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# Food safety evaluation of broccoli and radish sprouts

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**Abstract**

Three cultivars of broccoli seeds (*Brassica oleracea* var. *italica*), cv. Tiburon, cv. Belstar and cv. Lucky, and two cultivars of radish seeds (*Raphanus sativus*), cv. Rebel and cv. Bolide, were germinated for three and five days and safety aspects such as microbiological counts and biogenic amines were investigated. Cytotoxicity evaluation was also carried out. Broccoli and radish sprouts contained numbers of mesophilic, psychrotrophic, total and faecal coliform bacteria which are the usual counts for minimally processed germinated seeds. Putrescine, cadaverine, histamine, tyramine, spermidine and spermine increased during sprout production although these levels were below those permitted by legislation (5 mg/100 g of edible food). Broccoli and radish sprouts demonstrated no toxic effects on proliferation and viability of HL-60 cells and should be included in our diets as healthy and safe fresh foods.


*Keywords:* Germination; Radish; Broccoli; Biogenic amines; Food safety; Sprouts; HL60 cytotoxicity

## 1. Introduction

The use of seed sprouts as food has spread in the past few decades from Far Eastern countries to parts of the Western world. Consumers can find on the market an extraordinary variety of different types of sprouts in which the *Cruciferae* family is well represented. Several studies have demonstrated that cruciferous sprouts such as broccoli (*Brassica oleracea* L. var. *italica*) and radish (*Raphanus sativus*) are very rich in health-promoting phytochemical constituents such as glucosinolates related to cancer prevention as well as having antioxidant properties (Fahey et al., 1997; Tian et al., 2005; Barillari et al., 2005), phenolic compounds and ascorbic acid in these vegetables (Zielinski et al., 2002, 2003; Takaya et al., 2003). Moreover, a recent study performed *in vivo* showed that Japanese radish sprouts have the potential to alleviate hyperglycemia in diabetes cases and are effective in the primary prevention of diabetes mellitus in animal models (Taniguchi et al., 2006). Eating the fresh sprouts is the best way of gaining all of the health benefits claimed for cruciferous sprouts because only minor losses in health-promoting components are likely to occur.

Most consumers and retailers do not cook sprouts, and since bacteria on the seed surface can become internalized during sprouting, washing sprouts is probably an ineffective way to eliminate spoilage and pathogenic bacteria (Mohle-Boetani et al., 2001). The importance of this problem is reflected by a number of specific recommendations that were developed in 1997 by US National Advisory Committee of Microbiological Criteria for Foods (NACMCF, 1999). Also the US Food and Drugs Administration (USFDA) issued guidance to enhance the safety of sprouts (1999). It is generally accepted that microbial populations exceeding $5 \times 10^6$ cfu/g may produce measurable metabolites, not only in terms of spoilage parameters but also in relation to toxic metabolites.

Biogenic amines are usually generated by decarboxylation of amino acids or by amination and transamination

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of aldehydes and ketones (Askar and Treptow, 1986). Biogenic amines can be naturally present in plant food since they are required in cellular metabolism and in growing tissues (Matilla, 1996; Santos, 1996). However, they are also a consequence of microbial activity (Glória et al., 2005; Hallàsz et al., 1994). The presence of biogenic amines in foods and beverages is considered important because of their influence on physiological activities, which may cause symptoms such as skin irritations, headache, dizziness, vomiting and diarrhoea (Pechanek et al., 1983). The presence of some mono-, di- and polyamines has been suggested as a supplementary criterion to indicate the freshness and quality of food. In particular, putrescine, cadaverine, spermidine, spermine, histamine and tyramine have been suggested as indicators of food deterioration (Ramantanis et al., 1985; Paulsen et al., 1997). On the other hand, amines are also investigated as a possible mutagenic precursor, since some of them may be nitrated or act as precursors for other compounds capable of forming nitrosamines, which are carcinogenic for various species of animals and a potential hazard to humans (Shalaby, 1996). Moreover, in order to ensure the food safety of these cruciferous sprouts it would be interesting to determine the effect of seed germination on human cell viability.

Therefore, the aim of this work is to determine the hygienic status, biogenic amine content and cytotoxicity of three cultivars of broccoli (*B. oleracea* var. *italica*) and two cultivars of radish (*R. sativus*) after sprouting.

## 2. Materials and methods

### 2.1. Plant material

Broccoli seeds (*B. oleracea* L. var. *italica* cv. Tiburon, cv. Belstar and cv. Lucky) were purchased at Bejo Iberica (Spain). Radish seeds (*R. sativus* cv. Rebel and cv. Bolide) were purchased at Bejo Iberica and Man Fong Pacific Trading (Spain), respectively.

### 2.2. Seeds germination

Seeds (10 g) was soaked with 50 mL of 0.07% sodium hypochlorite for 30 min. These seeds were drained and washed with distilled water until they reached neutral pH. Afterwards, seeds were soaked in 50 mL distilled water for 5½ h with shaking every 30 min. The imbibed seeds were germinated at pilot scale by layering seeds over moist filter paper in a germination tray. The tray was placed in a seed germinator G-120 model (ASL Snijders International S. L., Holland) and seeds were continuously watered by capillary. Germination of broccoli seeds was carried out at 25 °C under photoperiod of 16 h light and 8 h darkness and germination of radish seeds was carried out at 25 °C in darkness. Sprouted seeds were collected after three and five days. The germination process was carried out in triplicate and the rate was higher than 90%.

### 2.3. Microbiological determinations

Microbial counts were carried out using the poured plate technique. Five grams of fresh samples were aseptically placed into a flask with 45 mL of peptone water (Scharlau Chemie, Spain) to achieve a $10^{-1}$ dilution and shaken with vortex for 1 min. Serial dilutions were made in 0.1% buffered peptone water (Scharlau Chemie, Spain) in tubes.

To determine total mesophilic and psychrotrophic aerobic bacteria counts, appropriate serial dilutions were surface-plated on Triptic Soy Agar (TSA, Scharlau Chemie, Spain). Plates were incubated at 32 °C for mesophilic bacteria counts for 48 h and at 8 °C for psychrotrophic bacteria counts for 10 days. Total and faecal coliforms were determined on Violet-Red Bile Agar (VRBA, pH 7.4, Scharlau Chemie, Spain) and incubated at 37 °C (for total coliforms) or 44 °C (faecal coliforms) for 24 h under anaerobic conditions. Faecal *Streptococci* were surface-plated on Slanetz Bartley Agar (Scharlau Chemie, Spain) and incubated at 37 °C for 24 h.

### 2.4. Biogenic amine determination

Determination of biogenic amines was carried out by acid extraction, derivatization with dansyl chloride and HPLC quantification according to Frías et al. (2007).

Putrescine dihydrochloride, cadaverine dihydrochloride, histamine dihydrochloride, tyramine, spermidine trihydrochloride, spermine tetrahydrochloride were purchased from Fluka (Spain). A stock standard aqueous solution of amines was prepared by adding an accurately weighed amount of each standard (ca. 80 mg) to a 25 mL volumetric flask. Standards were derivatized as described for the samples.

The chromatographic system consisted of an Alliance Separation Module 2695 (Waters, Milford, USA), a Photodiode Array detector 996 (Waters) and a personal computer running the Empower II for windows chromatographic software (Waters). The sample (20 μL) was injected onto a $C_{18}$ Kromasil 250 × 4.6 mm i.d., 5 μm size (Symta) column equipped with a $C_{18}$ guard column (Symta) both thermostatted at 30 °C. The mobile phase for DCl-derivatives separation consisted of bidistilled water (solvent A) and HPLC-grade acetonitrile (solvent B). The elution programme was held at 65% of B for 1 min, ramped at 80% (10 min), 90% (12 min), 100% of B (16 min) and held until the end of the run (23 min) with a flow rate of 0.8 mL/min. Calibration curves were obtained for standard amines and "*r*" was always above 0.990.

### 2.5. Cytotoxicity evaluation

To obtain the extracts, 50 mg of the seed flour were subjected to extraction with 2.5 mL of deionized water and sonicated for 30 min (Sonorex AK103H). Then, the extracts were centrifuged for 15 min at 12,000 rpm. The supernatant was filtered through 0.22 μm membranes into sterile test tubes. One mL of each filtered supernatant was evaporated in tarred vessels and the obtained dry masses were weighed after 24 h desiccation with $P_2O_5$. The residues were dissolved in sterilized water to a final concentration of 1 mg/mL.

The human leukemic cell line (HL-60), derived from a patient with acute promyelocytic leukemia, was obtained from American Type Culture Collection (ATCC). The culture was maintained on RPMI medium containing 10% fetal calf serum and 1% penicillin G (Sigma) and streptomycin (Sigma), at 37 °C in a humidity-controlled incubator at 90% relative humidity and 5% $CO_2$. After a few passages, cells were centrifuged, resuspended in fresh medium at the concentration of $0.30 \times 10^6$ cell per millilitre and transferred onto several plates in 2 mL volumes. The cells were exposed to 100 μg/mL of raw and germinated seed extracts and control (sterilized water) during 24, 48 and 72 h. Then, trypan blue dye (Sigma) was added to cell cultures in a ratio of 1:1 and left for 10 min. Cell suspension (20 μL) was then loaded by micropipette into Bürker chambers. The cells were counted under a microscope at 100× magnification. The number of viable cells was determined for cell proliferation approach.

The tetrazolium reduction assay (MTT) was performed in duplicate following the method of Denizot and Lang (1986). Briefly, 100 μL of the cultured medium was transferred into Eppendorf tubes and centrifuged for 5 min at 1.600 rpm. The supernatants were removed and cells resuspended in 100 μl of fresh medium. The cells were exposed to 100 μg/mL of raw and germinated seed extracts and control (sterilized water) for 24 h. After this time, 20 μL of MTT (Sigma) at a concentration of 5 mg/mL in PBS, were added to each sample. All plates were incubated for 4 h at 37 °C