Agricultural Sciences Journal
Available online at http://asj.mnsuam.edu.pk/index.php
ISSN 2707-9716 Print
ISSN 2707-9724 Online
https://doi.org/10.56520/asj.v7i2.507

Research Article

ENHANCING NUTRITIONAL PROFILE OF SOYBEAN FERMENTED PRODUCT TEMPEH BY INCORPORATING BARLEY

Ali Hamza[1]*, Muhammad Shahbaz[1], Shamas Murtaza[1], Umar Farooq[1]

[1]*Department of Food Science and Technology, MNS-University of Agriculture Multan, Pakistan.*
**Corresponding Authors: alihamza6560@gmail.com*

Abstract

Fermented foods are gaining importance among a large group of populations worldwide. Tempeh is a fermented soy-based product. Boiled soybeans are subjected to aerobic fermentation to obtain tempeh. It can be used as an alternative source of proteins for vegetarians, owing to high amounts of protein, as well as valuable prebiotics. The objective of the current study was the development of fermented soybean product combined with barley and determined nutritional profile as well as the storage condition of the product. The proximate composition of soybean indicated that it contained 10.6±0.58%, 35.00±0.58%, 3.8±1.15%, 7.2±0.58% and 10±0.58% of moisture, crude protein, crude fat, ash and crude fiber, respectively. The proximate composition of barley showed 9.6±0.58%, 2.52±0.58%, 2.64±0.58%, 13.8±1.45% and 1.4±0.58% of moisture, crude fat, crude fiber, crude protein and ash, respectively. Fermentation of soybeans with barley with different ratio (10%, 20% & 30%) was carried out to develop the product (tempeh) by using the *Rhizopus oligosporus*. Some proximate analysis and sensory evaluation were done at an interval of 7 days for 14 days. Results showed that the crude dietary fiber increased as the concentration of incorporated barley enhanced. Product T3 has a higher content of fibers that are beneficial for probiotics in the gut. There was a decline in protein concentration due to the replacement of soybeans with barley. The antioxidant activity of soybeans was much better than incorporated products. Sensory evaluation showed that the texture was good for all incorporated product, but the color was not much appealing as the standard product. In the context of nutritional value, T3 was best having the higher value of dietary fiber which serve as prebiotics.

Keywords: *Prebiotics, Soy-based product, dietary fiber, gut health.*

(Received: 10-June-2025 Accepted: 22-Aug-2025) Cite as: Hamza. A., S. Muhammad., Murtaza. S., Farooq. U. 2025. Enhancing nutritional profile of soybean fermented product tempeh by incorporating barley. Agric. Sci. J. 7(2): 19-31

1. INTRODUCTION

Food that contains a sufficient amount of biologically active microbes as well as meeting the basic nutrition values and show positive effects on consumer health, are known as fermented foods. Food despite the provision of energy, also helps in body structure forming. Interest and research on fermented foods is increasing due to health benefits (Kalui *et al*., 2010). Fermented foods are a good source of basic nutrients as well as having an adequate amount of biologically active components Achi and Ukwuru, (2015). These foods enhance beneficial organisms and prevent diseases, also known as medicinal foods. Main reason behind the production of functional foods is to maximize the number of probiotics and prebiotics for better gut health (Nyanzi and Jooste, 2012). Fermented foods act as antioxidant, anti-hypertensive, bioactive, anti-diabetic and FODMAP-reducing components formed after fermentation. Antioxidant activity of milk, fruits, vegetables, cereals, meat and fish increase during the fermentation process. Fermentation of milk and cereal produced

anti-hypertensive peptides. There is also an increase in vitamin content in fermented cereal and milk (Frias *et al*., 2016). After cereal fermentation, products contain bioactive ingredients just like functional and dietary fibers. Various variable factors affect the fermentation of cereals that can be eliminated by different methods for obtaining the standard product. These factors involve fermentation time, storage duration, pH and temperature of fermentation, grain's moisture, extent for grain size reduction, alcohol production level, variety of cereal, required growth factor, cereal nutrient, sources of enzymes, starter quality and material for fermenting substrate (Blandino *et al*., 2003).

Soybean (*Glycine max*.) a legume, local in East Asia, is mostly grown for production of edible beans because of their several beneficial characteristics (Bedani *et al*., 2015). Plants that are used as a source of protein, among them isolated protein from Soy, are considered "Complete protein". Protein digestibility corrected amino-acid score (PDCAAS) is 1.00 in soybean (Hughes *et al*., 2011). Soybeans can be fermented to produce traditional foods, known as tempeh, miso and natto. Iso-flavones present in soybean improve bone health. Soybean seeds are rich sources of oil, minerals like calcium and iron but carbohydrate is low. Lysine content is maximum in soybean seeds. Soybeans can be converted into other value-added products to fulfill human dietary requirements in developing countries. Soybean is considered as the perfect substitute to utilize protein for preventing malnourishment (Kumar *et al*., 2007). Moreover, chemical constituents of soybean prevent risk of diseases like cancer, as it contains sufficient amount of antioxidants (Nill, 2016).

Barley grains have approximately 65-68% starch, 4-9% B-glucan, 1.5-2.5% minerals, 10-17% protein and 2-3% lipids (Izydorczyk *et al*., 2001). Use of barley according to product formation breakfast cereals, soups, noodle, stews and pasta, for substitution of coffee and especially in baked products (bread) (Noaman, 2017). Due to the nutritional value and physiological benefits of barley, consumers are emerging rapidly. Barley is an excellent source of dietary fiber, which makes this cereal an important and health beneficial food component for humans Arend and Zannini, (2013). Barley is used for many fermented products just like the process of malting (Rimsten, 2003). Barley is helpful in treatment of diseases due to its functional ingredients and their molecular mechanisms. B-glucans, arabinoxylan, phytosterols, polyphenols, tocols, and starch are functional ingredients of barley grain (Shimizu *et al*., 2008). It is rich in dietary fiber and bioactive components like enzymes, vitamins and phenolic contents. Levels of B-glucan is maximum (9%) that is easily solubilize polysaccharides (Fastnaught, 2001).

Tempeh is a fermented product produced from soybean and excessively consumed by humans because of its remarkable health benefits Nout and Kiers, (2005). Tempeh is prepared by fermentation of soybeans using the fungal strain Rhizopus oligosporus. Tempeh is basically Indonesian traditional food, considered as healthy food because it prevents disease e.g. chronic diseases (Bavia *et al*., 2012). Tempeh can be prepared with varieties of cereals and beans by fermentation. Starter culture plays a vital role in tempeh preparation by converting cooked soybeans into mycelial-knitted product, e.g. compact cake (Feng, 2006). Tempeh is mostly a good food alternative for vegetarians because of its nutritional value (Ahnan *et al*., 2021). Tempeh can be served in various forms i.e. roasted, fried, grilled and baked and its flavor can be changed by marinating it with different ingredients like garlic, ginger etc. After Soybean, tempeh is a

rich source of protein even though it can be used as meat alternative, addition of barley was to enhance the fiber content of the product. The current research work was planned for detailed investigation on; production of fermented soybean-barley products and to evaluate the nutritional quality of value-added products (tempeh).

2. MATERIALS AND METHODS

This research was completed in the laboratories of the Department of Food Science and Technology and Central Lab System of MNSUAM. The details of materials and methods are as follows:

Procurement of raw material

Soybeans and barley grains were purchased from the local grain market of Multan. All the required chemicals, reagents and materials were obtained from the Department of Food Science and Technology and Central Lab System of Muhammad Nawaz Shareef University of Agriculture Multan. Fungal strain (*Rhizopus oligosporus*) was used for fermentation and purchased from ebay store. The purchased raw material was cleaned, washed and stored at room temperature till further use.

Proximate analysis of raw material

The proximate analysis (crude fat, crude fiber, crude protein, moisture, crude ash) of soybean and barley was determined according to the methods of AACC (2000).

Treatment plan for product development

Treatment	Soybean %	Barley %
T_0	100	--
T_1	90	10
T_2	80	20
T_3	70	30

Fermentation

The standard fermentation procedure was followed for the fermentation of hulled soybean and dehulled barley for product formation and checking the nutritional changes. The soybean and barley sample were inoculated with 2-gram cultures of *Rhizopus oligosporus* and incubated at a temperature of 35±2ºC. The product was incubated for 2 days to produce the mass compact form product (tempeh).

Physicochemical analysis of product

Moisture analysis

The percentage of moisture content in tempeh samples was estimated by using oven drying method no. 44-15, according to AACC (2000). The sample was run in triplicate as R1, R2, and R3. Moisture percentage was analyzed by taking 5-gram sample in each china dish. The temperature of hot air oven was set at 105 °C and was placed for 24 hours. Dried samples were placed in the desiccator for cooling to avoid re-absorbance of moisture from the atmosphere. After cooling, the china dishes were removed from the desiccator and placed on a weighing balance for weighing the sample. Repeat the procedure again. The process was sustained till constant weight. Then calculate the moisture percentage.

Crude fat

Soxhlet apparatus was used to determine the crude fat according to the method no. 30-25.01 of AACC (2000). Weigh the sample and place it into the thimble. N-Hexane was used as a solvent to extract the fat content. After completing the 6 cycles remove the thimble and place it into the hot air oven. Weigh the sample again and calculate the fat content.

Crude fiber

Crude fat was determined according to the method no. 32-10 given in AACC (2000). Moisture free sample (3g) was digested with H_2SO_4 and filter then risen with water and digest again with NaOH. Repeat the filtration process and place the filtrate into the hot air oven at 110°C. Weigh the sample and place it into the muffle furnace for ignition. Calculate the fiber percentage according to formula.

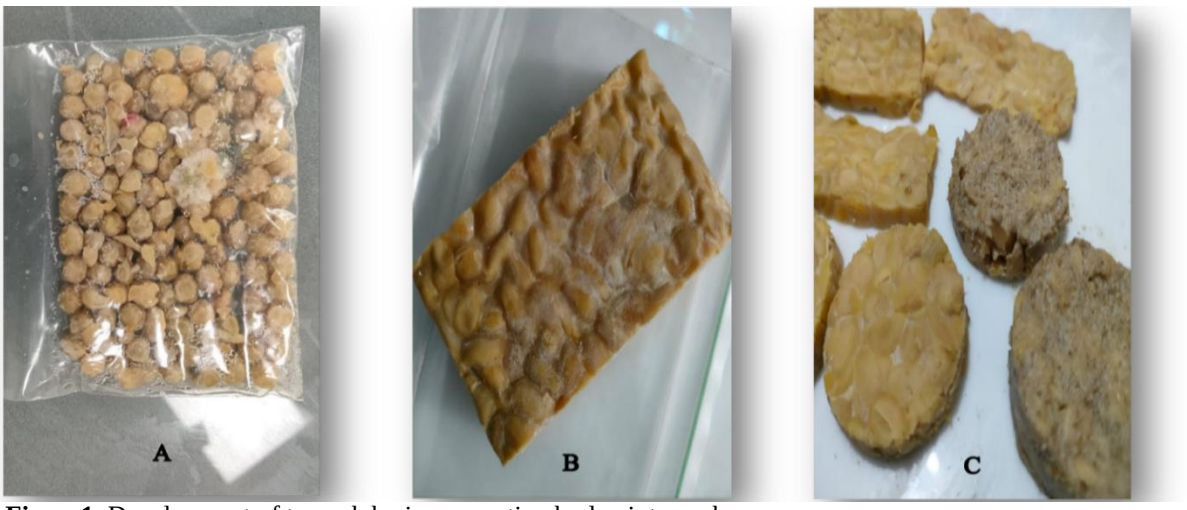

Figure1. Development of tempeh by incorporating barley into soybean
A: (Initial process of fermentation), **B**:(Product developed, ready to fry or storage), **C**: (Fried product)

Crude protein

Kjeldahl apparatus was used for protein determination according to the method of AACC (2000). Sample (5g) was mixed with digestion mixture and 30 ml H_2SO_4. Place the tubes in digester and run the process. After digestion shake the tubes on cooling and dilute it with distilled water. Diluted sample was transferred to distillation unit and mix with boric acid solution. On completing the distillation, sample was titrated with 0.1 N H_2SO_4 solution. The color of solution was changed from white to pink representing the quantity of protein then note the value of H_2SO_4 that was used to determine the nitrogen percentage. Use the nitrogen percentage for protein calculation.

Determination of ash

The incineration technique outlined in method no. 08-01 AACC (2000) was used for crude ash determination in products. For charring, 4g of sample was taken in crucible, it was heated until the sample became black and smoke ended. After it, crucibles were placed in desiccator. After 10 to 15 mins removed them from desiccator and weighed. After weighing, the crucibles were placed in the muffle furnace and set the temperature at 650°C for 5 hours. After cooling, the crucibles were removed and placed in desiccator. Then samples were removed, and ash content was calculated by using formula.

Determination of color (L* a*, b*) value of the product

Developed products were analyzed for color with the help of equipment named as "Color Tec" colorimeter, using the procedure described by Gul *et al*., (2018). Data for "L*", "a*" & "b*" was recorded by placing samples under the colorimeter color sensor and digital reading shown on the screen. Readings of "L* value" for white and dark appearance of subjected sample. "a* value" for green and red shade. "b*" as a function of blue and yellow shading.

Sensory profiling

Panel of food specialists from the Food Science and Technology department done the sensory evaluation of products, for color, flavor, aroma, texture, appearance, after taste and overall acceptability by using the 9-point hedonic scale. The prepared products were served in labeled plates as T0, T1, T2, T3 in sensory evaluation lab with separate cabin. The trial followed the instructions of Lawless and Heymann (2010).

Storage stability

Sensory properties depend highly on the stability of the product as well as the ability of the product to resist deterioration by

multiple factors. Tempeh were stored on refrigeration temperature in zipper bags for two weeks to check the nutritional profile with respect to time.

Antioxidants activity

Extraction of sample (supernatant) for DPPH

The extraction mixture of 5 mL was made by combining methanol, acetone and HCl at a ratio of 90:8:2 to homogenized 1 g of tempeh sample. Pestles and mortar were used to homogenize the mixture. After extraction, supernatants were poured into the Eppendorf tubes and centrifuged at 4 °C for 3-5 min at 12000 rpm.

DPPH Scavenging Activity

For antioxidant assay tempeh were determined by following the protocol described by Ozcelik *et al*., (2003) using 1-l-diphenyl-2- picrylhydrazyl (DPPH) method. 50 µL supernatant was taken from each sample as prepared in treatment plan and transferred in test tube having 5mL DPPH solution and incubated for 30 minutes under ambient conditions. 200 micro litters sample was poured in 96 microwell plate and Epoch Eliza reader was used to read the absorbance at 517 nm (Bio-Tek Instruments, Inc., Winooski, USA).

Statistical analysis

The data obtained from research was subjected to analysis of variance (ANOVA) by using STATISTIX (Version 8.1) software as recommended by Steel *et al*., (1997).

3. RESULTS AND DISCUSSION

The present research was aimed at the production of fermented soybean products with barley addition and to evaluate the nutritional quality of value-added product tempeh. The details of the results obtained during these studies are as under:

Analysis of raw material:

According to obtained results shown in table 1, soybeans contained 35±1.52 crude protein, 10.6±0.72 moisture content, 3.8±0.94 ash content, 18.6±0.10 crude fat and 7.2±1.32 crude fiber. Soybean tempeh was prepared by the addition of different ratio of barley. The proximate composition of barley showed 9.6±0.58%, 2.52±0.58%, 2.64±0.58%, 13.8±1.45% and 1.4±0.58% of moisture, crude fat, crude fiber, crude protein and ash, respectively. Proximate composition of raw materials were cross match with the research of (Etiosa *et al*., 2017) for soybeans and Abeshu and Abrah, (2017) for barley, minute difference was present due to use of different varieties.

Analysis of product:

Storage stability

Developed products were subjected to shelf-life study at 0, 7th and 14th day by considering following parameters i.e. moisture, crude fat, crude fiber, crude protein, ash, color (l*, a*, b* value), antioxidant value and sensory parameters.

Moisture

The combined effects of treatment and storage showed highly significant results P=0.00 as well as individually it showed significant results P=0.00. By increasing the percentage of incorporated barley moisture content of the products decreased as shown in table 2. Moreover, the moisture content of products changed a little bit during storage conditions. Table shows that the highest moisture content was observed at 1st day in T0 (38.58A±0.02%), while lowest moisture content was observed in T3 (36.30D±0.06 %) at 14th day of storage, because the moisture content in barley is lower than the soybean. Increase in barley concentration decreases the moisture content. Tan et al., (2024) also work on tempeh production with different beans in their research, they develop the soybean fermented product as well as chickpea and red bean addition and result revealed that the findings of both research were approximately similar with a difference of ±3 values. These variations are due to the environmental conditions and variety differentiation.

Crude protein

Crude protein content in tempeh analyzed at prescribed intervals showed highly significant results P=0.00 during individual effects of treatment and storage and non-significant P=0.441 during combine effects as shown in table no. 2. Maximum protein content was analyzed at day 1 while minimum protein content was observed at 14^{th} day. High protein content was noted at T_0 while lowest was observed when barley percentage increased. Crude protein content findings were compared with the study of Ahnan-Winarno *et al*., (2021) and Tan *et al*., (2024) and the obtained results were always different from each other due to variety of soybean. Decrease in protein content of products were due to the presence of barley instead of soybean because it contained less protein as compared to soybeans.

Crude fat

The statistical analysis result of tempeh showed non-significant results P=0.61 during the combined effect of treatment and storage and significant results with storage and treatment separately. Results showed that crude fat content decreased with increase in storage time and barley content (treatment) as shown in table 2. Maximum fat content (17.20AB±0.05) was noted on day 14^{th} of soybeans-based tempeh while lowest (16.45E±0.05) on day 14 of tempeh prepared with 30% of barley addition. Present results were matched with Vital *et al*., (2018) that concluded the fat content of 24.88 0.30% and Bavia *et al*., (2012) that showed 22.13% of fat and the obtained results of this research are below these percentages, reason behind this was storage condition, temperature variation in environment and soybean legume fat contents.

Crude fiber

Statistical analysis for addition of barley into soybean tempeh showed significant results P=0.00 while the combined effect of treatment and storage was non-significant P=0.29 as shown in table no. 2. Crude fiber content during storage became lower with the passage of time and increased with increased barley percentage. Maximum crude fiber was observed at day 0 while lowest on day 14. Lowest crude fiber was analyzed at T0 while highest at T3 as shown in table 2. Current findings were in collaboration with Tan et al., (2024) that showed almost same crude fiber content (10.69%) of soybean tempeh and the highest value of fiber content was in product, which was incorporated with 30% barley, addition of barley was mainly used to enhance the fiber content of products. A little difference may be due to change in cultivar or environmental difference.

Ash

Statistical results of ash content during mutual effect of treatment and storage showed significant results between treatment and storage. Increase in ash content during storage and when barley percentage was increased as shown in table 2. Highest ash value (2.17 A±0.09) was analyzed on day 14 of product with 30% barley, while lowest value was of standard tempeh made with soybeans. Current results were almost like the study of Erkan *et al*., (2020) that showed 1.91%-2.00% ash content in tempeh.

Color (L*, a*, b* value)

Results of L* value of products showed that lowest L* value was observed in T_1 (47.19) at 14^{th} day of storage, whereas the highest value was noted in T_0 at 0 day (55.35). The statistical analysis showed significant effect of treatment and storage as well as individual parameter on tempeh as shown in figure no. 1. Mean values showed that L* value of tempeh decreased with increase in storage time and barley percentage. Because enzymatic browning of barley cause production of darker pigment. Results showed that a* value decreased with increase in storage time

Table 1: Proximate analysis of soybean and barley (%)/ 100 gram

Parameters	Soybean (%)	Barley (%)
Moisture content	10.6±0.72	9.6±0.54
Ash content	3.8±0.94	1.4±0.15
Crude Fat	18.6±0.10	2.5±1.08
Crude Fiber	7.2±1.32	2.6±0.55
Crude Protein	35±1.52	13.8±0.75
NFE (Nitrogen free extract)	24.8±0.03	70.1±0.37

Table 2. Influence of storage and treatment on different parameters of tempeh

Moisture (%)/ 100 gram				
Treatment	**0 day**	**7 days**	**14 days**	**Mean ± SE**
T_0	38.58^{A}±0.02	38.15^{AB}±0.02	38.17^{AB}±0.69	38.30^{A}±0.14
T_1	37.49^{A-D}±0.16	37.42^{A-D}±0.02	38.06^{ABC}±0.77	37.65^{AB}±0.18
T_2	37.18^{A-D}±0.16	37.11^{A-D}±0.28	36.83^{BCD}±0.35	37.04^{BC}±0.15
T_3	36.39^{CD}±0.23	36.35^{CD}±0.09	36.30^{D}±0.06	36.35^{C}±0.07
Mean	37.41^{A}±0.14	37.26^{A}±0.10	37.34^{A}±0.47	
Crude protein (%)/ 100 gram				
T_0	31.84^{A}±1.09	30.90^{AB}±0.34	30.65^{AB}±0.81	31.13^{A}±0.43
T_1	31.00^{AB}±0.25	30.09^{AB}±0.21	29.84^{AB}±0.12	30.31^{AB}±0.11
T_2	29.97^{AB}±0.25	29.40^{AB}±0.20	28.76^{B}±0.25	29.38^{B}±0.13
T_3	29.87^{AB}±0.69	29.33B±0.23	28.70^{B}±0.09	29.30^{B}±0.19
Mean	30.67^{A}±0.57	29.93^{AB}±0.25	29.49^{B}±0.32	
Crude fat (%)/ 100 gram				
T_0	17.67^{A}±0.25	17.08^{B-D}±0.13	17.20^{AB}±0.05	17.31^{A}±0.08
T_1	17.14^{BC}±0.15	16.94^{B-E}±0.01	16.85^{B-E}±0.01	16.98^{B}±0.03
T_2	16.94^{B-E}±0.01	16.68^{C-E}±0.01	16.58^{DE}±0.08	16.73^{C}±0.02
T_3	16.84^{B-E}±0.01	16.44^{E}±0.06	16.45^{E}±0.05	16.58^{C}±0.02
Mean	17.15^{A}±0.11	16.79^{B}±0.05	16.77^{B}±0.05	
Crude fiber (%)/ 100 gram				
T_0	9.33^{CD}±0.33	10.00^{A-D}±0.58	8.67^{D}±0.88	9.33^{C}±0.35
T_1	11.00^{A-D}±0.58	10.33^{A-D}±0.67	11.67^{A-D}±0.88	11.00^{B}±0.41
T_2	11.07^{A-D}±0.29	12.01^{A-C}±0.31	11.54^{A-D}±0.33	11.54^{AB}±0.18
T_3	13.33^{A}±0.88	12.33^{A-C}±0.33	12.53^{AB}±0.53	12.73^{A}±0.34
Mean	11.18^{A}±0.52	11.17^{A}±0.47	11.10^{A}±0.66	
Ash content (%)/ 100 gram				
T_0	1.84^{C}±0.02	1.87^{C}±0.01	1.93^{BC}±0.04	1.88^{C} ±0.01
T_1	1.94^{BC}±0.02	1.95^{BC}±0.02	2.0^{A-C} ±0.05	1.96^{BC} ±0.02
T_2	1.97^{A-C} ±0.04	2.03^{A-C}±0.04	2.14^{AB}±0.02	2.04^{AB} ±0.02
T_3	2.05^{A}-C±0.05	2.10^{AB}±0.06	2.17^{A}±0.09	2.11^{A} ±0.04
Mean	1.95^{B}±0.03	1.99^{AB}±0.03	2.06^{A}±0.05	

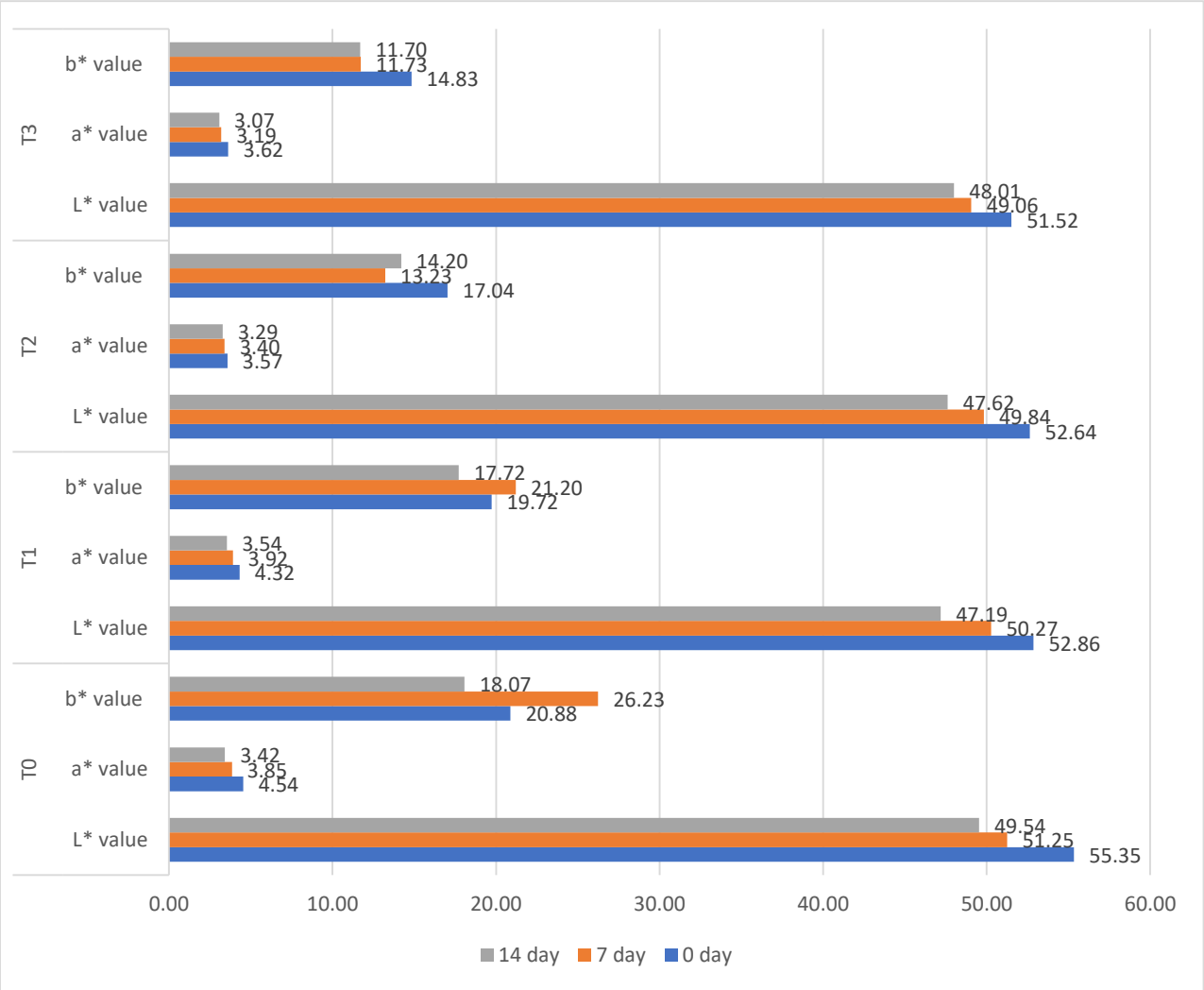

Figure:1. Effects of storage and treatment on color parameters of tempeh

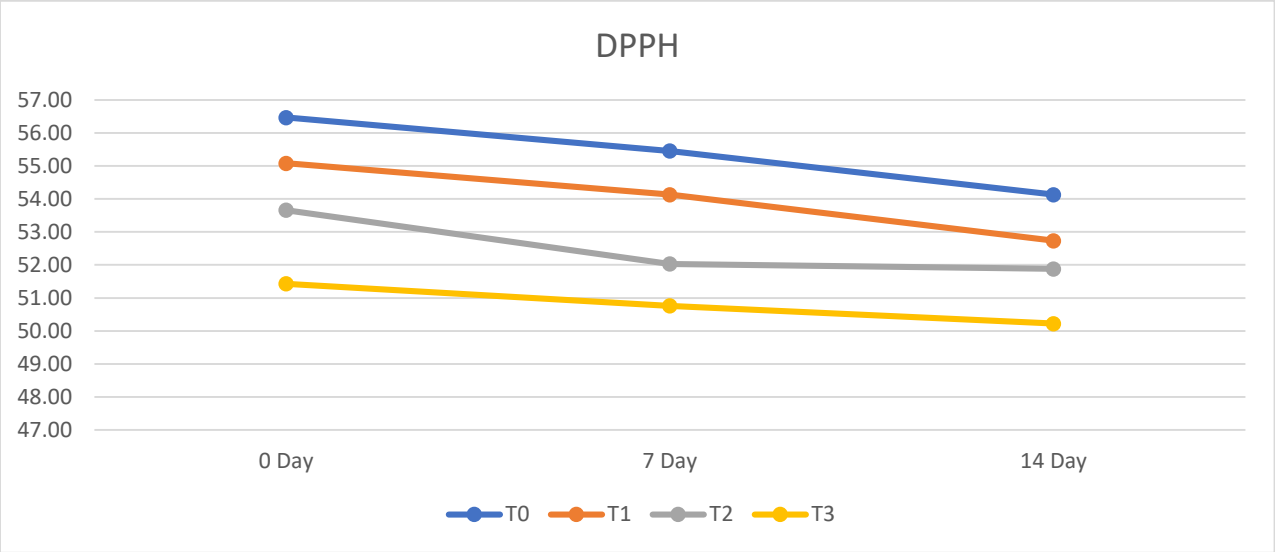

Figure:2. Impact of storage and treatment on DPPH radical scavenging activity of tempeh

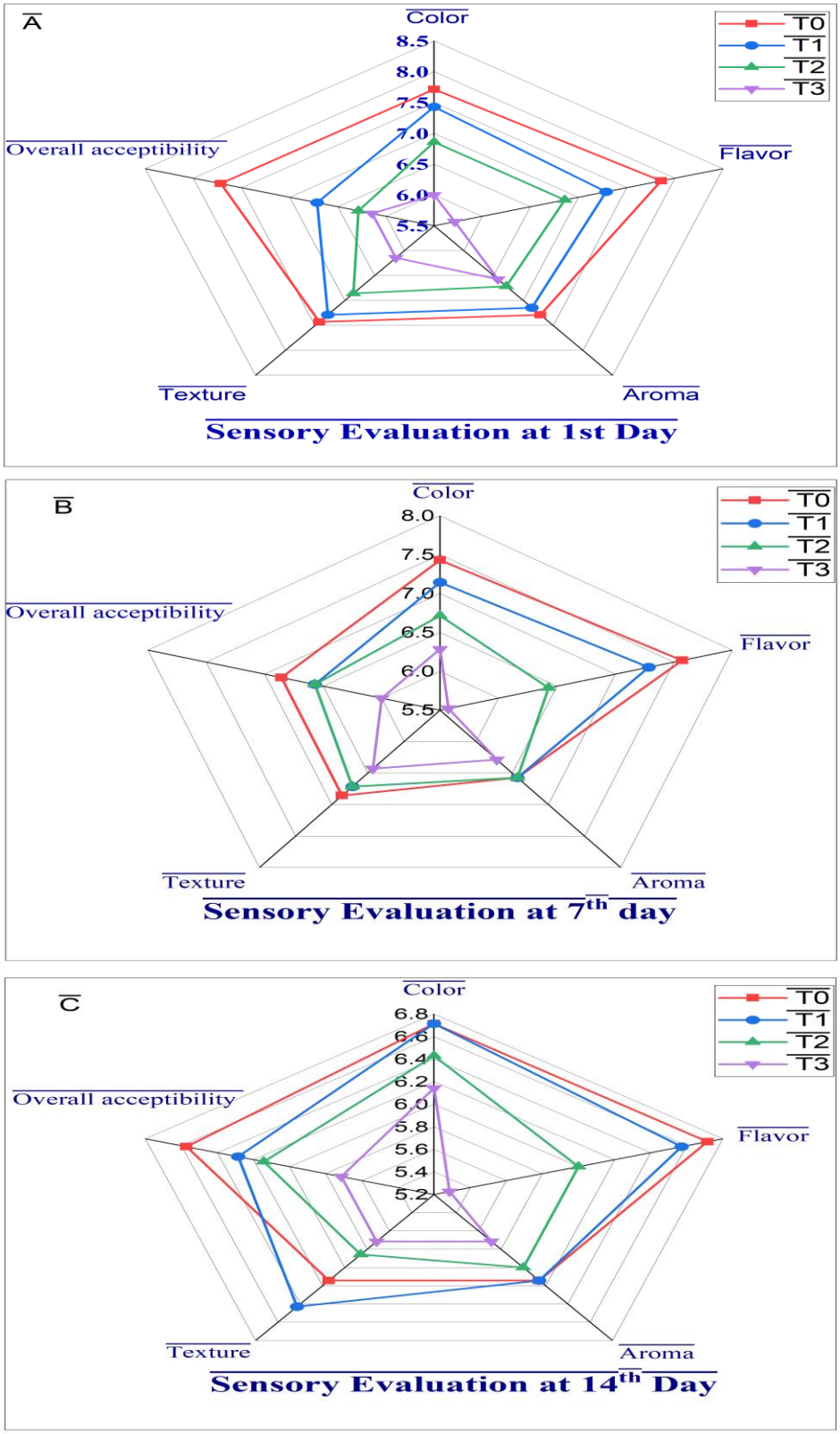

Figure:3. Effect of treatment and storage on sensory parameters of tempeh at Day 1st (A), Day 7th (B) and Day 14th (C)

treatment (barley) percentage. As shown in Figure 1 lowest a* value was observed in T_3 (3.07) at 14th day of storage, whereas the highest score was noted in T_0 at 0 day (4.57). Barley have less quantity of red pigment than soybean which cause a*reduction. Statistical analysis of b* the value of tempeh showed significant results P=0.00 during the combined effect of treatment and storage. Results showed that b* value decreased with an increase in storage time and barley percentage due to the degradation of carotenoids.

DPPH Analysis

1,1-diphenyl-2-picrylhydrazyl stable radicals (DPPH) analysis showed significant results with respect to treatment and storage study and showed non-significant result on combine effects of treatment and storage. Increase in storage duration causes the increase in microbial activity that affects the radical scavenging activity of the product. According to the result, DPPH value was higher at 0 day of storage of T0 (56.47) and the lower value of DPPH was observed at 14th of T3 (50.22) as shown in Figure 2. These results were matched with the study of Ahmad *et al*., (2015).

Sensory parameters; Aroma, Color, Flavor, Texture and Overall acceptability

Sensory analysis for aroma, color, flavor, texture and overall acceptability of product T_0, T_1, T_2 and T_3 was conducted at 0, 7 and 14 days. The combine effect of treatment and storage on tempeh (T_0, T_1, T_2 and T_3) showed non-significant results. Statistical analysis of aroma showed significant results with respect to storage conditions as well as treatment variations. With the passage of time, a significant difference was shown in the aroma of tempeh. On day 0, the aroma score of T_0 was highest (7.29) and T_3 showed the minimum value (5.71) at 14th day of storage. Aroma score decreased with an increase in storage time as shown in Figure 3 (A, B, C). With the passage of time, the color score of tempeh was decreased as shown in Figure 3 (A, B, C). On day 0, the value of color for T_0 was highest (7.71) and T_3 had the minimum value (6.14) on the 14th day. Separately the treatment and storage showed significant results, but the combined effects were non-significant. Results showed that flavor value decreased with increased storage time and treatment percentage. On day 0, the value of flavor at T_0 was highest (7.86) while lowest at T_3 (5.29) on 14th day. Significant result was obtained for storage and treatment for the texture of products. At 0 day, the value of texture was highest at T_0 (7.43) and lowest on 14th day for T_3 (5.71). Results showed that the score decreased with increase in storage time and treatment percentage. Statistical analysis of overall acceptability of tempeh showed that effect of storage was highly significant, effect of treatment was significant, while the joint effect of storage and treatment on overall acceptability were found to be non-significant (P>0.05). As shown in Figure 3 (A, B, C), the lowest score of overall acceptability was observed in T_3 (5.71) at 14 day, whereas the highest score was observed in T_0 at 0 day (7.71). The lower scores of T_3 may be attributed to the higher percentage of incorporated barely which may have disrupted the sensory properties. The results of this study closely relate to the findings of Erkan *et al*., (2020) and Tan *et al*., (2024).

CONCLUSION:

Chemical composition of soybean showed 35±1.52 crude protein, 10.6±0.72% moisture content, 3.8±0.94% ash, 18.6±0.10% crude fat and 7.2±1.32 crude fiber as shown in table1. Composition of barley was analyzed as 13.8±1.45% crude protein, 9.6±0.58% moisture content, 1.4±0.58% ash, 2.5±0.58% crude fat and 2.64±0.58% crude fiber. In this research, *Rhizopus oligospours* was used for fermentation process, several researchs were done in which the fermentation was done with fungal strains for better product development but other species of *Rhizopus*

give black or dark color. The factors i.e. radical scavenging capacity, dietary fibers and fat content etc. associated with the products are mainly influenced by the concentration of barley. As evident from the results the crude fiber content of the prepared tempeh increased with the increase in concentration of barley, its percentage increased from 9% to 13.33% in 100 grams of tempeh. The antioxidant potential of the product was significant; however, it decreased as the percentage of barley enhanced. Moreover, storage study showed significant results for products by increasing treatment (barley) percentage, some parameter showed non-significant result that can be overcome by using the preservatives.

The fermentation enhanced the nutritional properties of the developed soybean-barley tempeh. A shortcoming was observed in the sensory properties of the product which may be attributed to the presence of barely. Further research studies are required to fully understand the effects of combination of soybean and barely for the development of fermented products like tempeh. The industrial applications must also be explored to gain maximum benefit from barley added tempeh.

CONFLICT OF INTEREST

The manuscript has been read and approved by all listed authors and there is no conflict of interest exists. We assure that the research is original, and this manuscript is not under any submission. All the procurements were made by the department.

REFERENCES

AACC. 2000. Approved methods. St. Paul, MN: American Association of Cereal Chemists. Methods 46-13.

Abeshu, Y., and E. Abrha. 2017. Evaluation of proximate and mineral composition profile for different food barley varieties grown in central highlands of Ethiopia. World Journal of Food Science and Tech. 1(3):97-100.

Achi, O. K. and M. Ukwuru. 2015. Cereal-based fermented foods of Africa as functional foods. Int. J. Appl. Microbiol. 2(4): 71-83.

Ahmad, A., K. Ramasamy, A. B. A. Majeed and V. Mani 2015. Enhancement of β-secretase inhibition and antioxidant activities of tempeh, a fermented soybean cake through enrichment of bioactive aglycones. Pharmaceutical Biology. 53(5):758-766.

Ahnan, W. A. D., L. Cordeiro, F. G. Winarno, J. Gibbons and H. Xiao. 2021. Tempeh: A semicentennial review on its health benefits, fermentation, safety, processing, sustainability, and affordability. Compr. Rev. Food Sci. Food Saf. 20(2): 1717-1767.

Ahnan-Winarno, A. D., L. Cordeiro, F. G. Winarno, J. Gibbons and H. Xiao 2021. Tempeh: A semicentennial review on its health benefits, fermentation, safety, processing, sustainability, and affordability. Comprehensive Reviews in Food Science and Food Safety. 20(2):1717-1767.

Arendt, E. and E. Zannini. 2013. Barley'. Cereal Grains for the Food and Beverage Industries. Elsevier.

Bavia, A.C.F., C. Eduardo Da Silva, M. Pires Ferreira, R. Santos Leite, J. Marcos Gontijo Mandarino and M. Concordia Carrao-Panizzi. 2012. Chemical composition of tempeh from soybean cultivars specially developed for human consumption. Food Sci. Technol. 32(3): 613-620.

Bedani, R., E. A. Rossi, D. C. U. Cavallini, R.A. Pinto, R.C. Vendramini, E. M. Augusto, D. S. P. Abdalla and S. M. I. Saad. 2015. Influence of daily consumption of synbiotic soy-based product supplemented with okara

soybean by-product on risk factors for cardiovascular diseases. Int. Food Res. J. 73: 142-148.

Blandino, A., M. E. Al-Aseeri, S. S. Pandiella, D. Cantero and C. Webb. 2003. Cereal Based Fermented Food and Beverages. Int. Food Res. J. 36(6): 527-543.

Cempaka, L., N. Eliza, A. Ardiansyah, D. D. Handoko and R. M. Astuti. 2018. Proximate composition, total phenolic content and sensory analysis of rice bran tempeh. Makara J. Sci. 22(2): 5.

Erkan, S. B., H. N. Gurler, D. G. Bilgin, M. Germrc and I. Turhan. 2020. Production and characterization of tempehs from different sources of legume by Rhizopus. LWT. 119:108880.

Etiosa, O. R., N. B. Chika, and A. Benedicta. 2017. Mineral and proximate composition of soya bean. Asian Journal of Physical and Chemical Sciences. 4(3):1-6.

Fastnaught, C. E. 2001. Barley fibre. In: Cho S, Dreher M, editors. Handbook of dietary fibre. New York: Marcel Dekker. 519-542.

Feng, X. 2006. Microbial dynamics during barley tempeh fermentation. (59): 1652-6880.

Frias, J., C. Martinez-Villaluenga, and E. Penas. 2016. Fermented Foods in Health and Disease Prevention, 1st Ed. Academic Press: Boston, MA, USA.

Hughes, G. J., D. J. Ryan, R. Mukherjea and C.S. Schasteen. 2011. Protein Digestibility-Corrected Amino Acid Scores (PDCAAS) for Soy Protein Isolates and Concentrate: Criteria for Evaluation. J. Agric. Food Chem. 59(23): 12707-12712.

Izydorczyk, M. S., A. Hussain, and A. W. MacGregor. 2001. Effect of barley and barley components on rheological properties of wheat dough. J. Cereal Sci. 34(3): 251-260.

Kalui, C. M., J. M. Mathara, and P. M. Kutima. 2010. Probiotic potential of spontaneously fermented cereal based foods–A review. Afr. J. Biotechnol. 9(17): 2490-2498.

Kumar, V., A. Rani and G.S. Chauhan. 2007. A Comparative Study of Oligosaccharides in Immature and mature Seeds of Soybean Genotypes. J. Food Sci. Technol. 44(1): 49-51.

Nill, K. 2016. Soy Beans: Properties and Analysis. Encyclopedia of Food and Health. 54-55.

Noaman, M. 2017. New Utilization of Barley as Human Healthy Food. Journal of Plant Biology and Agriculture Science. 1: 2.

Nout, M. J. R. and J. L. Kiers. 2005. Tempeh fermentation, innovation and functionality: Update into the third millennium. J. Appl. Microbiol. 98(4): 789-805.

Nyanzi, R. and P. Jooste. 2012. Cereal Based Functional Foods'. In Everlon Rigobelo, Probiotics 161-197.

Ozcelik, B., J. H. Lee and D. B. Min. 2003. Effects of Light, Oxygen and pH on the 2,2-diphenyl-1-Picrylhydrazyl (DPPH) method to evaluate antioxidants. J. Food Sci. 2003. 68:487-490.

Rimsten, L. 2003. Extrac Table cell-wall polysaccharides in cereal, with emphasis on B-glucan in steeped and germinated barley. Doctoral thesis, Department of Food Science, Swedish University of Agricultural Sciences, Uppsala. 1-49.

Shimizu, C., M. Kihara, S. Aeo, S. Araki, K. Ito, and K. Hayashi. 2008. Effect of high B-glucan barley on serum cholesterol concentrations and visceral fat area in Japanese men. A

randomized double blinded, placebo controlled trial. Foods Hum Nutr. 63: 21-25.

Tan, Z. J., A. Bakar, M. F. Lim and H. Sutimin. 2024. Nutritional composition and sensory evaluation of tempeh from different combinations of beans. Food Research. 8(2):138-146.

Vital, R. J., P. Z. Bassinello, Q. A. Cruz, R. N. Carvalho, J. C. De Paiva and A. O. Colombo 2018. Production, quality, and acceptance of tempeh and white bean tempeh burgers. Foods. 7(9):136.