conditions applied during the manufacturing of these soybean fermented products. Keep in mind that the microorganisms involved in the fermentation process cannot be solely responsible for the amine formation. Deficient hygienic manufacturing

Table 3
Mean (\pm SD) and maximum histamine content of soybean products (mg HI/kg), mg of histamine per serving and grams of product necessary to achieve the safety levels.

		HI content	HI per serving	Grams to achieve 50 mg HI
Miso	Mean (SD)	3.4 (1.7)	<0.1	≈15,000
	Max.	4.7	<0.1	≈10,000
Soy sauce	Mean (SD)	0.6 (0.5)	<0.1	≈84,000
	Max.	1.0	<0.1	≈50,000
Tamari	Mean (SD)	49 (10)	0.7	≈1000
	Max.	58	0.9	≈860
Soy paste	Mean (SD)	17 (30)	0.3	≈3000
	Max.	52	0.8	≈1000
Sufu	Mean (SD)	448 (303)	27	≈100
	Max.	738	44	≈67

Table 4

Mean (\pm SD) and maximum tyramine content of soybean products (mg TY/kg), mg of tyramine per serving and grams of product necessary to achieve the safety levels in healthy individuals (600 mg), in individuals taking third generation MAOI drugs (50 mg) and in individuals taking classical MAOI drugs (6 mg).

		TY content	TY per serving	Grams to achieve 600 mg TY	Grams to achieve 50 mg TY	Grams to achieve 6 mg TY
Tempeh	Mean (SD)	3.6 (6.1)	0.7	\approx 170,000	\approx 14,000	\approx 1600
	Max.	10.7	2.1	\approx 56,000	\approx 4600	\approx 560
Soy sauce	Mean (SD)	1.3 (1.3)	<0.1	\approx 470,000	\approx 38,500	\approx 4700
	Max.	1.6	<0.1	\approx 370,000	\approx 32,000	\approx 4000
Tamari	Mean (SD)	63 (20)	0.9	\approx 10,000	\approx 800	\approx 95
	Max.	78	1.1	\approx 7700	\approx 650	\approx 78
Soy paste	Mean (SD)	52 (89)	0.8	\approx 11,000	\approx 1000	\approx 110
	Max.	158	2.4	\approx 3700	\approx 300	\approx 38
Sufu	Mean (SD)	1190 (468)	71	\approx 500	\approx 42	\approx 5
	Max.	1741	104	\approx 350	\approx 30	\approx 3

practices could originate a contamination with microorganisms owing histidine and tyrosine decarboxylase activity. Different species isolated from fermented soybean products of genera of *Clostridium*, *Bacillus*, *Enterococci*, *Enterobacteria* and *Pseudomonas* have been reported as TY and HI producers (Nout, Ruiker, & Bouwmeester, 1993; Han, Beumer, Rombouts, & Nout, 2001; Tsai, Chang, & Kung, 2007; Moon et al., 2010; Bai, Byun, & Mah, 2013). According to Guan et al. (2013), to minimise the biogenic amine contents, sufu should be produced under strict hygienic conditions. In this sense, it would be necessary to investigate the evolution of biogenic amines throughout the production and storage of sufu, including the effects of temperature, NaCl concentration and herbal extracts, in order to implement the good manufacturing practices (GMP) guide and the hazard analysis and critical control point (HACCP) system.

3.3. Risk assessment of histamine and tyramine

The objective of this study was to assess whether the consumption of the soybean products available in the Spanish market could imply a health risk for their HI and TY contents. The determination of the toxicity threshold for biogenic amines in humans is extremely difficult since the toxic dose is strongly dependent on the efficiency of the detoxification mechanisms of each individual and the presence of other factors. Then, other amines, alcohol or some MAOIs and DAOIs drugs, being some of these drugs usually taken, can saturate the enzymatic system.

Soybean products were not included in the scientific opinion of the EFSA (EFSA, 2011), due to the lack of suitable data. On the other hand, there is an increase of the consumption of this kind of products in Europe. In this sense, a preliminary risk assessment of HI and TY for fermented soybean products was made. To estimate the HI and TY consumption, two scenarios of each soybean product were considered: (a) the mean HI and TY values and (b) the maximum HI and TY values. A serving size of 15 g for miso, soy sauce and soybean paste, 60 g for sufu (common size serving as hard cheese), and 200 g for tempeh (used as meat products substitute) was taken (CESNID, 2005). The results of exposure assessment of HI contents in fermented soybean products (Table 3) show that, in all cases, HI amount per serving were bellow of the 50 mg proposed maximum ingestion by meal for healthy population. However, less than two servings of sufu would be enough to exceed the safety level by considering the average HI content (448 mg/kg). Taking the maximum HI value (738 mg/kg), this level would be reached with almost one serving. In the other soybean products, the safety level of HI for healthy individuals would only be reached with unusual consumptions. It is important to notice that it would be easier to surpass the safety level in a meal because the fermented soybean products are not the only contributors of HI. Likewise, the consumption of any of these fermented soybean products could cause adverse effects in histamine intolerant individuals.

Results of the exposure assessment of TY contents are shown in Table 4. For all soybean fermented products, the estimated TY contents per serving are far away of the 600 mg per meal proposed as safe consumption for healthy population. To reach the 50 mg of TY marked for patients taking third generation MAOIs, it would be necessary to eat 42 g of sufu (less than one serving) containing the mean value of TY. However, the maximum dose of 6 mg of TY reported for patients under classical MAOIs therapy could be easily reached by consuming a tenth part of an usual serving of sufu or even with a twentieth part of sufu with highest TY content (with a content of 1740 mg/kg). Based on these results, it should take into account that patients who receive a treatment with classic and third generation MAOIs drugs should be strongly recommended to avoid fermented soybean products in order to avoid adverse health effects.

In summary, adverse effects of TY and HI as a result of the consumption of the studied soybean fermented products, in general, would be unlikely, except for individuals under MAOIs and DAOIs drugs therapies. It is important to remark that other foods in the same meal, such as cheese, wine, sauerkraut, fish and meat products, could be additional sources of TY and HI, enhancing their adverse effects. Moreover, an increase in the intake of soybean products marketed in Spain, such as those of the Asian people, could considerably enhance the risk of suffering biogenic amine health-related disorders.

4. Conclusions

To our knowledge, this is the first report to determine the occurrence of biogenic amines and polyamines in soybean products from the Spanish market. The fermented soybean products, such as sufu, tamari and soybean paste, showed high biogenic amine contents, especially TY and HI. In agreement with Guan et al. (2013), minimising the biogenic amine content of sufu would increase its acceptability worldwide, especially in Western countries, where it could be consumed as healthy cholesterol-free condiment or as an alternative protein source for vegetarians and vegans. Adverse effects of biogenic amines as a result of consumption of tofu, soymilk, soybean sprouts, miso, tamari, soy sauce, natto, tempeh, soybean paste and sufu would be unlikely in healthy population, but some of them, especially sufu and soybean paste, should be taken into account in individuals taking MAOIs and DAOIs drugs. On the other hand, some fermented soybean products, such as tempeh and natto, showed to be an important source of polyamines, which appears to have a positive effect on health, except for patients suffering from cancer.

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