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SAFE SOY: A MICROBIOLOGICAL FACE-OFF BETWEEN ORGANIC AND CONVENTIONAL TOFU

S u m m a r y

Introduction: The global demand for soy and soy products continues to grow steadily, with forecasts indicating further increases in the production of this commodity. While soy is primarily used as animal feed, its consumption among consumers is on the rise. Both organic and traditional agriculture aim to achieve the same goal – producing food products. In accordance with the current European Union law, all food available on the market, not only that which is labeled as organic, must be safe and provide proper nutritional values. Soy products, such as tofu, are susceptible to the growth of microorganisms due to their high water and protein content, posing a health risk to consumers. The aim of the study was to compare the microbiological quality of organic and conventional tofu. Total bacterial counts, total fungal counts, *Staphylococcus aureus* and *Escherichia coli* were determined.

Results and conclusions: *E. coli* was not detected in the tested tofu samples, while the presence of *S. aureus* was found in more than half of the samples, with a maximum of $5.4 \cdot 10^2$ cfu/g. A statistical analysis did not show statistically significant differences between the origin of tofu and the total counts of fungi ($p = 0.8293$) or *S. aureus* ($p = 0.5673$), but revealed significant differences in the total count of mesophilic bacteria contaminating tofu depending on their origin ($p = 0.0191$). In particular, a higher number of these microorganisms was found in organic tofu samples ($7.8 \cdot 10^2$ cfu/g) compared to conventional ones ($1.7 \cdot 10^2$ cfu/g). The highest level of fungi ($1.2 \div 4.8 \cdot 10^2$ cfu/g) was observed in 8 % of organic tofu samples. The results obtained emphasize the importance of maintaining appropriate hygiene standards during the production and storage of tofu to ensure food safety for consumers.

Key words: tofu, organic product, conventional product, food safety, microbiological contamination

Introduction

The demand for soy and soy products is steadily increasing worldwide [32]. According to a report published in November 2023 by the International Grain Council (IGC), world soybean production for the 2023/24 season is estimated to increase by 7 % compared to the 2022/23 season, reaching 395 million tons [6]. Soy is mainly used

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as animal feed in the form of soybean meal. Approximately 20 % of the world's soy production is used for direct human consumption, and a small percentage is used as fuel [32].

The high nutritional value of soy and its products, especially protein and amino acids, makes them a dominant factor. Soy contains about 40 % protein, 35 % carbohydrates, 20 % soybean oil and 5 % ash (non-hydrated metal oxides). Additionally, it is rich in minerals, particularly calcium, phosphorus and iron, and abundant in vitamins such as thiamine, niacin and riboflavin [1, 24]. Soy is rich in phytoestrogens, which exhibit a range of estrogen-like effects [13]. The isoflavone genistein has similar effects to human estrogens, which can counteract bone tissue resorption and also has anti-cancer properties [11, 13]. Soy is an excellent source of protein for people on cereal-based diets, those with lactose intolerance, those wishing to reduce meat consumption and older adults [1, 2]. Traditional soy products such as soy milk and tofu, prepared from whole soybeans, are rich in protein and vitamins, while being low in calories, carbohydrates, fat and omega-3 fatty acids (Table 1). Furthermore, they are cholesterol-free and easily digestible [21]. Tofu comes in various textures, from soft to very firm. It is often referred to as "boneless meat". Fresh tofu contains 86.7 % water, 7.2 % protein, 3.4 % fat, 2 % carbohydrates, as well as vitamins (B_1) and minerals (iron, copper, magnesium and zinc), antioxidants [1, 18, 19, 23, 27]. Increasing consumer awareness is leading to growing demand for organic products. Plant-based products from organic farming are observed to have a lower total protein content compared to their conventional counterparts, but they also have a higher protein quality, as measured by the content of key amino acids [26].

The market for organic food in Poland is steadily growing, evident not only in organic stores, but also in grocery discounters [25]. Sources report a lack of clear scientific evidence of differences in environmental impact, nutritional quality, safety and health effects between food from conventional and organic farming [5, 9]. The control of organic products, supervised by independent certifying organizations, ensures the highest quality. Products from organic farming are subject to specific requirements, different from standard product testing, including additional controls to ensure compliance with organic standards before they are introduced to the market [7, 30]. In organic farming, the main emphasis is on quality control during the production process, not just on the final product. It is assumed that the quality of food depends on the way and conditions of its production, and the final products are only checked when there are justified concerns about their quality [9]. Growing consumer awareness of environmentally friendly techniques accelerates the development of more sustainable agricultural practices. Organic labelling provides information about products, creating a positive cycle between producers and consumers. Literature data suggests that consumers prefer organic food, considering it safer, healthier and more environmentally friendly [5, 16].

However, consumers should be aware that labels on organic products only inform about production methods and do not guarantee their safety [15]. Under the current European Union (EU) law, which is in force in Poland as well, all food sold on the market must meet safety standards and provide appropriate nutritional values, regardless of whether it is labeled as organic. For this purpose, there is an integrated food control system covering all Member States, aimed at monitoring the presence of various types of contaminants, including chemical, physical and microbiological ones [7].

Tofu cheeses, due to their high water content, protein and pH value ranging from 6.0 to 7.0, are susceptible to microbial growth, especially if proper manufacturing and/or storage procedures are not followed [2, 21, 24]. Although there are articles in the international literature regarding the microbiological quality of tofu, there are still few reports on the microbiological safety of these products from the Polish market. The aim of the study was to compare the microbiological quality of organic and conventional tofu.

Materials and methods

Materials

The research material consisted of tofu cheese of conventional origin (CT, n = 25) and organic origin (OT, n = 25). The organic products were labeled as such and had organic production certification. The composition of the examined products is presented in Table 1. The tofu cheese came from various producers and batches. The transportation time of the examined products from purchase to delivery to the laboratory did not exceed 1 hour. The research material was transported in a thermally insulated container. The products, sourced from both the EU and non-EU countries, were purchased between February and July from organic food stores or discount shops in Gdynia.

Methods

Twenty grams of each tofu sample were collected in a laminar airflow chamber and then homogenized with 180 mL of Ringer's solution using a Stomacher lab-blender 400 (Seward, Worthing, the United Kingdom). The homogenates were further diluted. In the analyzed dietary supplements:

- mesophilic aerobic bacteria counts (AMC) were conducted on Merck's nutrient agar (incubated at 30 °C for 72 hours),
- yeast and mold counts (YMC) were performed on Merck's YGC chloramphenicol agar (incubated at 25 °C for 120 hours),
- the number of *Staphylococcus aureus* was determined using bioMérieux's Baird Parker + RPF medium (incubated at 37 °C for 48 hours),
- the number of *Escherichia coli* was determined using the selective medium Coli ID by bioMérieux (incubated at 37 °C for 48 hours).

Microbiological analyses were performed using the dilution method, and microorganisms were counted according to PN-EN ISO 7218:2008.

Statistical analyses

The normality of the samples was checked using the Shapiro-Wilk test. The differences between groups in terms of bacterial counts for the samples of different origins (organic and conventional) were tested using the Mann-Whitney U test. The significance level was set at 0.05. The data was processed using Statistica v.13 software (StatSoft, Inc.).

Results

The presence of *E. coli* was not detected in any of the samples analyzed. In over half of the analyzed tofu samples, the presence of *S. aureus* was observed. The maximum level of these bacteria for CT and OT was $3.3 \cdot 10^2$ and $5.4 \cdot 10^2$ cfu/g, respectively. The mean number of *S. aureus* was higher for OT than for CT (Table 2). There were 20 % of CT samples and 32 % of OT samples with *S. aureus* counts between $10^2 \div 10^3$ cfu/g. Approximately one-third of the tested products were contaminated with fungi. A statistical analysis showed no statistically significant differences between the origin of tofu and the total counts of fungi (z = -0.22; p = 0.8293) or *S. aureus* (z = -0.57; p = 0.5673). The highest level of fungi ($1.2 \div 4.8 \cdot 10^2$ cfu/g) was found in 8 % of the OT samples. For CT, the highest levels of fungi were lower and did not exceed 10^2 cfu/g. In 90 % of the analyzed tofu, the presence of AMC was found at levels ranging from 10^1 to $6.2 \cdot 10^3$ cfu/g. In 40 % of CT samples, only the presence of mesophilic microorganisms was observed, while for OT, the percentage of samples with only AMC was slightly lower (36 %). In the case of the second group of tofu, 4 % of the samples were free from any microorganisms. In 14 % of the analyzed tofu, AMC was found at levels $>10^3$ cfu/g (Fig. 1). The statistical analysis revealed significant differences in the total counts of mesophilic bacteria contaminating tofu depending on their origin (z= -2.34; p = 0.0191) (Fig. 2). Among the results obtained for CT, 25 % of the samples were characterized by results equal to or higher than $1.1 \cdot 10^2$ cfu/g. The degree of contamination of OT with the total number of microorganisms was higher because 25 % of the samples were characterized by results equal to or higher than $5.4 \cdot 10^2$ cfu/g.

Discussion

Plant-based products are an essential part of a human diet. It is predicted that organically grown fruit and vegetables contain fewer contaminants compared to their conventional counterparts [5]. The statistical analysis showed no statistically significant differences between the origin of tofu and the total counts of fungi (p = 0.8293) or *S. aureus* (p = 0.5673). Also, available data indicates no differences in contamination levels of organic and conventional dairy products, including bacteria such as *E. coli* and *S. aureus* [14, 28]. The high water and protein content of tofu makes it susceptible to bacterial spoilage [10]. However, in the food industry, cleaning and sanitation procedures are important to prevent environmental contamination of products and to prevent the presence and proliferation of pathogenic and saprophytic microorganisms [29]. In all tested tofu samples, the presence of *E. coli* was not detected. Similar results for total coliforms and *E. coli* were reported by Nazim et al [17] and El-Hadidi [3] during soy cheese storage. Smith-Spangler et al [28] demonstrated that *E. coli* occurred in 7 % of organic products and 6 % of conventional ones, suggesting that the former group is more susceptible to contamination due to the use of natural manure fertilizer. Additionally, poor personal hygiene among workers, along with the use of unclean devices, can contribute to the presence of *E. coli* [10, 22]. However, aerobic composting of manure effectively reduces or eliminates initial pathogenic microorganisms such as *Salmonella enteritidis* or *E. coli* [14]. Ananchaipattana et al. [2] found *E. coli* and *Staphylococcus* spp. contamination in 28 % and 26 % of tested tofu samples, respectively. In our own research, a twofold higher number of samples contaminated with *S. aureus* was observed. Ribeiro et al. [24] obtained results exceeding 10^4 cfu/g for *S. aureus* in their studies, while Kukułowicz and Pryczkowska [12] showed staphylococcal presence ranging from $0 – 2.2\cdot10^4$ cfu/g. As determined in the literature, acceptable limits for *S. aureus* in tofu should be accepted if lower than $10^2 \div 10^3$ cfu/g [8, 20]. In our own research, the highest levels of *S. aureus* did not exceed 10^3 cfu/g. The presence of *Staphylococcus* spp. in products may be due to improper hygiene practices during production or improper storage conditions [2]. The mesophilic aerobic bacteria counts in the tested tofu ranged from $0 \div 6.2\cdot10^3$ cfu/g and did not exceed the quality standards for soy products (max $2.0\cdot10^4$ cfu/g) [4]. These results were lower than those obtained by Ribeiro et al [24], who found AMC levels above $4.3\cdot10^5$ cfu/g. Poudel [21] obtained results over 1 log cycle higher than Ribeiro et al [24] after storing tofu in refrigerated conditions for six days. Nazim et al [17] found AMC presence below 10 cfu/g two months after tofu production, while El-Hadidi [3] found these microorganisms at a level of $3.0\cdot10^3$ cfu/g after four weeks of tofu storage. Available data suggests that during subsequent tofu production stages (adding water, grinding, coagulation, molding, etc.), conditions conducive to natural microorganism growth are created [24]. Literature sources also state that the number of microorganisms in tofu can be controlled by using modified atmosphere packaging, which maintains optimal conditions for longer freshness and prevents bacterial growth [21]. In our research, the presence of yeast and mold counts was detected in 30 % of the tested tofu cheeses. Nazim et al [17] found yeast and mold counts below 10 cfu/g two months after tofu production, while El-Hadidi [3] found these microorganisms at a level of $2.5\cdot10^2$ cfu/g after four weeks of tofu storage. In our own research, only 8 % of OT samples exceeded the quality standards (10^2 cfu/g) for soy products [4]. Ndife et al. [18] found YMC in tofu with various additives in a slightly lower range than El-Hadidi [3], ranging from 10 ÷ $5.5 \cdot 10^1$ cfu/g. Microorganisms such as bacteria, mold and yeast play significant roles in soybean fermentation and may be present in raw materials used for tofu production, such as soybeans, water and other ingredients. Additionally, mold can infiltrate tofu during the production process, especially in environments lacking sufficient hygiene. In some cases, mold may also develop during tofu storage, particularly if stored under improper conditions like high humidity or temperature. However, unwanted or harmful metabolites from mold (mycotoxins) can be released into the food during fermentation [3, 31].

Conclusion

1. Despite the absence of detectable *E. coli* and *S. aureus* counts not exceeding 10^3 cfu/g in the tested products, Good Manufacturing Practices (GMP) and maintaining appropriate hygiene standards are essential for achieving high-quality tofu and ensuring food safety.
2. It is also important to emphasize the need to raise public awareness about proper tofu storage and hygienic handling to minimize the risks associated with potential microbiological hazards and ensure safe consumption.

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BEZPIECZNA SOJA: STARCIE MIKROBIOLOGICZNE MIĘDZY TOFU EKOLOGICZNYM A KONWENCJONALNYM

S t r e s z c z e n i e

Wprowadzenie: Światowe zapotrzebowanie na soję i produkty sojowe stale rośnie, a prognozy wskazują na dalszy wzrost produkcji tego surowca. Mimo że soja jest głównie wykorzystywana jako pasza dla zwierząt, jej spożycie wśród konsumentów wzrasta. Zarówno rolnictwo ekologiczne, jak i tradycyjne, dążą do tego samego celu – wytwarzania produktów żywnościowych. Zgodnie z obowiązującym prawem Unii Europejskiej, wszelka żywność dostępna na rynku, nie tylko ta oznaczona jako organiczna, musi być bezpieczna i zapewniać właściwe wartości odżywcze. Produkty sojowe, takie jak tofu, są podatne na rozwój mikroorganizmów ze względu na ich wysoką zawartość wody i białka, co stwarza zagrożenie dla zdrowia konsumentów. Celem badań było porównanie jakości mikrobiologicznej tofu ekologicznego oraz konwencjonalnego. Badania obejmowały określenie ogólnej liczby drobnoustrojów, liczby grzybów, *Staphylococcus aureus* i *Escherichia coli.*

Wyniki i wnioski: W badanych próbkach tofu nie stwierdzono obecności *E. coli*, natomiast w ponad połowie badanych próbek tofu wykryto występowanie *S. aureus*, przy max. $5.4 \cdot 10^2$ cfu/g. Przeprowadzona analiza statystyczna nie wykazała istotnych statystycznie różnic między pochodzeniem tofu a ogólną liczbą grzybów ($p = 0{,}8293$) oraz *S.aureus* ($p = 0{,}5673$), natomiast ujawniła istotne różnice w ogólnej liczbie drobnoustrojów mezofilnych zanieczyszczających tofu w zależności od ich pochodzenia ($p = 0{,}0191$). W szczególności stwierdzono wyższą liczbę tych drobnoustrojów w próbkach tofu ekologicznego ($7.8 \cdot 10^2$ cfu/g) w porównaniu do konwencjonalnego ($1.7 \cdot 10^2$ cfu/g). Najwyższy poziom grzybów (1.2 ÷ $4,8 \cdot 10^2$ cfu/g) stwierdzono w przypadku 8 % próbek tofu ekologicznego. Uzyskane wyniki podkreślają znaczenie utrzymania odpowiednich standardów higieny podczas produkcji i przechowywania tofu, aby zapewnić bezpieczeństwo żywności dla konsumentów.

Słowa kluczowe: tofu, produkt ekologiczny, produkt konwencjonalny, bezpieczeństwo żywności, zanieczyszczenie mikrobiologiczne