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Fermented tofu: Enhancement of keeping quality and sensorial properties

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ABSTRACT

In this study a new way to produce tofu by means of soymilk fermentation by specific lactic acid bacteria, *Lactobacillus casei* and *Lactobacillus acidophilus* alone or in combination, and subsequent precipitation has been developed, in order to prevent undesired microbial and chemical spoilage, as well as improve the stability and the quality of the product. In particular, the combination *L. casei* and *L. acidophilus* generated tofu having shelf life exceeding 20 days and able to prevent the growth of the spoilage strains inoculated. This fermented tofu was characterized by the production of antimicrobial molecules, such as acetic acid, limonene, 2-nonen-1-ol, 1-nonanol, 2(5H)-furanone, benzyl alcohol, phenylethyl alcohol and heptanoic acid. Depending on the *Lactobacillus* species used, the fermentation process generated different metabolites profiles and sensorial properties. Another promising properties conferred by the lactic acid bacteria fermentation was the inhibition of unsaturated fatty acids (UFAs) peroxidation or reduction of the aldehydes originated to their corresponding alcohols.

1. Introduction

The high nutritional value and low cost of soybean products make them a suitable solution for malnutrition problems for poor people living on grain centered diets. Tofu, a fundamental part of Asian food culture, is a traditional oriental soybean food composed principally of protein and lipid. Currently, its consumption and popularity is growing rapidly in the West countries. Moreover, whole soy foods such as milk, tofu and meat analogues represent a big market segment in USA and Europe and innovative soy based products are being introduced in the market. Soy foods were also given a significant boost when FDA in 1999 approved a health claim linking soy foods with heart disease risk reduction (FDA, health claim petition, 2004). In fact, soy contains a number of biologically active compounds, including isoflavones, essential fatty acids, antihypertensive, antioxidative, opioid agonistic peptides and tocopherols (Adams et al., 2005; Andrade, Twaddle, Helferich, & Doerge, 2010; Martin et al., 2006). Tofu may be the most popular food made of soy. It is produced from water-extracted and salt- or acid precipitated soybean in the form of a curd, resembling a soft white cheese or a very firm yogurt. Tofu can also be further processed into various secondary tofu products, including deep-fried tofu, grilled tofu, frozen tofu, dried tofu, fermented tofu, and more. In most cases, these processed tofu products have different characteristics, such as taste, texture, end uses, and commercial identities with respect to the original plain tofu (Li & Hsieh 2004). Tofu is a perishable highly hydrated, gelatinous product having relatively high pH (5.8–6.2) and moisture content (80–88%) (Liu, 1997, pp. 1–532). Its water content can be varied to produce an array of tofus with different characteristics. The development of spoilage bacteria such as *Pseudomonas* spp., Coliforms, *Bacillus* spp., *Klebsiella* spp., *Leuconostoc* spp., and *Staphylococcus* spp., in tofu

Abbreviations: C, traditional tofu prepared according to the company flow chart; Lac, tofu produced with soymilk acidified by addiction of lactic acid; Fca, tofu obtained by soymilk fermentation by *Lactobacillus casei* and *Lactobacillus acidophilus*; Fc, tofu obtained by soymilk fermentation by *L. casei*; Fa, tofu obtained by soymilk fermentation by *L. acidophilus*; CPs, control tofu inoculated with *Pseudomonas fragi*; CLn, control tofu inoculated with *Leuconostoc lactis*; CEn, control tofu inoculated with *Enterococcus faecium*; FcaPs, fermented tofu inoculated with *P. fragi*; FcaLn, fermented tofu inoculated with *Ln. lactis*; FcaEn, fermented tofu inoculated with *E. faecium*; LacPs, acidified tofu inoculated with *P. fragi*; LacLn, acidified tofu inoculated with *Ln. lactis*; LacEn, acidified tofu inoculated with *E. faecium*.

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products has been described in some papers (Matsuzawa et al., 1998; No, Lee, & Meyers, 2000; Tuitemwong & Fung 1991). However, the microbial ecology of tofu has not been specifically investigated. Moreover some pathogens are able to grow in this food system which has been associated with several food-borne diseases (Anbarasu & Vijayalakshmi, 2007; Szabo et al., 1989). Some researchers and enterprises have developed specific approach for quality and shelf life improvement. Commercial tofu is often pasteurized or packed under *vacuum*. Different methods of tofu stabilization include preparation of tofu with ozone treated soybeans (Park, Kim, & Kim,1994), application of electrolyzed water (Zhao, Saito, Yoshihashi, Nakahara, & Tatsumi, 2002), high pressure (Prestamo, Lesmes, Otero, & Arroyo, 2000), packaging in biopolymers such as kitosan (No & Meyers, 2004). The use of acetic acid as a coagulant (Lee, Kim, Baek, & Choun, 1990), use of natural coagulants, like specific fruit juice, the use of chemical preservatives in brines solutions and smoking were also reported (Lee et al., 1990; Miskovsky & Stone, 1987). These procedures negatively affect the sensorial quality of the resultant tofu (Anbarasu & Vijayalakshmi, 2007). Moreover, due to the intrinsic perishability of tofu products, these treatments are not sufficient to prevent the growth of spoilage or pathogenic microorganisms. In addition, due to the high content of polyunsaturated fatty acids (PUFAs), and specifically linoleic acid, in the phospholipids of soy lecithin (Cherry & Kramer, 1989), tofu and soy products are particularly subjected to enzymatic and chemical oxidation with consequent sensorial quality and shelf life reduction (Anbarasu & Vijayalakshmi, 2007).

Spontaneous fermentative process of the brine surrounding tofu has been used to produce more stable fermented pickled tofu and "stinky" tofu. The first one is a dry tofu superficially slowly fermented with aerobic bacteria added with Chinese wine, vinegar or red yeast rice (Shurtleff & Aoyagi, 2008), while the "stinky" tofus are produced by soaking ready tofu in naturally fermented stinky brine where the fermentation metabolites permeates it. Chao, Tomii, Watanabe, and Tsai (2008) identified in stinky brines mainly *Lactobacillus fermentum*, *Lactobacillus delbruekii*, *Lactobacillus salivarius*, *Leuconostoc* spp. (*citerum*, *mesenteroides* and *pseudomesenteroides*) and *Pediococcus* spp.

In this study a new process to produce fermented tofu, by means of soymilk fermentation with selected lactic acid bacteria (LAB) strains and subsequent protein precipitation has been developed in order to prevent or delay undesired microbial and chemical spoilage, improve the stability and the quality of the final product. Moreover, to test the stability of the tofu obtained with fermented soymilk, a challenge testing, inoculating strains previously isolated from spoiled tofu, has been developed. The quality and stability of the various samples has been evaluated on the basis of volatile metabolites production, electronic nose and sensorial analysis.

2. Materials and methods

2.1. Microorganism isolation, identification and enumeration

Seventy-five different *vacuum* packaged tofu from daily production, delivered by a Company ConBio (Santarcangelo, Italy), were collected during a month and stored at temperature of 6 ± 2 °C for one month and were used for the isolation and identification of the spoilage flora. All the samples, stored at 6 ± 2 °C, were characterized more or less by bloated package, sour smell or both before the expiry date of 40 d. The total mesophilic bacteria and the LAB were counted respectively by using PCA (Oxoid, England), incubation time 24 h at 30 °C, and MRS (Oxoid, England), incubation time 24–48 h at 37 °C, the bacterial endospores were counted after thermal treatment at 80 °C, 10 min, in PCA (Oxoid, England), incubation time 48–72 h at 30 °C.

From each plate different colonies, corresponding to different morphological types, were randomly isolated. Genomic DNA was extracted from pure cultures of the isolates by the procedures previously described (Serrazanetti et al., 2011). Forty-one representative isolates were identified by RAPD PCR (primer M13) and sequencing of the 16S rRNA region.

2.2. Fermentation of soymilk

A screening on nine different LAB strains belonging to the collection of Department of Agri-Food Science and Technology (DISTAL) of Bologna University, belonging to the species *Lactobacillus plantarum* (LP1, LP2, LP3, LP4), *Lactobacillus casei* (LbCD2), *Lactobacillus acidophilus* (LA1), *Lactobacillus helveticus* (LH1) and *L. fermentum* (MR16), was performed in order to select suitable starters. Strains were grown in MRS medium (Oxoid, England) at 37 °C for 24 h. Each LAB strain were inoculated in 20 ml of soymilk at a 2 logCFU ml^{-1} and incubated at 32 °C. This temperature has been selected, on the basis of preliminary experiments (data not shown), in order to attain the end of the exponential phase in the time of one night. Growth of the LAB and pH decrease (Crison Instruments, S.A., Barcelona Spain) of the medium were monitored at 0, 5, 15 and 20 h. The data were analysed according to Gompertz equation (modified by Zwietering, Jongenburger, Rombouts, & van 't Riet, 1990). The fermentation efficiency was evaluated on the basis of the growth parameters (λ: incubation extension in hours; μmax: maximum growth rate as $hours^{-1}$; A: maximum growth extent as logCFU g^{-1}), as well as pH decrease after fermentation.

The two most performing strains, in terms of acidification, were chosen for the further experiment; these strains were: LbCD2, belonging to the *L. casei* species, and LA1 which belongs to the *L. acidophilus* species.

2.3. Tofu preparation at laboratory scale

Soymilk was provided by Company ConBio (Santarcangelo, Italy). The soymilk was obtained by soaking soybeans overnight and grinding adding hot water (30% soy and 70% water), and heating the whole mass until reaching 105 °C (under pressure cooking), then it was filtered separating the liquid part (soymilk) from the solid part called okara. The soybeans (*Glycine max* (L.) Merrill) used belong to the PR91M10 variety. As coagulation agent Nigari (Nigari composition: $MgCl_2 + 6H_2O$: more than 95%; Zn: less than 70 ppm; As_2O_3: less than 4 ppm; provided by *Soluzione Naturale*, Brescia, Italy) was added in order to standardize the curd formation. For Nigari preparation: 243 g of Nigari salt was solved in 1 L of distilled water; 24 ml of that solution was added for every litre of soymilk after the heating step (80 °C). The curd was pressed mechanically with a filter press in order to obtain firm and regular tofu, which has been cut in small portions (125 g).

Inoculum preparation: *L. casei* and *L. acidophilus* were grown in MRS medium and incubated at 37 °C for 24 h. Two ml of the cultures (9 logCFU ml^{-1}) were centrifuged (11,000 × *g* for 5 min) and the cells collected and washed with saline solution (0.09%), centrifuged again and suspended in soymilk. The cells suspension was added to 1 L of soymilk (6 logCFU ml^{-1}) and was incubated at 28 °C for 20 h.

Five types of tofu were prepared at laboratory scale according to the flow diagrams above indicated (Fig. 1) to evaluate the effect of selected starters fermentation on spoilage of naturally occurring population. In particular, 1) traditional tofu prepared according to the company flow chart (C); 2) tofu obtained by fermentation with *L. casei* (Fc); 3) tofu obtained by fermentation with *L. acidophilus*