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Comparison of Physicochemical Characteristics and Microbial Quality between Commercially Available Organic and Conventional Japanese Soy Sauces

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Abstract: The article aims to compare the properties and quality of two types of organic Japanese soy sauce from the market, declared by manufacturers as koikuchi, and tamari, both conventional and organic, along with an attempt to determine the differentiating factors using modern statistical methods. The amino acid profile showed the highest proportions were glutamic acid and aspartic acid. Tamari sauces could be distinguished from koikuchi by an elevated content of glutamic acid and alanine, while conventional and organic products differed the most in the shares of arginine, aspartic acid, and serine. The total polyphenol content was higher in conventional soy sauces and better antioxidant properties were found in koikuchi. Organic tamari sauces were characterized by higher antioxidant capacities and total flavonoid content. The volatile profile showed a significant difference between organic and conventional sauces. The research did not confirm that the quality of sauces declared as organic was significantly enhanced, and the overall quality of all tested sauces was high, both in terms of microbiological safety and physicochemical parameters.

Keywords: soy sauce; organic food; volatile chemicals; amino acids content; antioxidant activity

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1. Introduction

Soy sauce is a traditional condiment originating in China. In Japan, the technique of making soy sauce (shoyu) developed based on miso paste [1]. Currently, there are a total of five types of traditional soy sauce according to the Japan Agricultural Standards for the honjozo fermenting method: koikuchi shoyu (made with 50% soybeans and 50% wheat), usukuchi shoyu (lighter in color with milder aroma), saishikomi shoyu (with a full aroma and full-bodied taste), tamari shoyu (a large number of soybeans and 10% or less wheat), and shiro shoyu (made with a very high wheat/soybean ratio) [2,3]. The traditional process of producing Japanese soy sauce—the honjozo fermenting method—consists of five major steps: treatment of raw materials, koji-making process, moromi fermentation and aging, moromi pressing, and refining [1]. The steamed soybeans or defatted soybean flakes and roasted wheat are combined in the proper proportion and inoculated with a pure culture of koji mold (*Aspergillus oryzae* or *Aspergillus sojae*). This mixture is incubated for two to three days at 30 °C and high humidity to allow the koji mold to spread throughout the mixture, and the resulting dry mash is known as koji. In the second step, koji is combined with a 22–25% saline solution and fermented for a few months. The moromi mash is then allowed to mature for some time, during which a range of chemical reactions occurs among the various constituents of the moromi [1]. The final step of soy sauce production is refining, constant pressing, and then pasteurizing using heat.

Soy sauce is widely used in households and food processing, as well as the restaurant, catering, and food service industry as a spice to season a wide range of cuisines, from Asian to fusion dishes and even deserts, and as a flavor enhancer for food. In the food processing market, soy sauce is an ingredient of many food products, such as sauces, marinades, snacks, and RTE (ready-to-eat) meals. According to Cognitive Market Research, in 2024, the global soy sauce market size will reach USD 1352.2 million and European countries account for over 30% of the global market volume. EU markets of soy sauce are expected to grow rapidly in the coming years. Despite the unwavering demand for soy sauce, producers are refining the sauce formulation, expanding the range of products (including soy sauce powder), reducing the sodium content, optimizing the microflora that form the basis of individual stages, developing technologies to shorten the fermentation time, improving the aroma and taste characteristics, or using organic raw materials.

The global organic food market reached nearly USD 215,350 million in 2022, where the largest segment of the organic food market was the organic fruits and vegetables market [4]. In line with the growing market for organic food and the huge interest of consumers, soy sauce producers have also developed organic products, which are already available in European Union countries including Poland. In the European market, recognizing a product as organic means that all stages of its production are carried out in strict accordance with the rules set out in Regulation (EU) 2018/848 of the European Parliament and of the Council of 30 May 2018 on organic production and labeling of organic products. It mainly concerns the ban on the use of chemicals and mineral fertilizers during the growth of soya and wheat, as well as the exclusion of genetically modified organisms (GMOs) in food production. Here, the manufacturer of shoyu sauces must not use genetically modified soy and wheat, or microorganisms at fermentation stages. Some producers declare that they are certified by QAI (Quality Assurance International) and USDA Organic (US Department of Agriculture).

The properties and taste of soy sauces have been widely researched in terms of the region of origin and fermentation method, but there are few publications about the products available on the market. Research on the quality of products available on the market is considered to be not very innovative, and the discussion of the results is often controversial due to the limited amount of information on the details of the production technology. On the other hand, organic food producers encourage consumers to eat organic products, which are perceived as being more nutritious and tastier, and having health-promoting properties. There is therefore a justified need to instrumentally verify measurable differences between products declared as standard and organic.

The article aims to compare the properties and quality of two types of organic Japanese soy sauces from the Polish market, declared as koikuchi and tamari, and conventional and organic, by manufacturers, along with an attempt to determine the differentiating factors using modern statistical methods.

2. Materials and Methods

Japanese koikuchi shoyu and tamari shoyu, both conventional and organic soy sauces, are designated in this publication (KS_C), (TS_C), (KS_O), and (TS_O), respectively. The samples were available in the Polish market (n = 3) and came from different producers (sauce products were randomly chosen from one manufacturer but with different expiration dates, which indicated that they came from different batches of production).

2.1. Physicochemical Characteristic

The density of soy sauce samples was determined at a temperature of 20 °C using a portable densimeter (Densito, Mettler Toledo, Columbus, OH, USA) and the pH value was measured by a pH meter (Metler Toledo, Singapore). The dry weight content was determined using of moisture analyzer (Radwag WPS 30S, Radom, Poland), where 5 cm^3 of each sample was dried at 105 °C until reaching a constant weight, and the dry weight was calculated automatically. The total nitrogen content of the soy sauces was determined by the Kjeldahl method [5]. Soy sauce samples (5 mL) were mineralized with 12 cm^3 of 96% of sulfuric acid and two catalyst tablets (Kjeldahl tablets Missouri, Milton Keynes, UK). Then, the samples were alkalized using 30% NaOH (Poch.S.A., Gliwice, Poland) and the resulting ammonia was determined by distilling into a measured volume of 4% boric acid (Poch S.A., Poland) using a KjeFlex (Büchi, Flawil, Switzerland) apparatus with an automatic titrator, where 0.1 M HCl (Poch S.A., Poland) was used to determine the excess ammonia by titration to pH 4.3. The result is expressed as g $\times$ 100 cm^{-3}.

2.2. The Amino Acid Composition

The content of amino acids (except for tryptophane) was determined using an AAA-500 chromatographic amino acid analyzer (INGOS, Prague, the Czech Republic) according to [6] with changes. The samples of soy sauces were hydrolyzed in 60 mL 6M HCl for 23 h (previously purged with nitrogen) at 110 °C and cooled. Then, the hydrolyzates were filtered through Whatman 3 paper into volumetric flasks (100 mL) and filled with demineralized water. Next, HCl was removed under reduced pressure (35 bar/60 °C). After evaporation, the dry residues were dissolved in a citric buffer of pH 2.6. The samples were analyzed using ion-exchange chromatography with a post-column ninhydrine reaction and LED detector at wavelengths at 440 nm (for proline) and 570 nm (for other amino acids). An analytical column having a length of 250 mm was filled with Poly 8 INGOS cation ion exchange resin (the Czech Republic). Buffers of pH 2.6, 3.0, 4.25, and 7.9 were used. The temperature of the column was 55–74 °C, and the reactor temperature was 121 °C. The determination of the sulfur-containing amino acids, methionine and cysteine, was carried out by means of oxidative hydrolysis. A 2.5 mL mixture of formic acid and hydrogen peroxide (9:1) was added to liquid samples of soy sauce and incubated at 4 °C for 16 h. The reaction was stopped by adding 0.5 mL of concentrated hydrochloric acid. Then, acid hydrolysis was performed with 40 mL of 6 M HCl at a temperature of 125 °C for 23 h. After cooling, the hydrolyzate was filtered through Whatman 3 paper into volumetric flasks (100 mL), which were filled with demineralized water. Evaporation of hydrochloric acid took place at a pressure of 35 mbar in a water bath having a temperature of 50 °C. Then, the dry residues were dissolved in a citric buffer of pH 2.6. This time, the buffers of pH 2.6 and 3.0 were used; the temperature of the column was 58 °C and that of the reactor was 121 °C. The calculations were carried out with reference to external standards using the Clarity program.

2.3. Analysis of Volatile Compounds

Solid-phase microextraction (SPME) coupled to gas chromatography mass spectrometry (GC-MS) were used for volatile compounds determination. Analytical methods used for experiments have been described previously [7]. Briefly, a CombiPal autosampler (CTC Analytics AG, Zwingen, Switzerland) was used to condition/extract samples and to introduce them into a GC instrument. Five grams of soy sauce was placed in a 20 cm^3 glass autosampler vial closed with screw caps sealed with Teflon and conditioned for 10 min at 50 °C. Headspace was extracted for 180 min using SPME fibers coated with 50/30 µm thick Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) films. Before use, the fibers were conditioned for 60 min at 270 °C in the GC injector port. The fibers were desorbed for 10 min at the injector port operated at 260 °C in the split-less mode. An Agilent 5975 C/6890 GC/MS instrument (Santa Clara, CA, USA) was used for analysis. Chromatographic separation was performed on a ZB-WAX fused-silica capillary column 60 m $\times$ 025 mm id $\times$ 0.25 µm film thickness. Helium flowing at a 1.2 cm^3 min^{-1} constant rate was used as the carrier gas. The oven was programmed for 6 min hold at 40 °C, ramped at 4 °C/min to 150 °C, ramped at 20 °C/min to 250 °C, and held for 5 min. Samples were analyzed in triplicate. Urethane was used as an internal standard. The results are expressed as the relative peak area of individual quantified peaks.

2.4. Total Polyphenol Content Determination

The total phenolic content was determined quantitatively using the Folin–Ciocalteu reagent, with gallic acid as the standard [8]. In a well of a 96-well plate, 10 µL of the soy sauces was placed, 40 µL of Folin–Ciocalteu reagent (5 times diluted with distilled water) was added, and after 3 min, 250 µL of 7% sodium carbonate solution was added [9]. The samples were incubated for 60 min at 20 °C, protected from light, and absorbance was measured at a wavelength of 765 nm (Multiskan Sky, Thermo Fisher Scientific, Waltham, MA, USA). The standard curve ($y = 261.67x - 0.3028$, $R^2 = 0.9995$) was prepared based on the measured absorbance values of gallic acid (Merck Life Science, Darmstadt, Germany) at a wavelength of 765 nm. Results are expressed as micrograms of gallic acid equivalents per 1 mL of soy sauce sample (µg GAE mL).

2.5. Total Flavonoid Content

The total content of flavonoids in soy sauces was determined using the aluminum chloride colorimetric method [10]. A quantity of 150 µL of soy sauce was mixed with 2% (*w/w*) $AlCl_3$ (100 µL) in a 96-well microplate and then incubated at 37 °C for 30 min. The absorbance (415 nm) was recorded with a spectrophotometer (Multiskan Sky, Thermo Fischer Scientific, Waltham, MA, USA) in the presence of a blank (a sample without aluminum (III) chloride). A standard curve of a standard (quercetin, Merck Life Science, Germany) was prepared at concentrations of 5–120 µg/mL ($y = 47.765x + 0.4675$, $R^2 = 0.9986$). The results are expressed as micrograms of quercetin equivalents per 1 mL of soy sauce sample (µg QAE mL).

2.6. The Antioxidant Activity

To assess the antioxidant properties of samples, spectrophotometric methods were used, which consisted of determining the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and the cation radical 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS) [11–13].

Reactions were performed in 96-well plates. Soy sauce (10 µL) and radical solution (250 µL) were added to each well, shaken, and measured for the absorbance of DPPH after 30 min at 515 nm and for ABTS after 6 min at 734 nm (Multiskan Sky, Thermo Fisher Scientific, Waltham, MA, USA) versus 80% ethanol. The equivalent antioxidant capacity of the soy sauce was expressed in Trolox equivalent (Merck Life Science, Germany) per mL of soy sauce (mg TE/mL and µmol TE/mL).

2.7. Microbial Quality

The preparation of samples for microbiological analysis followed the procedure described in ISO 6887-1:2003 [14]. To determine the total number of bacteria, the standard plate count agar (PCA; BTL, Warsaw, Poland) was used. The plates were incubated at 30 ± 1 °C for 24–48 h and counted according to ISO 4833-1:2013 [15]. For the determination of the number of fungi, DRBC medium (Biomaxima, Lublin, Poland) and incubation at 25 ± 1 °C for 72 h for yeast counting [16] and for 120 h for mold [17] enumerating was used. *Escherichia coli* was determined using Violet Red Bile-MUG Agar (Sigma-Aldrich, Poznań, Poland) at 37 ± 1 °C for 48 h [18]. The presence of *Salmonella* [19] and coagulase-positive *Staphylococcus* [20] was determined.

2.8. Statistical Analysis

The statistical analyses were performed using ANOVA, where a *p*-value of <0.05 was considered significant (TIBCO Statistica 13.3, Warsaw, Poland). Principal component analysis (PCA) was performed to reduce the dimensionality of the dataset and show the differences in amino acid composition among soy sauce samples, and heat map analysis of the relative content of volatile compounds in different soy sauce samples was performed in the R program (ver. R 4.1.1.).

3. Results and Discussion

3.1. Physicochemical Characteristics of Japanese Sauces

Throughout this publication, we refer to manufacturers' declarations specifying the type of sauce when analyzing and discussing the results. The lowest value of density characterized the sauce TS_O (Table 1). The significantly higher value was observed for TS_C and KS_O, and the highest density was detected for conventional koikuchi sauce. The results of dry weight correspond to high moisture content (over 69% w/v) and are comparable to results of different studies (65% [21] and 60% [22]). The differences can be caused by the boiling time and amounts of protein and starch in soy sauce [23]. However, in our data, there is no correlation between protein content and dry weight of the product.

Table 1. Physicochemical characteristics of conventional and organic Japanese shoyu sauces available on the Polish market.

	KS_C	KS_O	TS_C	TS_O
Density [g × 100 cm^{-3}]	1.1746 ± 0.0033 [a]	1.1685 ± 0.0013 [a]	1.6430 ± 0.0013 [c]	1.5471 ± 0.0005 [b]
Dry weight [%]	29.74 ± 0.08 [a]	31.22 ± 0.05 [b]	29.14 ± 0.06 [a]	29.76 ± 0.11 [a]
pH	4.78 ± 0.04 [b]	4.60 ± 0.01 [a]	4.45 ± 0.02 [a]	4.78 ± 0.05 [b]
Nitrogen [g × 100 cm^{-3}]	1.628 ± 0.016 [a]	1.661 ± 0.066 [a]	1.643 ± 0.014 [a]	2.112 ± 0.059 [b]

Values with different uppercase letters in verses are significantly different using Tukey's test ($p < 0.05$).

The pH values of traditional koikuchi shoyu and tamari shoyu should be 4.7 and 4.8 [24]. The significantly lower pH value of KS_O and TS_C (Table 1) can be explained by the higher content of organic acids as a result of the different microflora and enzymatic activities during the fermentation process of products. Several studies have pointed out that the organic acid contents (lactic acid, formic acid, and citric acid) in handcrafted tamari are higher than in koikuchi shoyu [22]. However, common industrial practice is the addition of vinegar or spirit vinegar to the final product, which corrects pH and enhances the preservation effect.

Koikuchi shoyu (KS) sauces are made with the same quantity of soybeans and wheat, and have a strong aroma and a deep reddish-brown color. Tamari shoyu-style products (TS) are made with a large number of soybeans and 10% or less wheat. The information available on the packaging provided information about the manufacturer, raw material composition, energy, and nutritional value. However, the information about the percentage share of raw materials of soya and wheat is not labeled.

The quality of naturally brewing soy sauces depends on microorganisms, which play a crucial role in koji and moromi fermentation processes. They secrete enzymes hydrolyzing starch, proteins, and lipids, into saccharides, peptides, free amino acids, and volatiles [25]. However, the flavor and color development during the production process is also attributed to non-enzymatic Maillard reactions occurring between reducing sugars and amino acids, and to other reactions such as lipid oxidation, esterification, or degradation of amino acids [26]. Japanese have developed the craft of using mold as a carrier of hydrolytic enzymes, which is significantly different from the use of malt in the European tradition. *A. oryzae* and *A. sojae* are the most popular for use in the koji step; however, *A. sojae* is more recommended. The genome sequencing of *A. oryzae* showed higher α-amylase productivity [27], which results in higher carbohydrate consumption (caused by high α-amylase activity), and consequently can slow the moromi fermentation [1]. *A. sojae* characterizes the higher activity of endopolygalacturonase. The immersion of koji in the saline solution creates a moromi, while enzymatic activities remain to digest the ingredients in the raw materials. This process occurs in parallel with the growth in halophilic microorganisms in the moromi mash [28]. Halophilic lactic acid bacteria (LAB) and yeast play a key role in the moromi stage [25]. The dominant yeast species are Tetragenococcus halophilus, Zygosaccharomyces rouxii, Candida etchellsii, and Candida versatilis [24,29–31]. LAB populations include strains of Lactococcus, Lactobacillus, Leuconostoc, Weissella, and

Streptococcus [31]. However, some authors suggested a variable bacterial composition depends on minor changes in environmental conditions, such as differences in temperature in the koji production room, soybean lipid content, and the species of *Aspergillus* used in the previous step. The growth and activity of lactic acid bacteria rapidly reduces pH. When pH drops below 6.0, the bacterial community begins to decline, and when pH drops below 5.0, lactic acid fermentation ends [30]. This state facilitates the growth of salt-tolerant yeasts, which produce alcohol from the remaining sugars [1].

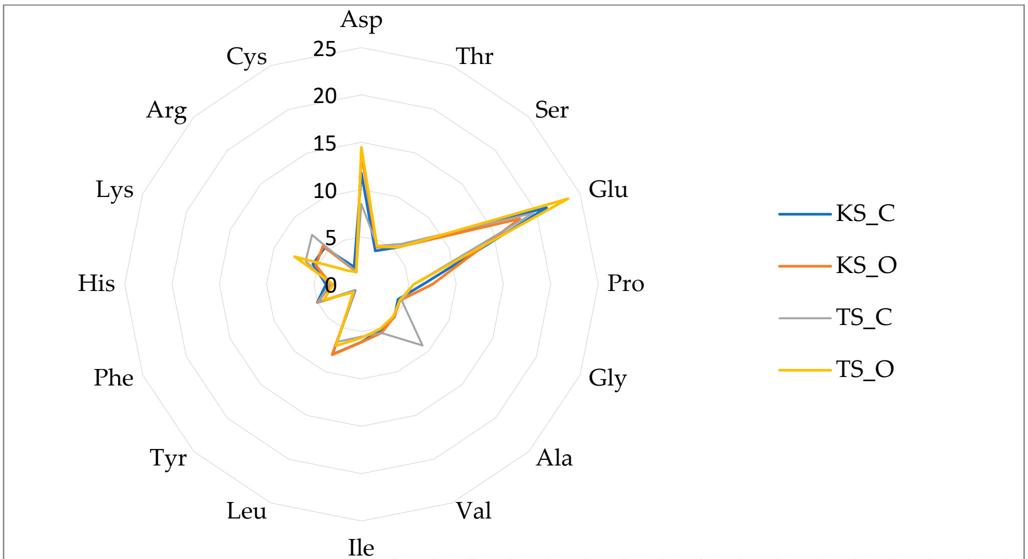
Nitrogen content in soy sauce is one of the most important indicators of the quality of soy sauce, and the higher the content, the better the quality of the final product [32–35] While the differences between the three kinds of soy sauces were not significant, TS_O was recorded as the one that contains the highest content of total nitrogen (2.112 w/v%) (Table 1). According to the requirements set out by Japanese Agricultural Standards for Soy Sauce with total nitrogen criteria, all tested samples of soy sauces can be qualified as Special grade. A study conducted in 2014 showed the protein content in organic soybeans was significantly higher than that of conventional soy [36]. This could suggest that organic soy sauce may contain higher total nitrogen, and hence better quality, in comparison to conventional soy sauce. It would be also in line with the results from another study that characterized and differentiated Chinese conventional and organic soy sauces and indicated an increased concentration of amino acids in the latter type compared to the former type [37]. However, for the tested samples in our studies, only organic tamari shoyu showed a higher content of total nitrogen. This result could be attributed to the reduced salt content in this sample, suggesting the method of low-salt organic tamari shoyu production is based on the elevation of total nitrogen content [24]. The total nitrogen content in soy sauce is composed of simple peptides (nearly 45%) and amino acids (50–75%) [34].

When analyzing and discussing the results in this study, we referred to the manufacturers' declarations specifying the type of sauce. Therefore, we know that differences in quality parameters may result from using different proportions of raw materials or production methods.

3.2. Amino Acid Profile of Shoyu Sauces

Glutamic acid was the dominant amino acid in all tested samples (Figure 1). The highest content of glutamic acid was found in TS_O (over 23%), and the lowest in KS_O (18.09%). The average share of aspartic acid ranged from 8.49% for TS_C to 14.46% for TS_O. Samples of the same sauce were characterized by an average high content of lysine (7.63%), and the lowest content of arginine (2.04%), compared to other sauces (where the range was 5.47–7.37%). The organic koikuchi sauces were distinguished by a higher content of proline (over 7.5%) compared to an average of 5.5% for tamari sauces, and the lowest content of lysine (5.21%). TS_C was characterized by a significantly higher proportion of Ala (9.12%), while the average contents were less than 5% for the rest of the sauces. In all samples of all sauces, the lowest content of tyrosine was observed (approx. 0.93 ÷ 1.21%).

Principal component analysis (PCA) was performed on amino acid content to obtain a small number of factors that accounted for variance in the observed variables. Two principal components (PC1 and PC2) were considered meaningful and together accounted for 95.08% of the total variance in the dataset (Figure 2). Component 1 explained 66.2% of the variance in the dataset and presented meaningful loadings for the variables. It is visible that TS_O and TS_C differed the most in the shares of arginine, aspartic acid, and serine, and KS_O and KS_C were impossible to distinguish according to PC1. Component 2 explained 28.88% of the variance and revealed the differences between KS_O and both TS_O and TS_C, mainly due to proline, arginine, and aspartic acid. Factor scores for PC1 and PC2 were obtained from the statistical output for each of the soy sauce samples and plotted together with the significant variable loadings.



	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ile	Leu	Tyr	Phe	His	Lys	Arg	Cys
KS_C	11.8	3.82	5.54	21.2	6.52	4.19	4.92	5.25	6.12	8.01	0.93	5.03	3.74	5.56	5.47	1.97
KS_O	13.9	4.24	5.6	18.1	7.5	4.43	4.88	5.55	6.12	8.05	1.01	4.99	3.21	5.21	5.74	1.46
TS_C	8.49	4.39	6.03	19.6	5.53	4.65	9.12	5.58	5.53	6.58	0.97	4.93	3.37	6.38	7.37	1.41
TS_O	14.5	4.34	5.52	23.6	5.53	4.4	4.78	5.13	5.65	7.02	1.22	4.29	2.98	7.63	2.05	1.39

Figure 1. Average share (%) of individual amino acids in soy sauces.

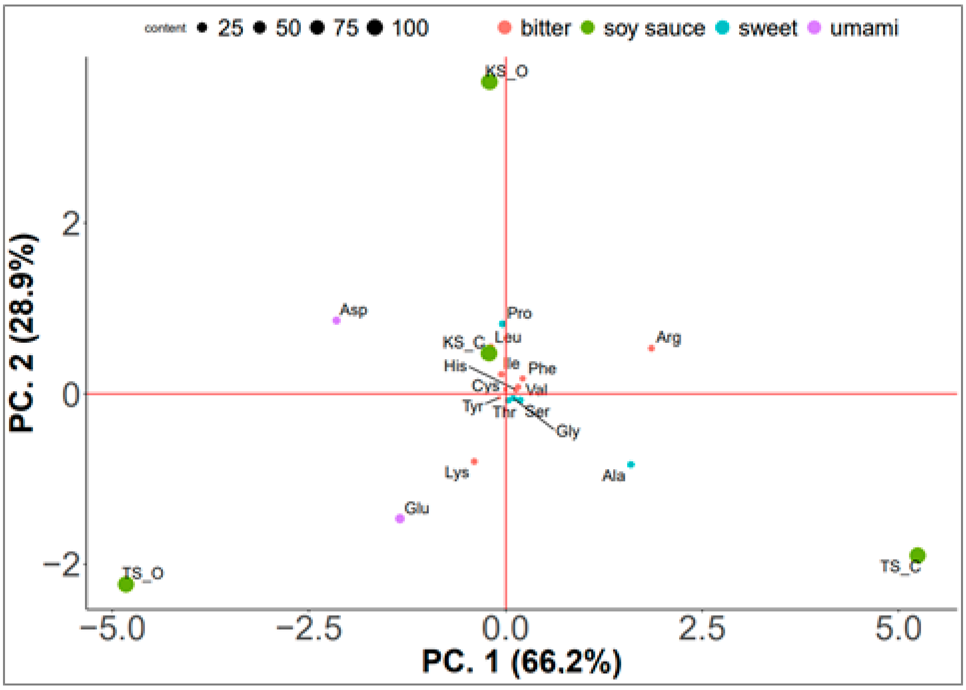


Figure 2. Principal component analysis (PCA) of soy sauces using contents of amino acid and their impact on taste (umami, sweet, bitter). The diameter of the dots is correlated with the av. content of amino acids in all samples.

The flavor of soy sauce is characterized by a distinct strong umami taste, accompanied by complex salty, sweet, and sour flavors [25]. The type and number of amino acids have a strong effect on the taste, and both aspartic and glutamic acids are responsible for the umami taste [38]. Serine, proline, glycine, threonine, and alanine are associated with a sweet taste, and valine, methionine, isoleucine, phenylalanine, lysine, leucine, arginine, histidine, and tyrosine give a rather bitter taste [26,38]. According to this, we mapped the quality characteristic (taste) of individual amino acids and analyzed the principal components once again (Figure 3). The analysis revealed that tamari sauces (both conventional and organic) could be distinguished from koikuchi sauces by variables relating to a richer umami and sweet taste, which was the consequence of the elevated content of glutamic acid and alanine, respectively. However, organic shoyu sauces are characterized by a higher content of umami taste carried by amino acids such as aspartic and glutamic acids. In practice, the consumer might perceive tamari sauces as sweeter and organic sauces as having a more intense umami flavor.

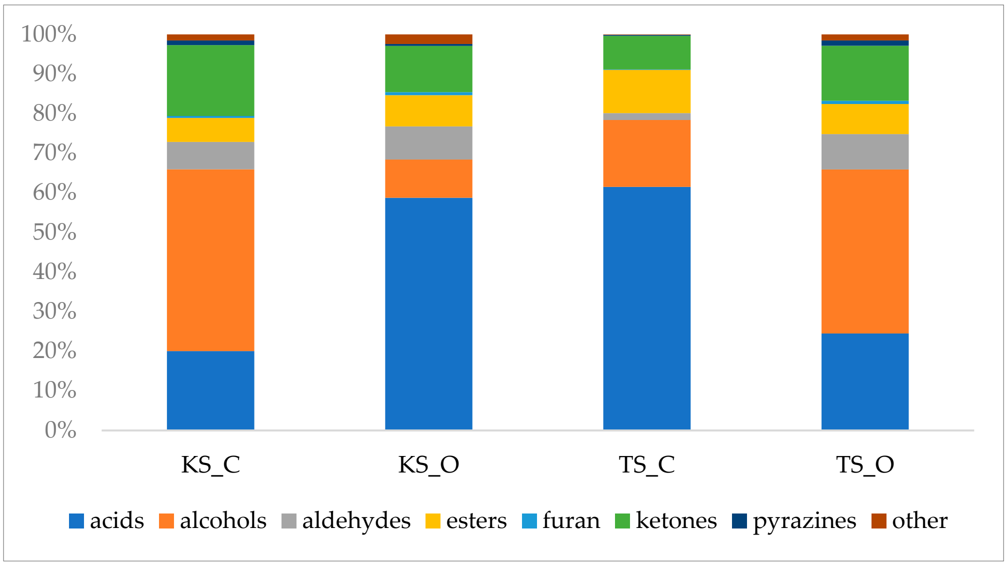


Figure 3. The relative concentration of the different types of aroma compounds in soy sauces (without ethanol).

Apart from amino acids, a large number of compounds have been reported to be linked to taste attributes in soy sauces. The taste of soy sauces is influenced by the presence of peptides, some of which give bitterness and astringency. Acidic peptides and organic acids' presence have an impact on the sour taste. Kokumi peptides are known to act with specific receptors in the mouth, (calcium-sensing receptor—CaSR) [1], leaving a kokumi taste, described as complex, mouthful, long-lasting, and enhancing the intensity of other basic tastes, such as umami. Therefore, the enzymatic activity of microorganisms creates the taste of the product, for instance, through the activity of L-leucine aminopeptidase (koji molds), which reduces the bitterness of soy sauces by removing hydrophobic amino acids from the N-terminus of peptides [39].

3.3. The Antioxidant Activity of Soy Sauces

Polyphenols have been associated with many health-benefiting properties. They act as antioxidants in chronic diseases and are positively related to cardiovascular events, osteoporosis, diabetes, and neurodegenerative diseases [40]. Many studies confirmed that some are associated with bitterness and astringency in many types of food, including soy sauces [41].

The results of the current study (Table 2) showed that higher total polyphenol content (GAE) was found in conventional soy sauces ($p < 0.05$). The total flavonoid content ranged from 32.39 to 46.31 µg quercetin/mL, with higher content observed for TS_O and KS_C. These values were generally lower compared to non-pasteurized soy sauces; for instance, Korean industrially prepared sauce contained 13.3 mg of GAE per ml (original units are converted to SI units here and in the whole paragraph). Nevertheless, the use of heat treatment (pasteurization) reduces the total polyphenol content of the final products. The temperature influenced this parameter; after application at 75 °C, the GAE content was 86% of the initial value, and after 100 °C, it was only 59% [42]. Similarly, several times higher content was observed for the traditional soy sauces fermented with *Monascus*, commercial or lab-cultivated red fungi. The total phenolic content in the products was 800.2 and 885.7 µg gallic acid/mL, respectively [43].

Table 2. The total polyphenol and flavonoid content and the antioxidant capacities of soy sauces.

Soy Sauce	Total Polyphenol Content	Total Flavonoid Content	The Antioxidant Capacities Measured by DPPH		The Antioxidant Capacities Measured by ABTS	
	µg Gallic Acid/mL	µg Quercetin/mL	mg TE/mL	µmol TE/mL	mg TE/mL	µmol TE/mL
KS_C	226.26 ± 7.81 [b]	41.55 ± 2.95 [b]	0.316 ± 0.008 [b]	1.258 ± 0.030 [b]	3.689 ± 0.124 [c]	14.900 ± 0.500 [c]
KS_O	202.43 ± 10.10 [a]	34.02 ± 3.33 [a]	0.284 ± 0.022 [b]	1.129 ± 0.087 [b]	3.162 ± 0.119 [b]	12.771 ± 0.481 [b]
TS_C	225.87 ± 7.65 [b]	32.39 ± 1.81 [a]	0.201 ± 0.033 [a]	0.799 ± 0.131 [a]	2.848 ± 0.119 [a]	11.501 ± 0.481 [a]
TS_O	206.43 ± 2.56 [a]	46.31 ± 3.37 [b]	0.399 ± 0.018 [c]	1.590 ± 0.071 [c]	3.470 ± 0.082 [c]	14.015 ± 0.332 [c]

Each value is the average of three analyses' standard deviation. Values with different uppercase letters in columns are significantly different using Tukey's test ($p < 0.05$).

Soybean as a raw material is the main source of polyphenols and flavonoids in shoyu sauce. The initial content of raw soy seeds depends on many factors. For example, black and brown soya seeds varieties contain a lot of total polyphenols, ranging from 4.94 to 6.22 mg of gallic acid equivalents/g, and can be an effective source of anthocyanins. The yellow seed variety has high total isoflavone content (>3.6 mg/g), whereas the black seeds are rich in total daidzein (>2.0 mg/g) [44]. The fertilizers have a significant effect on the antioxidant activity and phenolic metabolites [45]. Germination and sprout formation are accompanied by a significant increase in total flavonoid content with antioxidant potential [46,47]. Several studies indicated that fermentation processing increases the antioxidative potential of soybean products [48–51]. A high concentration of polyphenols (>5 mg/g) was observed in tempeh-fermented soybean via the *R. oligosporus* NRRL 5905 strain [52]. Moreover, during fermentation, the polyphenol glycosides are typically converted to their aglycone forms, which are significantly readily absorbed from the small intestine [4,53]. Some of the polyphenols found in soy sauce are daidzein (0.20 mg/100 mL), glycitein (0.15 mg/100 mL), 6″-O-acetylgycitin (0.15 mg/100 mL) [54], and genistin (0.75 mg/100 mL) and genistein (0.23 mg/100 mL) [55]. However, daidzein and genistein were not found in soy sauces available at the retail level in Australia and Indonesia [56]. Likewise, in dark soy sauces, daidzein and genistein were not detected [57]. Generally, soy sauce is regarded as a poor source of isoflavones. The leading hypothesis is that during the fermentation, microorganisms can degrade them [56,57]. Prolonged fermentation also leads to additional modifications to the polyphenols, and the presence of 6-hydroxygenistein, 8-hydroxygenistein, and genistein-7-tartaric acid ether [58] has been observed.

When analyzing the antioxidant activity of the current study's sauce samples, a higher antioxidant activity was found in the system with the ABTS radical than with the DPPH radical (Table 2). The highest antioxidant activity was demonstrated for KS_C. The results are expressed as Trolox equivalent antioxidant capacity (TEAC). The lowest antioxidant activity of sauces was approx. 0.799 µmol TE / mL (DPPH) and 11.50 µmol TE/mL (ABTS) for TS_C. Generally, the Trolox equivalent antioxidant capacities of seasoning were low compared to other studies. Dark soy sauce (Singapore brands) values ranged from 47.1 to 147.33 TEAC mM (ABTS) [59], and our results are more similar to Oyster or plum sauces from the same country. According to [48], the soy sauce showed strong DPPH radical-scavenging activity (87.7% scavenging activity).

According to TEAC of commercial seasonings, it was indicated that common additives used in commercial soy sauces, such as fructose syrup, caramel, malt syrup, and monosodium glutamate, show no antioxidant effect or even act as a pro-oxidant. However, the preservative sodium benzoate did not react significantly with $ABTS^{\bullet+}$ and did not lead to an overestimation of the sauces' TEAC [59]. The observation of decreasing antioxidant activity after acidic hydrolysis of dark soy sauces suggests that melanoidins as colored components are formed by Maillard reactions between reducing sugars and free amino acids and the amino groups of peptides can influence that parameter [57]. However, the color of soy sauce was not proportional to its antioxidant capacity in different studies [60].

The current results do not indicate that shoyu sauces commercially available in Poland, both standard and organic, show excellent in vitro antioxidant capacity.

3.4. Aroma Volatile Compounds of Soy Sauces

Volatile compounds in soy sauces were analyzed by SPME-GC-MS. According to their chemical structure, 39 volatile compounds were detected in different samples including 10 acids, 8 alcohols, 6 aldehydes, 5 esters, 5 ketones, and 2 pyrazines, furan, phenol, and sulfur-containing compounds. Their relative concentration is shown in Figure 3.

Among the volatile components, the ethanol content was the highest in all product samples: 3.99 ± 0.27 ^{a} g/kg in KS_C, 6.94 ±0.57 ^{c} in KS_O, 4.61 ± 0.17 ^{b} for organic tamari, and 7.21 ± 1.90 ^{c} for standard tamari sauce. The ethanol content was comparable to the results of koikuchi sauces [61] determined by the same method with an internal standard, where it amounted to 4.08 µg/kg. The presence of ethanol is a characteristic quality marker for natural koikuchi soy sauces as the effect of alcoholic fermentation. A larger share of wheat results in a larger pool of carbohydrates, which are hydrolyzed into saccharides by the enzymatic activity of active microflora and then fermented by yeast into alcohol. Despite tamari sauce being produced with a low proportion of wheat flour, and thus expected to contain less ethanol content, the analyzed samples were characterized with a higher content of ethanol compared to koikuchi.

The relative contents of these volatile compounds in different treatments are analyzed in the heat map in Figure 4; however, ethanol was excluded due to its high concentration. In general, the presence of alcoholic compounds in soy sauces (the effect of yeast and lactic acid bacteria fermentation) results in alcoholic and malty notes. However, 1-octen-3-ol gives a mushroom-like fragrance [62]. The malty effect depends also on the presence of short-chain, branched aldehydes, like 2-methylbutanal and 3-methylbutanal. The relative content of 3-methylbutanal in all soy sauces was low (0.973 ÷ 6.01 mg/kg; Figure 4).

Another volatile compound with a large relative content (Figure 4, red color) was acetic acid (up to 774 mg/kg), which represents a sour taste. It is worth mentioning that producers sometimes add acetic acid for preserving, and then label the proper information. The content of 3-methyl-1-butanol (malty, rancid odor attributes) differs between standard and organic sauces, and significantly so for the koikuchi style [26]. While the general profile of these sauces was similar, tamari sauces differed depending on the origin of the ingredients. For the fruity note, the presence of the ester is necessary, which can additionally mask the unpleasant odors from the metabolism of sulfur compounds (Met) [63]. The presence of ethyl carbamate in all soy sauce samples was observed (4.47 ÷ 7.32 mg/kg). This genotoxic and carcinogenic compound can accumulate in soy sauces during thermal processes [64]. Technologies are being developed to reduce the concentration of ethyl carbamate via optimization of the moromi fermentation step [39], charcoal filtration [65], and the use of low precursor-secreting or high precursor decomposition bacteria [66–68]. The presence of pyrazines may indicate the roasted (2-ethyl-6-methylpyrazine) and burnt (2,3,5–trimethylpyrazine) [61] aromas, and their concentrations were 9.82–17.92 and 3.01 ÷ 11.84 mg/kg, respectively.

Among volatile compounds, [69] suggested that the most important aroma compounds in koikuchi shoyu are 3-methyl butanal, sotolone, 4-HEMF, 2-methyl butanal, methional, ethanol, and ethyl 2-methyl propanoate. Another study [2], which examined the key aroma compounds in different types of Japanese soy sauces, revealed 25 key aroma compounds, in which methional and sotolone appear in all types. In our studies, we did not compare the presence of sotolone or furano(one)s carrying the caramel-like fragrance. A lot of furans (one)s create a caramel-like or sweet note; however, our analysis confirmed the presence of 3-phenylfuran (green bean-like attributes, unlike most aldehydes).

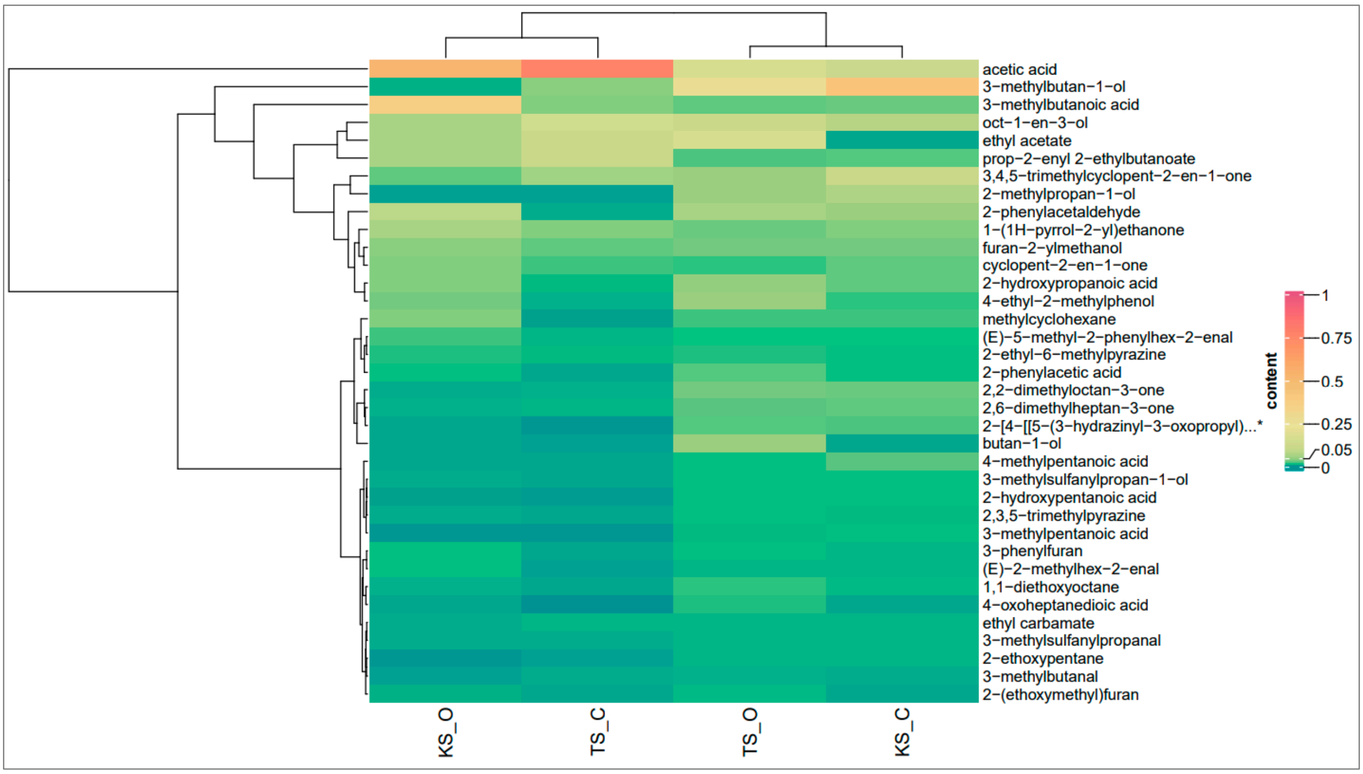


Figure 4. Heat map of relative contents (g/kg) of volatile compounds in Japanese soy sauces (without ethanol) * 2-[4-[[5-(3-hydrazinyl-3-oxopropyl)-1,3,4-thiadiazol-2-yl]amino]-4-oxobutanoyl]butanedioic acid.

Recent years have deepened the knowledge of chemical compounds that determine the taste and aroma of soy sauces and their impact on the taste experience. In 2023, trained panelists tested dozens of samples of Chinese soy sauces. They indicated a spicy, sauce-like aroma and a sour, smoky, and bean aroma in all soy sauce samples. However, the fruity characteristics of the products were rarely noticed [70]. The characteristic volatiles of Chinese low-salt solid-state soy sauce (fermented with a 13–15% brine concentration), and high-salt diluted-state soy sauce fermented with higher brine concentration (18–22%) were different. The main components in the first type of sauces were acids, alcohols, and sulfur-containing compounds, whereas alcohols, acids, and esters dominated in the standard salt products [71].

3.5. The Microbial Quality of Soya Sauces

Our goal in the microbiological analysis was to check the safety criterion and the overall load of the product. *Salmonella*, coagulase-positive staphylococci, and *E. coli* cells were not found in any of the sauce samples. All products were free from fungal microflora. A trace presence of bacteria was found, and tamari-type sauces contained only 10 CFU/mL and organic koikuchi contained 20 CFU/mL. The sauce in which the presence of both bacteria and fungi could not be detected was KS_C. The microbial quality of all soy sauces (including low-salt product) was excellent and only residual bacterial microflora were detected. We did not determine the salt content in soya sauces; however, one of the sample products TS_O characterized a lower salt content according to the manufacturer's declaration. The presence of salt, the pasteurization, and the aseptic packing process mainly contribute to good microbiological quality. It is worth adding that instead of practicing a dilution process after high-salt fermentation, the low-salt fermentation process can be used to reduce the salt concentration. For example, treatment with a high hydrostatic pressure process (HPP) can efficiently eliminate harmful bacteria that could survive during low-salt fermentation [72].

4. Conclusions

In this research, we compared selected parameters that are important for the quality of tamari and koikuchi soy sauces, both organic and conventional. All commercially available products could be classified as Special grade due to their total nitrogen content, and their microbial quality was excellent. Analysis of the amino acid profile showed the highest proportions were of glutamic acid and aspartic acid. Tamari sauces could be distinguished from koikuchi sauces by an elevated content of Glu and Ala, and consequently a richer umami and sweet taste, while conventional and organic sauces differed the most in their shares of arginine, aspartic acid, and serine. The organic koikuchi sauces were distinguished by a higher content of Pro. The total polyphenol content was higher in conventional soy sauces. Organic tamari sauces were characterized by higher antioxidant capacities and total flavonoid content. In the case of koikuchi sauces, better antioxidant properties were observed in conventional samples. All sauces contained ethanol and acetic acid, and the analysis of the heat map of the volatile profiles revealed a significant difference between organic and conventional types of both styles of sauce. The microbial quality and safety of the tested soy sauces should be assessed as high in the light of the conducted research. It was not possible to identify factors that significantly differentiate products of organic and conventional origin from the same type.

The research did not confirm that the quality of sauces declared as organic was significantly better. The overall quality of all tested sauces was high, both in terms of microbiological safety and physicochemical parameters. Therefore, consumer decisions might be the result of individual beliefs that organic foods promote healthier and more sustainable use of natural resources, rather than the advantages of organic products themselves. Future research extended to include the analysis of residue levels of pesticides or other chemicals used during the cultivation of soybeans and wheat would provide a clear basis for assessing the quality of products such as soy sauce.

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