Physicochemical Properties and Antioxidant Potential of Tateishi Kazu Vegetable Soup

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Many industrialized areas of the world demand for the nutraceuticals due to rapidly growing health risks linked with higher consumption of processed foods. Tateishi Kazu vegetable soup or miracle soup is widely consumed around the world because of its nutraceutical properties. In the current research, the Tateishi Kazu vegetable soup was made from both organic and nonorganic sources, such as carrot, burdock root, shiitake mushroom, daikon radish, and radish leaves. We analyzed colour, antioxidant properties, cell viability, and mineral and free amino acid content of both soups. KY/he∗a∗b and pH values revealed no drastic changes in the colour of the organic soup stored for 96 hours. The essential amino acids were present in higher amounts in an organic soup compared to the nonorganic soup. Similarly, the total mineral contents of the organic soup were higher than the nonorganic soup; however, potassium was the major mineral in both soups. Higher phenolic contents with elevated 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and superoxide dismutase (SOD) activity were noticed in organic soup. Moreover, both soups showed considerable reduction in cell viability of HepG2 cells tested through the MTT assay. From the present study, we concluded that the organic Tateishi Kazu vegetable soup can be of great importance to food industry due to the presence of viable nutrients and pharmacological properties.

1. Introduction

Vegetable soups can be served as a great source of nutrients that are required for normal growth, development, acid-base balance, and other health benefits [1, 2]. Vegetable soups or plant extracts contain various essential constituents (minerals, dietary fibers, antioxidants, fatty acids, vitamins, and amino acids) that are vital for physical and mental health of humans [3–7]. In fact, vegetable soup is a staple diet for billions of people around the globe that exists in many traditional varieties and generally consumed for health and nutritional benefits [8–10]. Soup is often served before the meal because of quick nourishment and for improvement in hunger and gastrointestinal responses [11].

Tateishi Kazu vegetable soup is commonly used for health benefits and anticancer properties. It was produced on the principles of the five elements theory in Chinese medicine. The five elements are wood, fire, earth, metal, and water. Dr. Kazu studied and tested over 1500 types of herbal plants to come up with this unique healing vegetable soup. The vegetable comprised of daikon radish leaves (green), daikon radish (white), carrots (red), burdock root (yellow), and shiitake mushroom (black). Previous research studies reported the importance of vegetable soups in terms of reducing risk of major diseases [12]. Likewise, the five
elements of the soup are supposed to treat hypertension, diabetes, and cancer [13].

Cancer is a major life threatening diseases that caused 9.6 million deaths in 2018 all over the world [14]. Certainly, the synthetic drugs and radiotherapy are very expensive and have side effects. The best alternate to the synthetic drugs and radiotherapy is plant extracts/soups. The use of plant species to treat degenerative diseases is not new to men, yet their use has been practiced for thousands of years [15]. Some types of plants extracts or soups can work like medicine against cancer, hypertension, and so on [5, 15, 16]. Vegetable soups contain higher amounts of nutrients and bioactive compounds, such as dietary fibers, vitamins, minerals, and phytochemicals. The vegetable soup also provides considerable quantities of minerals such as calcium, iron, potassium, sodium, and copper [17, 18]. Besides, vegetable soups can serve as a rich source of several essential amino acids, which are frequently required as our bodies cannot synthesize it. The current study was designed to investigate the antioxidant activity, minerals, and amino acid contents of the organic and nonorganic Tateishi Kazu vegetable soups. Furthermore, the efficacy of the organic and nonorganic vegetable soups was planned to test against the viability of HepG2 cells.

2. Materials and Methods

2.1. General Procedure. We purchased organic (vegetable grown without the application of synthetic chemicals) or nonorganic (synthetic chemicals were applied for optimum production) vegetables, i.e. radish, carrot, burdock root, and mushroom from the local market in Daegu, South Korea. The Tateishi Kazu vegetable soup was then prepared from the organic or nonorganic vegetables purchased from the market as per standard recipe. The vegetables (organic and nonorganic) were washed and cut into pieces without peeling. The cut pieces of vegetable were poured into 3 L of water in two separate pans for the production of soups. The contents of the pans were heated for 30 minutes at 100°C. After achieving boiling, the temperature was reduced to 40°C, and the contents of the pans were left to cook for another hour. The flame was then turned off, and the soups were allowed to cool at room temperature. The soups were filtered through net mesh and kept at 4°C before further analysis.

2.2. Assessment of Soup Colour and pH. Both organic and nonorganic soups (45 mL each) were analyzed at various time points (i.e., 0, 24, 48, 72, and 96 h) for fluctuation in colour and pH. After 0 h reading, the soups were stored at 4°C till 96 h. For colour measurement of organic and nonorganic vegetable soups, a chroma meter (CR-300, Minolta Corp., Osaka, Japan) was used. For pH measurement, the PHS-3BW pH meter was used following the protocol of Cho et al. [19]. The electrode of the pH meter was carefully washed and dried prior to dipping in each standard and soup.

2.3. Antioxidant Assays. The total phenolics of the vegetable soup were analyzed according to the method of Singleton et al. [20]. Sample (0.5 mL) was mixed with 0.2 M of Folin–Ciocalteu reagent (2.5 mL), and the mixture was allowed to stand for 4 minutes. Sodium carbonate solution (2 mL of 75%) was then added to the mixture, and the absorbance was measured by a spectrophotometer at 750 nm using a spectrophotometer. Gallic acid was used as a standard to construct a standard curve.

For DPPH scavenging activity, the method of Blois [21] was used with some modifications. Sample (3.0 mL) was mixed with 1.0 mL of methanolic DPPH (0.1 mM) solution. The mixture was allowed to stand for 30 minutes at room temperature. The absorbance was finally measured at 517 nm. Ascorbic acid was used as a standard to construct a standard curve.

For ABTS activity of organic and nonorganic vegetable soups, the method of Bilal et al. [22] was adopted. Sample (10 μL) was mixed with 190 μL of ABTS solution, and the mixture was incubated at 25°C in a dark. The absorbance was measured by a spectrophotometer at 735 nm. Methanol was used as a blank and ascorbic acid as a positive control. The following equation was used to calculate the ABTS radical scavenging activity.

\[
\text{ABTS activity (\%)} = \left[ 1 - \left( \frac{\text{sample absorbance} - \text{control absorbance}}{\text{control absorbance}} \right) \right] \times 100.
\]

2.4. Analysis of Mineral Contents in Vegetable Soup. Mineral contents of the organic and nonorganic vegetable soups were measured according to the method of Skujins [24] using inductively coupled plasma atomic emission spectroscopy (ICP AES).

2.5. Amino Acid Analysis. Amino acid contents (threonine (Thr), alanine (Ala), methionine (Met), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), histidine (His), lysine (Lys), tyrosine (Tyr), arginine (Arg), proline (Pro), aspartic acid (Asp), serine (Ser), glutamic acid (Glu), glycine (Gly), and valine (Val)) of both organic and nonorganic vegetable...
2.6. Determination of Cytotoxicity by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) Assay. To determine the antitumor activity of our vegetable soups, an MTT assay was carried out by following the method of Rehman et al. [16]. The MTT assay (an index of cell viability) is based on the principle that viable cells convert MTT into an insoluble formazan salt. To evaluate the efficacy of our vegetable soups, we performed a series of soup dose-response assays. HepG2 cells were randomly assigned to the control (untreated group), organic vegetable soup, and nonorganic vegetable soup groups at various concentrations (1, 2, 3, and 4 ?L). We used 96-well plates for seeding the cells (5000 cells/well) in 100 ?L RPMI (Roswell Park Memorial Institute 1640) medium. After seeding the cells, the 96-well plates were left overnight to let the cells adhere the surface. Various fractions of organic or nonorganic soup extracts were then added to individual well, and the plate was incubated. Following the incubation at 37°C for 24 h, each well was supplemented with 10 ?L of MTT reagent, and the mixture was incubated for another 6 h. A 100 ?L of dimethyl sulfoxide (DMSO) was then incremented for the stabilization of MTT crystals. The plates were again incubated overnight at 37°C. The inhibition percentage was calculated using the following formula [25].

\[
\text{Cell viability (\%)} = \frac{\text{The absorbance of the experimental group}}{\text{The absorbance of the blank untreated group}} \times 100.
\] (3)

2.7. Statistical Analysis. The data generated from the current study were subjected to statistical analysis. The differences between the two treatments were computed by Student’s t-test at \( p = 0.05 \). The significant means from various treatments represented by different letters were compared by Duncan’s multiple range test (DMRT) at \( p = 0.05 \) using SAS (Statistical Analysis System) version 9.3 statistical software program. For graphical presentation of results, GraphPad Prism-6 was used. All experiments were carried out in triplicates.

3. Results and Discussion

3.1. Colour and pH of Soups during Storage. Table 1 provides the \( L \times a \times b \) values of the organic and nonorganic vegetable soups. The \( L \) (lightness) value of nonorganic vegetable soup was high (60.9) at day first, but went down during the storage (53.2). The \( L \) value of organic vegetable soup was also high (54.3) at day first that fluctuated during the storage period. However, the changes were nonsignificant at \( p = 0.05 \). The \( a \) value (degree of redness) for organic soup at day first was 0.61, which was significantly \( (p = 0.05) \) higher than the \( a \) value for nonorganic vegetable soup (0.3). The \( a \) value of both kinds of soups was significantly \( (p = 0.05) \) decreased after day one of the storage. Though the \( a \) value of organic and nonorganic vegetable soups stayed unchanged for the rest of storage, but the \( a \) value of organic vegetable soups was higher (0.30) than that of nonorganic vegetable soup (0.08). On the contrary, the \( b \) value or yellowness of the soup was significantly \( (p = 0.05) \) increased after 24 h of storage in both organic and nonorganic vegetable soups. The \( b \) value of organic and nonorganic vegetable soups increased nonsignificantly after 24 h–96 h of storage time. Moreover, the \( b \) value of organic vegetable soup was significantly \( (p = 0.05) \) lower (3.09) as compared to the \( b \) value of nonorganic vegetable soup (4.73) after 96 h of storage (Table 1). Colour measurement has been used as an alternative measure of food quality such as flavor, aroma, nutritional, sensory, and other pigments because it is simpler, faster, and correlates well when compared with other physicochemical properties [11, 26]. Change in colour can be used to evaluate the shelf life of vegetable during storage [27]. Visual appearance has a strong influence on consumer decision on buying the food products [26]. In the present study, we found that the \( a \) value (degree of redness) of the organic vegetable soup after storage for 96 h was high, where the \( b \) value or yellowness was significantly \( (p = 0.05) \) low as compared to the nonorganic vegetable soup. This means that the appearance of the organic vegetable soup after storage was significantly \( (p = 0.05) \) higher than the nonorganic vegetable soup. So better the colour of the soup, greater will be the chances to attract the consumers.

Inadequate storage conditions may negatively affect the quality of food. The pH of nonorganic soup extract was slightly higher than organic soup. However, the overall changes in pH were nonsignificant \( (p = 0.05) \) during the storage duration (Figure 1). Kilinc [28] and Öztürk et al. [29] observed that the value of pH fluctuated at different periods of storage time. Certainly, the pH depends on the type of soup and its composition [30, 31]. In our case, the pH of both organic and nonorganic vegetable soups remained stable during the 96 h of storage time that might be due to the low production of organic acids in vegetable soup during the storage.

3.2. ABTS Activity. The ABTS scavenging activity has extensively been used for antioxidant activity of food extract containing hydrophilic compounds [32]. This assay is based on the determination of the discoloration in ABTS assay solution [33]. In the current study, the ABTS radical scavenging activity of the fresh organic vegetable soup was significantly \( (p = 0.05) \) higher than that of nonorganic vegetable soup (Figure 2(a)). More interestingly, a gradual increase in ABTS radical scavenging activity was observed during the 48 h of the storage in both soups. After 96 hours of storage, the ABTS value of the organic vegetable soup reached 28.4% and nonorganic vegetable soup reached 32.78% that were significantly higher than the fresh vegetable soups of both kinds (Figure 2(a)). Similarly, higher ABTS activity was recorded in organic beetroot cultivars.
**Table 1**: Colour measurement of both organic and nonorganic vegetable soups at 0, 24, 48, 72, and 96 hours of storage.

| Colour value | $L$       | $A$       | $B$       |
|--------------|-----------|-----------|-----------|
| 0 h          | 54.31±2.4 | 0.60±0.04 | 2.04±0.54 |
| 24 h         | 53.89±3.23| 0.26±0.06 | 2.46±0.61 |
| 48 h         | 55.05±2.13| 0.32±0.17 | 2.31±0.74 |
| 72 h         | 53.99±3.20| 0.21±0.03 | 2.65±0.36 |
| 96 h         | 55.07±2.10| 0.30±0.03 | 3.09±0.43 |

**Nonorganic vegetable**

| Colour value | $L$       | $A$       | $B$       |
|--------------|-----------|-----------|-----------|
| 0 h          | 60.91±1.62| 0.30±0.28 | 0.99±0.10 |
| 24 h         | 53.24±2.19| 0.09±0.04 | 4.14±1.21 |
| 48 h         | 54.65±2.07| 0.09±0.05 | 4.13±2.04 |
| 72 h         | 53.62±2.08| 0.07±0.02 | 4.34±0.92 |
| 96 h         | 54.49±2.17| 0.08±0.03 | 4.73±0.73 |

Each data point is the mean of three replicates followed by ± standard error. The data presented with different letters as superscript (i.e., a, b, and c) are significantly different from their respective data points as analyzed by Duncan’s multiple range test (DMRT) at $p = 0.05$.

**Figure 1**: pH value of both organic and nonorganic vegetable soups at 0h, 24h, 48h, 72h, and 96h. Each data point is the mean of triplicated data with ±SE. The bars are not significantly different from each other at $p = 0.05$ as evaluated by DMRT.

**Figure 2**: DPPH and ABTS activities of organic and nonorganic vegetable soups at 0h, 24h, 48h, 72h, and 96h. (a) Change in ABTS radical-scavenging activities. (b) Change in DPPH radical scavenging activity. Each data point is the mean of triplicated data with ±SE. The bars represented with different letters are significantly different from each other at $p = 0.05$ as evaluated by DMRT.
Both organic and nonorganic vegetable soups contain moderate to high levels of amino acids (Table 2). Among the amino acids, the amounts of Thr, Ala, Ile, Phe, Arg, Pro, Asp, Ser, Glu, and Val were the most abundant amino acids in both organic and nonorganic vegetable soups. According to Bilal et al. [22], Val is essential for muscle development and

3.3. DPPH Radical Scavenging Activity. The DPPH radical scavenging assay is one of the most widely used and efficient methods for assessing the antioxidant potential of plant extracts [23]. This free radical is reduced in the presence of antioxidant and form a colourless solution [36]. Our results showed a significantly \((p = 0.05)\) higher DPPH activity was observed in organic vegetable soup as compared to the nonorganic vegetable soup at all the tested time points (Figure 2(b)). Furthermore, the significant \((p = 0.05)\) increase in DPPH activity was recorded at 48 h of storage in both soups, but even at this point, the DPPH activity of organic soup was significantly \((p = 0.05)\) higher in organic vegetable soup (80.95%) as compared to the nonorganic vegetable soup (58.86%). However, a gradual decrease in DPPH activity was recorded in both kinds of soups after 72 h and 96 h of storage (Figure 2(b)). 

3.4. Superoxide Dismutase (SOD) Activity. The superoxide dismutase, one of the bioantioxidant enzymes, plays an important role in maintaining superoxide radical and removing active oxygen that causes oxidative damage to lipid and proteins [22, 43]. Our results showed that SOD activity was higher in the nonorganic vegetable extract at 0 h (103.6 U/ml) and 96 h (118 U/ml), while least at 24 h (84.9 U/ml). In case of nonorganic vegetable soup, a similar trend was observed in SOD activity as in organic vegetable soup but a gradual increase on preceding days (Figure 3). SOD is an essential antioxidant enzyme that has the ability to protect cells from the reactive oxygen species (ROS) by converting them into molecular oxygen and hydrogen peroxide [44].

3.5. Total Phenolics. Total phenolic compounds are important secondary metabolites that have beneficial biological activities [43, 45]. Phenolic compounds play a vital role in food industries for improving food quality due to lipid peroxidation ability [43, 45]. The current study showed that the total phenolic content of our vegetable soups was the highest in nonorganic soup (3.02 mg GAE/g) at 24 h, while in organic vegetable soup (3.58 mg GAE/g) at 48 h. Substantial variation in total phenolic content of both organic and nonorganic vegetable soups were observed at 48, 72, and 96 h, while the lowest total phenolic content was observed at 0 h for both vegetable soups (Figure 4). Higher phenolic content was considered beneficial for human health [22] and is known for a variety of physiological activities such as anticancer, antioxidant behavior, and cholesterol degradation [43]. Higher phenolic content in both organic and nonorganic vegetable soups indicated that this soup could be a good source of phenolic compound and could be used in food and nutraceutical industries [22].

3.6. Amino Acid Contents. The results in Table 2 provide the amounts of free amino acids in organic and nonorganic vegetable soups. The total amino acids (TAA) of organic and nonorganic vegetable soups were 294.7 µg/mL and 294.6 µg/mL. Similarly, the essential amino acid (EAA) of organic and nonorganic vegetable soups was 133.1 and 130.7 µg/mL, whereas the nonessential amino acids were 161.6 and 163.9 µg/mL, respectively (Table 2). Both organic (45.2%) and nonorganic (44.3%) vegetable soup samples showed optimum amounts of EAA. The WHO and FAO recommend food that has the best protein category must have an EAA/TAA ratio above 40% [46, 47]. In our current study, both the organic and nonorganic vegetable soups showed amino acid values higher than the set limits of the WHO and FAO, which suggests that the soups are good sources of free amino acids.

Both organic and nonorganic vegetable soups contain moderate to high level of amino acids (Table 2). Among the amino acids, the amounts of Thr, Ala, Ile, Phe, Arg, Pro, Asp, Ser, Glu, and Val were the most abundant amino acids in both organic and nonorganic vegetable soups. According to Bilal et al. [22], Val is essential for muscle development and
central nervous system stimulant, while Thr plays an important role in the formation of glycine and serine. Leucine-rich α-2-glycoprotein 1 (LRG1) and small leucine-rich repeat proteoglycans helps in wound healing in the body [48, 49]. Among all free amino acids, Asp, Glu, and Ala were the three distinct amino acids in both organic and nonorganic vegetable soups that can play beneficial roles in human health. Layman and Walker [50] reported that Ala plays a vital role in the human body by maintaining balanced amounts of glucose and nitrogen via glucose-alanine cycle. Moreover, higher amounts of Asp in the soups can serve as a precursor for the synthesis of various important amino acids [51]. Besides, Glu are known for enhancing food taste and plays an important role in the brain metabolism and various neurological disorders [52, 53]. We also observed the highest level of Pro content in both organic (81.2 µg/mL) and nonorganic (83.2 µg/mL) vegetable soups (Table 2). Pro is a vital amino acid for human, livestock, and fish that has an important role in protein synthesis, immune responses, antioxidant reaction, and wound healing [54].

3.7. Mineral Contents. Minerals are vital in human nutrition for overall physical and mental health as well as for the maintenance of acid-base balance [55]. The organic vegetable soup had significantly (p = 0.05) higher amounts of K, Na, P, S, and Fe as compared to the nonorganic vegetable soup, whereas significantly (p = 0.05) higher amounts of Ca and Mg was recorded in nonorganic vegetable soup (Figure 5). Both vegetable soups (organic and nonorganic) have almost the same amounts of Cu. It was also observed that K was the most abundant mineral in both organic (441.3 mg/kg) and nonorganic (293.3 mg/kg) vegetable soups. K is very important mineral as it helps to regulate fluid balance, muscle contractions, and nerve signals in the body [56], while Na helps to maintain stable blood pressure levels [57]. P is important for the formation of bones and teeth [58]. Mg can decrease the risk of colorectal cancer and inhibit carcinogenesis [59].

3.8. HepG2 Cancer Cell Viability Test. Medicinal plants are used for curing various ailments since ancient times [60]. The use of nature provides rich sources of pharmaceutical compounds, including anticancer metabolites [61]. In recent years, most of the scientists are involved in the discovery of natural anticancer compounds and their mode of action [16]. Thus, in the present research, both the organic and nonorganic vegetable soups were tested against HepG2 cancer cell line through MTT bioassay. Our results showed that the organic vegetable soup exhibited a significant (p = 0.05) reduction in HepG2 cell viability at 2, 3, and 4 µL

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Table 2: Analysis of free amino acid composition of both organic and nonorganic vegetable soups.

| Essential amino acids (EAA)       | Organic     | Nonorganic |
|-----------------------------------|-------------|------------|
| Threonine                         | 19.1 ± 1.5  | 18.8 ± 1.4 |
| Alanine                           | 32.7 ± 3.3  | 32.7 ± 3.4 |
| Methionine                        | 1.6 ± 0.4   | 1.9 ± 0.02 |
| Isoleucine                        | 18.6 ± 1.5  | 18.6 ± 1.5 |
| Leucine                           | 9.1 ± 1.2   | 8.9 ± 1.14 |
| Phenylalanine                     | 19.5 ± 1.3  | 19.1 ± 1.8 |
| Histidine                         | 7.3 ± 0.4   | 6.9 ± 0.2  |
| Lysine                            | 5.7 ± 0.1   | 5.8 ± 0.1  |
| Arginine                          | 22.6 ± 1.5  | 21.09 ± 1.9|
| Total EAA                         | 133.1       | 130.7      |

| Nonessential amino acids (NEAA)   | Organic     | Nonorganic |
|-----------------------------------|-------------|------------|
| Tyrosine                          | 7.1 ± 0.3   | 6.9 ± 0.2  |
| Proline                           | 81.2 ± 4.8  | 83.2 ± 4.6 |
| Aspartic acid                     | 21.3 ± 1.8  | 21.5 ± 2.5 |
| Serine                            | 19.7 ± 1.8  | 19.8 ± 1.9 |
| Glutamic acid                     | 29.5 ± 2.8  | 29.6 ± 2.4 |
| Glycine                           | 2.8 ± 0.12  | 2.9 ± 0.14 |
| Valine                            | 25.6 ± 2.5  | 25.6 ± 2.4 |
| Total NEAA                         | 161.6       | 163.9      |
| Total free amino acid             | 294.7       | 294.6      |

Each data point is the mean of three replicates followed by ±SE. The data were nonsignificant as compared by the t-test at p = 0.05.
Figure 5: Mineral content of both organic and nonorganic vegetable soups at 0 h, 24 h, 48 h, 72 h, and 96 h. Each data point is the mean of triplicated data with ±SE. The bars represented with “**, ***”, or “****” are significantly different at $p = 0.05$, $p = 0.01$, and $p = 0.001$, respectively. The bars represented with “ns” are nonsignificant at $p = 0.05$. 
as compared to the nonorganic vegetable soup. Furthermore, the organic vegetable soup showed maximum reduction in cell viability of the HepG2 cancer cell line at a concentration of 3 µL as compared to the nonorganic vegetable soup and control (0 µL) (Figure 6).

4. Conclusion

The results revealed that the organic Tateishi Kazu vegetable soup had maintained a good aesthetic value during the storage. Moreover, both organic and nonorganic Tateishi Kazu vegetable soups were rich in antioxidants, essential minerals, and amino acids, but in most cases, organic soup performed better than the nonorganic soup. Also, the organic Tateishi Kazu vegetable soup showed significant reduction in the cell viability of HepG2 cancer cells through MTT assay. From the results, it can be concluded that the organic Tateishi Kazu vegetable soup can be of a higher commercial value because of its aesthetic value, high phytochemical contents, and possible health benefits.

Data Availability

All data generated or analyzed during this study are included within this article.

Consent

All authors have provided their consent to publish this study.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors’ Contributions

K, MAK, and YSP conducted the experiments and antioxidant analysis; AI and K analyzed the data; AI, IDK, and MH helped in writing the manuscript; DHS designed, supervised, and financed the research and finalized the manuscript.

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