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Evaluation of the efficacy of rice bran and sesame oil for producing nutritionally-superior quality functional tofu

S. Sengupta[a] and J. Bhowal[b,✉]

[a]West Bengal State University, Dept. of Food and Nutrition, North 24 Parganas, Malikapur Berunanpukuria, Barasat, Kolkata- 700126, West Bengal, India.

[b]School of Community Science and Technology, Indian Institute of Engineering Science and Technology, Shibpur, Howrah 711 103, West Bengal, India.

✉Corresponding author: jayatibhowal@gmail.com; jbhowal@socsat.iiests.ac.in

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SUMMARY: Paneer prepared from edible-quality de-oiled soy flour is known as tofu. Functional tofu was augmented with specific antioxidants such as oryzanol and lignan obtained from rice bran oil (RBO) and sesame oil (SO), respectively. All the fortified and control tofu were evaluated for physicochemical (proximate composition, oxidative stability, antioxidant activity, penetration, and color property), microbiological, and sensory properties. The total viable count and yeast and mold counts increased slowly in the samples stored at 4 °C in the refrigerator for 9 days. Fortified tofu showed higher oxidative stability and antioxidant activity than the control tofu during storage conditions. Additionally, tartaric acid-coagulated tofu retained its quality attributes, especially taste and overall acceptability, during refrigerated storage. Functional tofu aided in an innovative technological technique for making functional non-dairy products with boosted healthy constituents.

KEYWORDS: *Antioxidant Activity; Fortification; Proximate Composition; Rice Bran Oil; Sesame Oil; Tofu.*

RESUMEN: ***Evaluación de la eficacia del salvado de arroz y el aceite de sésamo para producir tofu funcional de calidad nutricional superior.*** El queso panir preparado con harina de soja desgrasada de calidad comestible se conoce como tofu. El tofu funcional se complementó con antioxidantes específicos como orizanol y lignanos obtenidos del aceite de salvado de arroz (RBO) y aceite de sésamo (SO), respectivamente. Se evaluaron las propiedades fisicoquímicas (composición proximal, estabilidad oxidativa, actividad antioxidante, penetración y propiedad de color), microbiológicas y sensoriales del tofu fortificado y del control. El recuento total viable y los recuentos de levaduras y mohos aumentaron lentamente en las muestras almacenadas a 4°C en refrigerador durante 9 días. El tofu fortificado mostró mayor estabilidad oxidativa y actividad antioxidante que el tofu de control durante las condiciones de almacenamiento. Además, el tofu coagulado con ácido tartárico conservó sus atributos de calidad, especialmente el sabor y la aceptabilidad general, durante el almacenamiento en refrigeración. El tofu funcional contribuyó a un estilo tecnológico innovador para elaborar productos no lácteos funcionales con componentes mejorados que aportan beneficios para la salud.

PALABRAS CLAVE: *Aceite de salvado de arroz; Aceite de sésamo; Actividad Antioxidante; Composición proximal; Fortificación; Tofu.*

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1. INTRODUCTION

Indian paneer is one of the most preferred and prevalent dairy products. It is prepared from cow or buffalo milk or their combination, precipitated with acidic ingredients such as lactic acid or citric acid. Recently, low-fat paneer has occupied a significant position in Indian nutrition. However, the soaring price of dairy substitutes including tofu has constrained its attractiveness, predominantly among the middle class and the low-income population (Sengupta *et al.,* 2023). Dairy fat is expensive and is a main contributor to the increasing incidence of coronary disease, particularly in the SARS-CoV-2 pandemic. Additionally, higher consumption of high-fat foods significantly raised the prevalence of COVID-19, whereas dairy items such as yogurt, cheese, paneer, etc. enhanced the risk of COVID-19 (Sengupta *et al.,* 2023). Concerns arise over replacing dairy fat in paneer with non-dairy ingredients like low-fat oilseed meal from non-conventional food solids like soybean, sesame, flaxseed, and sunflower, which are economical and rich in plant protein. Soy paneer is also recognized as Tofu. Tofu is an outstanding source of superior-quality proteins and contains abundant sources of B vitamins, calcium, and isoflavones, which support reducing cholesterol and the threat of osteoporosis and breast and prostate cancer (Sengupta *et al.,* 2016; Sengupta *et al.,* 2023; Bandyopadhyay *et al.,* 2005).

Since paneer contains more calories, fat, carbohydrate, vitamins C, B2, B12, and A, biotin, pantothenic acid, calcium, phosphorus, and iodine than other dairy products, it is regarded as being healthy and nutritious. Saturated fatty acids (SFA) are more prevalent among the more than 400 fatty acids that make up dairy fat. The highest dietary supply of natural trans-fatty acid isomers, namely vaccenic acid, which has been demonstrated to have anti-carcinogenic and anti-atherosclerotic properties, is milk fat. All of the benefits mentioned apply to paneer made from dairy sources. However, milk also lacks polyunsaturated fatty acids (PUFA) in addition to these advantageous properties. Moreover, dairy products are costly and less affordable for consumption by the vast majority of the Indian population. Recently, histidine has also been proposed as the first limiting essential amino acid (EAA), along with methionine and lysine. Long-standing concerns about the high levels of saturated fat in dairy products and deficiency in one or more essential amino acids, along with PUFA, are in line with international recommendations to reduce dietary intake of animal-origin saturated fatty acids (SFA) and increase PUFA and EAA content through supplementation in order to improve cardiometabolic health (Sengupta *et al.,* 2016). Advancements in technology are leading to the development of non-dairy food products, including value-added functional foods like tofu, offering balanced nutrition, superior functionalities, and wellness.

Dairy substitutes like milk, cheese, and yogurt have gained popularity due to allergies, environmental concerns, and health concerns. However, economic growth slows, reducing expendable income and potentially hindering non-dairy milk production. Low-cost production technology is needed to create non-dairy food products, including paneer, cheese, snacks, and desserts. Tofu, a cheese made from soy milk, is popular in vegan and lactose-intolerant cuisine. Companies in India are promoting non-dairy products to gain a competitive edge.

Paneer, a heat- and acid coagulated dairy product which is nutritious, low in calories, high in protein, comprises significant amounts of iron, and has no saturated fat or cholesterol (Hou *et al.,* 1997), receives an exceptionally significant position in the Indian food bazaar. Low-fat oilseed meal from soybean and sesame flour is being explored for producing functional tofu, an affordable, nutritious, and textural alternative to dairy paneer. Thus, it would become known as functional tofu.

There is no information regarding the incorporation of rice bran oil (RBO) and sesame oil (SO) into making nutritionally-superior quality functional tofu. This study hypothesizes that oryzanol-enriched RBO and lignan-enriched SO can enhance non-dairy tofu's functional characteristics, resulting in improved nutritional profile, coagulation, and sensory test acceptance. The aim was to create a fortified tofu that was coagulated by citric, lactic, tartaric, and calcium lactate, retaining the functional qualities of a typical non-dairy product. Therefore, the objectives of this work aimed to create a new antioxidant-enriched tofu by combining RBO and SO, evaluate the effects of different coagulants on the tofu's quality, and compare its physical-chemical, antioxidative, microbiological, oxidative stability, and sensory properties to control tofu.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Chemicals were purchased from Merck, soybean seeds were from local markets, edible soy flour was provided by Progressive Exim, refined rice bran oil was from Shethia Oil Mill, and refined sesame oil was from the local market.

2.2. Production of tofu from soy milk obtained from whole soy bean seeds

Soy milk from whole soy seeds was heated to 97 °C, covered with aluminum foil, and cooled to 87 °C. It was coagulated with different coagulating agents, formed a gel, compressed, and left overnight at room temperature. The resulting four different types of tofu were used as control and designated:

CIT TI:	citric acid coagulated tofu
LAC TI:	lactic acid coagulated tofu
TAR TI:	tartaric acid coagulated tofu
CAL TI:	calcium lactate coagulated tofu

2.3. Production of tofu from RBO–SO-incorporated soy milk obtained from edible-quality de-oiled soy flour

De-oiled soy flour provided by M/S Progressive Exim, Raipur, M.P., India, was used in the preparation of Tofu. Soy milk from edible soy flours (meal) was prepared according to the methods described by Sengupta *et al.* (2016). The production of RBO and SO incorporated soy milk from edible de-oiled soy flour was prepared by Sengupta *et al.* (2016) with slight modifications. Soy milk from edible soy flour was homogenized and pasteurized at 80 °C for 15 minutes. RBO and SO were added, blended in different ratios (20:80, 50:50, and 80:20), and stored in amber bottles. The fat content in the tofu was maintained by adding 5% soy milk, and mixtures of soy milk and oils were homogenized. Citric acid, lactic acid, tartaric acid, and calcium lactate were used as coagulants, each dissolved separately in distilled water to create a coagulant solution.

The RBO and SO-fortified soy milk prepared from the de-oiled edible soy flour was subsequently used in the preparation of fortified tofu following the same procedure described in the preparation of the control tofu from soy milk obtained from whole soy seeds. A schematic diagram for the preparation of fortified tofu is given in Figure 1. All the control and experimental tofu were stored at 4 °C in the refrigerator for 9 days for further analysis. Twelve different types of experimentally fortified tofu were manufactured and designated:

CIT TII:	citric acid coagulated tofu with RBO and SO (50:50)
LAC TII:	lactic acid coagulated tofu with RBO and SO (50:50)
TAR TII:	tartaric acid coagulated tofu with RBO and SO (50:50)
CAL TII:	calcium lactate coagulated tofu with RBO and SO (50:50)
CIT TIII:	citric acid coagulated tofu with RBO and SO (80:20)
LAC TIII:	lactic acid coagulated tofu with RBO and SO (80:20)
TAR TIII:	tartaric acid coagulated tofu with RBO and SO (80:20)
CAL TIII:	calcium lactate coagulated tofu with RBO and SO (80:20)
CIT TIV:	citric acid coagulated tofu with RBO and SO (20:80)
LAC TIV:	lactic acid coagulated tofu with RBO and SO (20:80)
TAR TIV:	tartaric acid coagulated tofu with RBO and SO (20:80)
CAL TIV:	calcium lactate coagulated tofu with RBO and SO (20:80)

2.4. Proximate compositions of tofu

The proximate compositions of all tofu samples prepared by using different coagulants were carried out in triplicate using the standard methods of AOAC (2005). The tofu sample's initial weight was determined, dried in an oven, and then desiccated. The moisture content was determined by subtracting the dry weight from the original weight, while ash content was estimated by incinerating the sample in a muffle furnace for 24 hours and was then expressed as g/100g dry weight of tofu. The protein level in the defatted tofu sample was evaluated using the Bradford (1976) method at 595 nm after extraction, centrifugation, and protein level quantification. Fat was determined according to Bligh and Dyer's (1959) method by some process modifications and

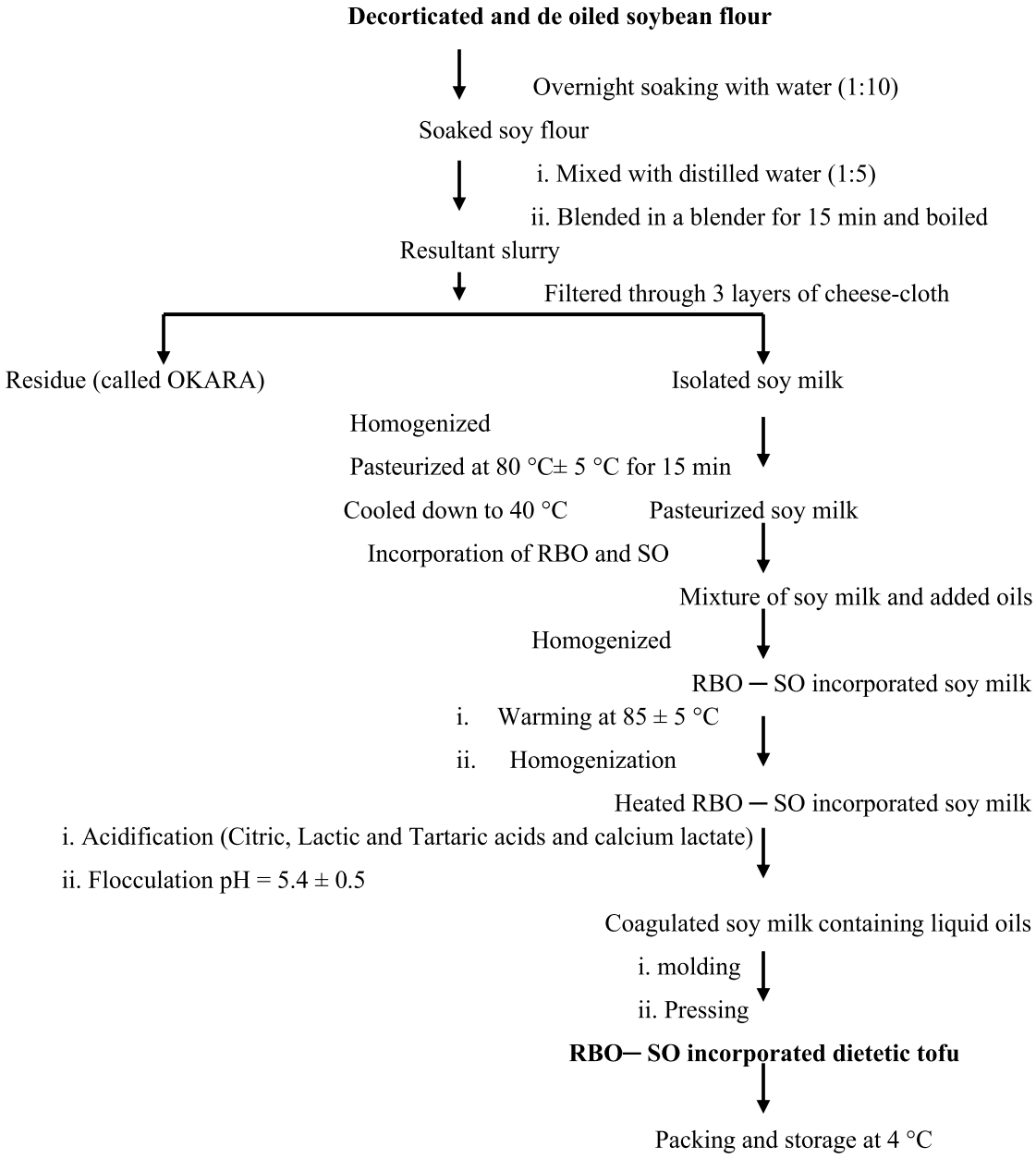

Figure 1. Flow diagrams for the production of RBO–SO fortified functional tofu from edible quality de-oiled soy flour. RBO: Rice bran oil; SO: Sesame oil.

the total lipid content was determined using the formula in Equation (1).

$$\text{Lipid content (\%)} = \frac{\text{Amount of lipid extracted (g)}}{\text{Weight of original sample (g)}} \times 100 \quad (1)$$

Carbohydrate content was calculated by difference [100 − (moisture + crude protein + lipid +ash)]. The Atwater formula was used to calculate energy values, with fat, protein, and carbohydrates contributing 9, 4, and 3.75 kcal g^{-1}, respectively (Merrill and Watt 1973).

2.5. Penetration property of tofu

The penetration properties of tofu were determined by a Penetrometer (Stanhope–Seta Surrey, England) using the cone–form penetration body with an apical angle of 45° and a weight of 72.5 g. The depth of penetration was measured at 5 s at a product temperature of 25 °C.

2.6. Color properties of tofu

The color intensities in the tofu were measured by use of the colorimeter (Konica Minolta CR 10), which gave the Hunter parameter (L^*, a^*, b^*) and also $c^*$and h^* values directly. L^* indicated lightness which describes the light reflecting or transmitting capacity of an object. Color analysis was also performed by the determination of a^* (−green to +red component), b^* (−blue to +yellow), c^*(chroma) and h^*(hue angle) values in triplicate.

2.7. Antioxidant properties of tofu

2.7.1. Preparation of tofu extracts

The tofu sample was homogenized with sterile water, pH was determined, acidified to pH 4.0, heated in a water bath, centrifuged, and NaOH was added to adjust the pH. The supernatants were then centrifuged again at 17000 x g for 10 minutes at 4 °C and stored in a -20 °C freezer for analysis.

2.7.2. Determination of antioxidant activity of tofu by 2,2–Diphenyl–1–Picrylhydrazyl (DPPH) radical inhibition assay of tofu

The homogenized tofu extract was mixed with 60 mM DPPH in ethanol, and the absorbance decrease was monitored at 517 nm until a constant reading was achieved. 250 µl distilled water instead of the extract (Shetty *et al.,* 1995) was used as blank. The % inhibition was calculated by Eq. (2):

$$\%\ \text{Inhibition} = \frac{(\text{Absorbance 517 control} - \text{Absorbance 517 extract})}{(\text{Absorbance 517 control})} \times 100 \quad (2)$$

2.7.3. Total phenolic assay of tofu

The total phenolic content of tofu was determined by the assay method modified by Shetty *et al.* (1995). The tofu extract was mixed with ethanol, water, Folin–Ciocalteu reagent, and 5% Na_2CO_3, then left to stand for 60 minutes. The absorbance was read at 725 nm, and total phenolics were expressed in microgram equivalents of gallic acid per g of sample.

2.7.4. Ferric reducing antioxidant power (FRAP) assay of tofu

The reducing power of tofu was determined using ferric reducing antioxidant power (FRAP) assay (Barahona *et al.,* 2011) with little modification. The FRAP reagent was added to freeze-dried tofu samples, consisting of 300 mM acetate buffer, 10 mM TPTZ, and 20 mM $FeCl_3.6H_2O$. The reaction mixture was kept in the dark for 10 minutes, and absorbance was measured. The reducing power of the tofu samples was calculated using Equation (3)

$$Reducing\ power = Absorbance\ at\ 593\ nm\ for\ sample - Absorbance\ at\ 593\ nm\ for\ FRAP\ reagent \quad (3)$$

2.8. Microbiological properties of tofu

According to APHA (2005), to assess the microbiological quality of tofu, ten grams of each sample were homogenized in a sterile saline solution until a 10^6-fold dilution was achieved. The dilutions were then distributed onto Petri dishes, and the total plate count was calculated using Plate Count Agar. Potato Dextrose Agar was used for yeasts and molds.

2.9. Sensory evaluations of tofu

Freshly prepared tofu samples were kept at 37 °C for sensory evaluations. 20 members were chosen from the School of Community Science and Technology, IIEST, Shibpur, Howrah, West Bengal**.** They developed a consensus evaluation for flavor attributes for tofu and the evaluation was carried out on a Nine-Point Hedonic Scale (ISO, 2014). The quality properties that were evaluated were color, taste, flavor and overall acceptance.

2.10. Oxidative stabilities of tofu

The oxidative stabilities of tofu were examined based on the determination of acid (FFA %) content, peroxide value (PV), thiobarbituric acid value (TBA) and p-anisidine value (p-AV) and Totox value (TV) of the oil extracted from tofu. The FFA content (%) of the tofu samples was determined using a method described by the AOCS Ca 5a–40 Official Method (1997) with some modification in the weight of sample used (5 g oil sample). Oil extracted from the samples was mixed with ethanol and phenolphthalein, titrated with NaOH, and analyzed to determine its color retention. The % FFA was expressed using the following Equation (4)

$$FFA(\%) = \frac{Normality\ of\ NaOH \times volume\ of\ titrant\ used\ (Ml) \times 28.2}{Weight\ of\ the\ sample(g)} \quad (4)$$

The PV (meq $O_2 \cdot Kg^{-1}$) of the oils was measured by the acetic acid–chloroform method AOCS Cd 8–53(1997) to determine primary lipid oxidation products. A 5 g oil sample was weighed, mixed with acetic acid, chloroform, starch, and potassium iodide, and titrated with thiosulfate until the blue color disappeared. The PV was determined according to Equation (5):

$$PV = \frac{(S-B)\times N\times 1000}{W} \quad (5)$$

where *B*, *S*, *N*, and *W* are ml of sodium thiosulfate titrated for the control, ml of sodium thiosulfate titrated for the sample, sodium thiosulfate normality, and weight of the sample, respectively.

The TBA analysis of tofu was conducted following the procedure described by Hekmat and McHamon (1997). The TBA reagent was prepared by dissolving 200 mg TBA in 1–butanol, filtered, and stored. The oil sample was dissolved, heated, and measured for absorbance. A reagent blank was also prepared and the TBA value was expressed using the following Equation (6)

$$\text{TBA value} = 50 \times \frac{\text{A}-\text{B}}{\text{M}} \quad (6)$$

Where, A, B, M are absorbance of the solution, absorbance of TBA reagent and mass of the mg of the oil sample, respectively.

The Official method AOCS Cd 18–90 (1997) was used to determine p-AV. The study involved dissolved p-anisidine in glacial acetic acid, diluted with isooctane, and measuring its absorbance using an isooctane-based spectrophotometer. The solution was then pipetted into separate test tubes, combined with 1 ml of *p*-AV solution, and the absorbance was measured after 10 minutes. Equation (7) was used to calculate *p*-AV

$$p\ AV = \frac{25(1.2As-Ab)}{W} \quad (7)$$

where As and Ab are absorbances of the solutions before and after the reaction with the p-AV solution, respectively. *W* is the weight of sample.

TOTOX values (TV) were calculated using the following Equation (8*)*.

$$TV = 2PV + p\text{-}AV \quad (8)$$

(PV: Peroxide Value; AV: Anisidine Value)

2.11. Statistical analysis

Data are presented as means ± SD. Statistical analysis was performed using multivariant analysis of variance (ANOVA) with the Origin Pro 8 software package for windows. The means were compared between groups by Tukey's post-hoc test. All analyses were carried out in triplicate. Values of $p < 0.05$ were considered significant.

3. RESULT AND DISCUSSION

3.1. Proximate compositions of control and fortified tofu

The study compared the composition of control tofu samples from soy milk and tofu samples from RBO and SO-fortified soy milk using the same coagulating agents. The proximate compositions of the control and the experimental tofu samples are shown in Table 1.

Table 1 revealed that there were significant differences in moisture content between the control and fortified tofu prepared by using the above-mentioned coagulants. The citric acid-coagulated tofu samples (both control and fortified) had the highest moisture content, and the tartaric acid-coagulated tofu had the lowest moisture content among the tofu samples. Among fortified tofu, CIT TII (4.59±0.04; $p < 0.05$) showed the highest moisture value, and TAR TIV (4.14±0.04; $p < 0.001$) showed the lowest moisture value. Joshi *et al.* (1991) also observed that the addition of tartaric acid as a coagulant increased moisture retention in chhana manufactured from animal sources, which is in contrast to our findings because we developed fortified tofu from plant sources. Additionally, the variation in moisture content of fortified tofu with different coagulants was probably due to the differences in gel networks within the fortified tofu particles which were influenced by different ratios of RBO and SO incorporation towards the water holding capacity of de-oiled soy protein gels. It may also be due to the unique coagulating properties of different coagulating agents (Yakubu *et al.,* 2013). The protein content of different tofu samples is given in Table 1, showing that there were significant differences ($p < 0.05$) in protein content between the fortified tofu and the control tofu. Fortified tofu prepared by using citric acid, lactic acid, and tartaric acid had a much higher

Table 1. Proximate composition (g/100g dry weight) of control and fortified tofu

Sample	Moisture#	Protein	Fat	Carbohydrate	Ash	Energy (Kcal·g^{-1})
			Tofu (control)			
CIT TI	64.15±0.48	34.29±0.51	22.54±0.26	35.26±0.65	3.34±0.05	4.72±0.02
LAC TI	64.35±0.56	28.95±0.29	25.29±0.20	38.66±0.24	2.75±0.06	4.88±0.04
TAR TI	64.57±0.25	32.48±0.40	21.25±0.36	38.87±0.36	3.25±0.05	4.66±0.05
CAL TI	63.75±0.54	43.29±0.25	24.29±0.42	25.43±0.48	3.24±0.02	4.87±0.06
			Vegetable oils fortified tofu			
CIT TII	71.59±0.34 ^{a}	44.24±0.75^{a}	22.45±0.69^{a}	25.33±0.29^{a}	3.39±0.02^{a}	4.73±0.07^{a}
LAC TII	71.36±0.74 ^{a}	38.96±0.34^{a}	25.27±0.47^{a}	28.69±0.43^{a}	2.72±0.03^{a}	4.90±0.02 ^{a}
TAR TII	71.88±0.82^{b}	42.39±0.41^{b}	21.20±0.34^{a}	29.00±0.20^{a}	3.25±0.04^{a}	4.69±0.05 ^{a}
CAL TII	70.45±0.72^{a}	43.47±0.58^{a}	24.26±0.29^{a}	25.54±0.16^{a}	3.28±0.05^{a}	4.68±0.06^{b}
CIT TIII	71.58±0.92 ^{a}	44.35±0.46^{a}	22.43±0.29^{a}	25.27**±0.19**^{b}	3.37±0.07^{a}	4.74±0.06 ^{a}
LAC TIII	71.34±0.73^{a}	38.64±0.33^{a}	25.22±0.48^{a}	29.01±0.24^{b}	2.79±0.05 ^{a}	4.90±0.03 ^{a}
TAR TIII	71.96±0.3^{8} a	42.40±0.42^{a}	21.31±0.59^{a}	28.88±0.38^{a}	3.23±0.04^{b}	4.69±0.05 ^{a}
CAL TIII	70.26±0.7^{5}a	43.38±0.68^{a}	24.09±0.67^{a}	26.05±0.33^{a}	3.22±0.02^{c}	4.38±0.06 ^{a}
CIT TIV	71.54±0.65 ^{a}	44.37±0.69^{a}	22.56±0.64^{a}	25.17**±0.26** ^{a}	3.36±0.06^{c}	4.74±0.05 ^{a}
LAC TIV	71.33±0.46^{a}	38.81±0.48^{a}	25.36±0.29^{a}	28.87±0.57^{c}	2.63±0.04^{a}	4.91±0.08^{a}
TAR TIV	4.14±0.04^{c}	42.51±0.51^{a}	21.24±0.17^{a}	28.83±0.96 ^{a}	3.28±0.04^{a}	4.69±0.09^{a}
CAL TIV	3.41±0.04^{c}	43.09±0.64	24.09±0.54^{a}	26.14±0.36^{c}	3.27±0.02^{a}	4.87±0.05 ^{a}

Results are expressed as mean ±SD (n=3). Statistically significant differences were determined by One Way ANOVA and Tukey's Post-hoc test. Mean values having different superscript letter in columns are significantly different $^{a}p < 0.05$, $^{b}p < 0.01$ and $^{c}p < 0.001$ vs. tofu (control).
- On wet weight basis
CIT TI: citric acid coagulated tofu; **LAC TI:** lactic acid coagulated tofu; **TAR TI**: tartaric acid coagulated tofu; **CAL TI**: calcium lactate coagulated tofu; **CIT TII:** citric acid coagulated tofu with RBO and SO (50:50); **LAC TII**: lactic acid coagulated tofu with RBO and SO (50:50); **TAR TII**: tartaric acid coagulated tofu with RBO and SO (50:50); **CAL TII**: calcium lactate coagulated tofu with RBO and SO (50:50); **CIT TIII**: citric acid coagulated tofu with RBO and SO (80:20); **LAC TIII**: lactic acid coagulated tofu with RBO and SO (80:20); **TAR TIII**: tartaric acid coagulated tofu with RBO and SO (80:20); **CAL TIII**: calcium lactate coagulated tofu with RBO and SO (80:20); **CIT TIV**: citric acid coagulated tofu with RBO and SO (20:80); **LAC TIV**: lactic acid coagulated tofu with RBO and SO (20:80); **TAR TIV**: tartaric acid coagulated tofu with RBO and SO (20:80); **CAL TIV**: calcium lactate coagulated tofu with RBO and SO (20:80) ; RBO: rice bran oil; SO: Sesame oil

protein content than the control tofu obtained by using the same coagulants. The protein content of fortified tofu was higher than that of the control tofu, except for the tofu prepared using calcium lactate. These values for proteins were higher than the values (13–17.6%) obtained by Yakubu *et al.* (2013) and lower than the values (56.89–59.98%) obtained by Shokunbi *et al.* (2011). Sengupta *et al.* (2016) also noted that the protein content of non-dairy soy yogurt with sesame and rice bran oil had a greater percentage of protein in comparison with the control soy yogurt without the addition of vegetable oil. Our research supports these observations. Table 1 indicated that there were no marked differences in fat content between the control and fortified tofu prepared by using the same coagulating agents. Lactic acid-coagulated tofu samples (both control and fortified) had the highest fat value, and tartaric acid coagulated tofu had the lowest fat content among the tofu samples. The decrease in fat content was due to the decrease in fat recovery from lactic acid, followed by calcium lactate, citric acid, and tartaric acid tofu (Khan *et al.,* 2014). It can be observed in Table 1 that the same trend in ash content was observed between the control and fortified tofu prepared by using different coagulating agents. Citric acid-coagulated tofu samples (both control and fortified) had the highest ash value, and lactic acid-coagulated tofu had the lowest ash content among all the tofu samples prepared by using different coagulating agents. Our findings are comparable to those of Khan *et al.* (2014), who found no significant differences in the ash level

of paneer coagulated by the three different forms of coagulant, although their citric acid-coagulated paneer had a greater value of ash content. Table 1 indicates that the same trend in moisture contents was observed between the control and fortified tofu prepared by using citric acid, lactic acid, tartaric acid, and calcium lactate. Table 1 reveals that the carbohydrate content of fortified tofu was lower than that of control tofu, except for tofu prepared using calcium lactate. The presence of carbohydrates is responsible for these energy values. The projected energy values increased when the amount of carbohydrates increased, and vice versa. When compared to the data from Shokunbi *et al*. (2011), the computed range of carbohydrates in the current study was from 25.17 to 38.17%, which was somewhat higher.

3.2. Penetration property of control and fortified tofu

The penetration properties of the different tofu samples prepared by different coagulating agents are shown in Table 2**,** showing that the penetration properties of the control tofu were lower than those of the fortified tofu. Each coagulant used in the preparation of fortified tofu influenced the texture of the tofu, particularly the hardness of the fortified tofu. The penetration of tofu indicated its resistance to compressive forces. The results showed that the hardness of the control tofu made from whole soy beans was very high and the addition of citric acid, lactic acid, tartaric acid and calcium lactate was not able to alter the hardness values. The difference in hardness in fortified tofu had a close correlation with the ratio of RBO and SO in the gel

Table 2. Penetration property of control and fortified tofu during storage at 4 °C in a refrigerator

Sample	0 day	3 days	6 days	9 days
		Tofu (control)		
CIT TI	180.45±34.29	181.29±32.15	184.54±2.15	180.27±2.63
LAC TI	185.36±26.47	184.45±54.26	182.14±5.29	180.29±3.25
TAR TI	182.25±25.45	186.26±45.29	194.36±32.54	190.25±34.29
CAL TI	185.45±15.56	186.48±14.26	182.15±13.25	179.89±15.65
		Vegetable oils fortified tofu		
CIT TII	202.47±16.29^{c}	210.48±26.54 ^{a}	204.67±19.54 ^{a}	206.98±15.26 ^{a}
LAC TII	222.26±26.41^{a}	215.29±22.65 ^{a}	212.65±24.87 ^{a}	209.49±14.98^{b}
TAR TII	230.46±25.41^{a}	235.41±43.15 ^{a}	245.29±32.56 ^{a}	255.48±15.64 ^{a}
CAL TII	220.26±15.42^{a}	219.19±13.24 ^{a}	216.35±26.25^{a}	210.25±2.39^{b}
CIT TIII	246.15±25.64^{a}	242.17±15.42^{a}	240.32±32.15^{b}	234.47±22.14^{b}
LAC TIII	239.16±33.25^{a}	259.48±10.29 ^{a}	238.48±36.25^{b}	230.16±32.15^{a}
TAR TIII	250.41±42.26^{b}	233.54±27.42 ^{a}	210.19±21.06^{b}	215.34±34.16^{a}
CAL TIII	244.26±25.45^{a}	230.54±13.24 ^{a}	229.25±12.26^{a}	225.85±20.24^{a}
CIT TIV	235.25±21.89^{a}	234.26±12.28^{c}	230.25±16.24^{c}	229.21±20.28^{c}
LAC TIV	248.24±20.05^{a}	217.15±10.48 ^{a}	220.36±15.47^{a}	220.47±21.47^{a}
TAR TIV	235.25±36.05^{a}	225.25±9.58 ^{a}	220.01±16.24^{b}	220.17±27.47^{a}
CAL TIV	234.26±11.25^{c}	240.26±29.22^{a}	239.55±16.25^{a}	238.57±16.24^{a}

Results are expressed as mean ±SD (n=3). Statistically significant differences were determined by One Way ANOVA and Tukey's Post-hoc test. Mean values having different superscript letter in columns are significantly different $^{a}p < 0.05$, $^{b}p < 0.01$ and $^{c}p < 0.001$ vs. tofu (control).

CIT TI: citric acid coagulated tofu; **LAC TI:** lactic acid coagulated tofu; **TAR TI**: tartaric acid coagulated tofu; **CAL TI**: calcium lactate coagulated tofu; **CIT TII:** citric acid coagulated tofu with RBO and SO (50:50); **LAC TII**: lactic acid coagulated tofu with RBO and SO (50:50); **TAR TII**: tartaric acid coagulated tofu with RBO and SO (50:50); **CAL TII**: calcium lactate coagulated tofu with RBO and SO (50:50); **CIT TIII**: citric acid coagulated tofu with RBO and SO (80:20); **LAC TIII**: lactic acid coagulated tofu with RBO and SO (80:20); **TAR TIII**: tartaric acid coagulated tofu with RBO and SO (80:20); **CAL TIII**: calcium lactate coagulated tofu with RBO and SO (80:20); **CIT TIV**: citric acid coagulated tofu with RBO and SO (20:80); **LAC TIV**: lactic acid coagulated tofu with RBO and SO (20:80); **TAR TIV**: tartaric acid coagulated tofu with RBO and SO (20:80); **CAL TIV**: calcium lactate coagulated tofu with RBO and SO (20:80) ; RBO: rice bran oil; SO: Sesame oi

of the protein network. Based on the penetration property, tofu made with tartaric acid was less rigid and stretchy than tofu cured with citric and lactic acids. Hanáková *et al.* (2013) made a similar study and discovered that gel hardness decreased with increasing vegetable oil concentrations.

3.3. Color properties of control and fortified tofu

The effects of different coagulants and the effects of vegetable oil fortification on the color (*L*, a*, b *, c*, and *h*) of the control and fortified tofu are shown in Table 3.

Table 3 indicates that brightness (*L**), redness (*a**), and yellowness (*b**) showed highly significant ($p < 0.05$) differences between the control and fortified tofu. It was observed that there was a decreasing trend for *L** values in the de-oiled seed flour-based fortified tofu compared to the control tofu. Citric acid-coagulated control and fortified tofu had the highest *L**, followed by lactic and tartaric acid-coagulated tofu, respectively. The lowest *L** values were obtained in the cases of calcium lactate-coagulated control and fortified tofu. With an increase in RBO content in blended oil-incorporated tofu, an increase in *L** values were observed. This may be due to the bleaching properties of the fatty acids present in the oil. There were significant differences in *a** values ($p < 0.05$) among the different fortified tofu (–0.35±0.01<a*<–0.15±0.05) and among different control tofu (–0.18±0.01<*a**<–0.12±0.15). The *a** values of fortified tofu were lower than those of

Table 3. Color properties of control and RBO–SO fortified tofu on day 0 of storage at 4 °C in a refrigerator

Sample	L^*	a^*	b^*	c	h
			Tofu (control)		
CIT TI	77.42±1.11	–0.16±0.02	+12.62±0.29	8.04±0.65	95.25±0.54
LAC TI	75.54±1.92	–0.12±0.15	+13.83±0.32	13.90±0.15	83.50±0.65
TAR TI	72.86±2.42	–0.14±0.02	+15.20±0.03	15.40±0.14	80.42±0.84
CAL TI	71.21±2.76	–0.18±0.01	+14.93±0.07	15.15±0.26	79.16±0.48
			Vegetable oils fortified tofu		
CIT TII	72.20±3.21[a]	–0.24±0.15[a]	+12.40±0.04[b]	14.84±0.34[a]	84.53±0.15[a]
LAC TII	70.90±3.60[a]	–0.27±0.01[b]	+13.51±0.35[b]	14.50±0.19[a]	84.16±0.74[a]
TAR TII	71.21±4.21[a]	–0.24±0.01[b]	+13.23±0.04[a]	13.93±0.05[a]	83.20±0.96[a]
CAL TII	70.65±4.24[a]	–0.35±0.01[a]	+14.80±0.29[a]	13.54±0.41[c]	80.15±0.54[a]
CIT TIII	73.64±1.74[a]	–0.20±0.02[a]	+11.95±0.03[a]	14.94±0.34[a]	85.71±0.36[a]
LAC TIII	71.70±2.21[a]	–0.24±0.01[a]	+13.10±0.36[a]	14.54±0.27[a]	76.52±0.47[a]
TAR TIII	72.53±2.90[a]	–0.21±0.01[a]	+13.82±0.04[b]	14.36±0.39[a]	75.24±0.19[a]
CAL TIII	72.14±3.65[b]	–0.26±0.02[a]	+13.24±0.60[b]	13.57±0.47[a]	75.24±0.25[a]
CIT TIV	70.34±3.14[a]	–0.23±0.05[a]	+11.36±0.33[c]	14.12±0.34[a]	83.50±0.18[a]
LAC TIV	69.51±3.14[a]	–0.15±0.02[a]	+13.27±0.05[a]	13.77±0.37[a]	74.74±0.37[a]
TAR TIV	68.96±3.50[a]	–0.16±0.01[a]	+12.90±0.03[a]	13.47±0.47[a]	74.44±0.19[a]
CAL TIV	67.18±2.88[c]	–0.19±0.01[b]	+11.90±0.01[a]	13.16±0.37[a]	73.36±0.57[a]

Results are expressed as mean ±SD (n=3). Statistically significant differences were determined by One Way ANOVA and Tukey's Post-hoc test. Mean values having different superscript letter in columns are significantly different [a]$p < 0.05$, [b]$p < 0.01$ and [c]$p < 0.001$ vs. tofu (control).

CIT TI: citric acid coagulated tofu; **LAC TI:** lactic acid coagulated tofu; **TAR TI**: tartaric acid coagulated tofu; **CAL TI**: calcium lactate coagulated tofu; **CIT TII:** citric acid coagulated tofu with RBO and SO (50:50); **LAC TII**: lactic acid coagulated tofu with RBO and SO (50:50); **TAR TII**: tartaric acid coagulated tofu with RBO and SO (50:50); **CAL TII**: calcium lactate coagulated tofu with RBO and SO (50:50); **CIT TIII**: citric acid coagulated tofu with RBO and SO (80:20); **LAC TIII**: lactic acid coagulated tofu with RBO and SO (80:20); **TAR TIII**: tartaric acid coagulated tofu with RBO and SO (80:20); **CAL TIII**: calcium lactate coagulated tofu with RBO and SO (80:20); **CIT TIV**: citric acid coagulated tofu with RBO and SO (20:80); **LAC TIV**: lactic acid coagulated tofu with RBO and SO (20:80); **TAR TIV**: tartaric acid coagulated tofu with RBO and SO (20:80); **CAL TIV**: calcium lactate coagulated tofu with RBO and SO (20:80) ; RBO: rice bran oil; SO: Sesame oil; L^* value represents lightness and darkness with a range from black (0) to white (100), a^* value represents the green–red spectrum with a range from green (–100) to red (+100), while b^* value represents blue–yellow spectrum with a range from blue (–100) to yellow (+100). c value represents chroma and h value represents hue angle.

the control tofu. Table 3 shows that citric acid-coagulated fortified tofu had higher *b** values, followed by lactic acid, tartaric acid, and calcium lactate, respectively. On the other hand, tartaric acid-coagulated control tofu had the highest *b** values compared to other control tofu. Fortified tofu showed a marked decrease in the yellow component (+11.90±0.01< *b**<+14.80±0.04) in relation to tofu (control) (+12.62±0.29<*b**<+15.20±0.03) and the *b** values of fortified tofu were lower than tofu (control). The results indicate significant differences among the color indices measured with respect to the different types of coagulants used. CIT TIII becomes more yellow. Food deteriorative processes, including the oxidation of fat, browning, and color oxidation, are all facilitated by oxygen. These findings highlight the need for effective packaging in order to preserve enriched tofu. Our findings are consistent with those of Jain *et al.* (2015), who similarly noted a decline in the color value of the Kalakand during an atmospheric oxygen attack. De-oiled soy flour would have increased the amount of amine compounds that combine with aldehydes during the Maillard process to create dark pigments (melanoidins).

3.4. Antioxidant properties of control and fortified tofu

3.4.1. Total phenolic assay of control and fortified tofu

The total phenolic content and antioxidant activity of the control and RBO-SO-fortified tofu are presented in Table 4. Oryzonal in RBO and sesamol-sesamolin in SO are the most potent antioxidants. Results revealed that the polyphenol content in fortified tofu was higher than that of the control tofu. TAR TIII recorded significantly high total polyphenols (15.06±0.48; $p < 0.001$) followed by the CAL PIII (14.60±0.639; $p < 0.05$), CAL TII (13.26±0.48; $p < 0.05$), CIT TIII (13.09±0.26; $p < 0.05$) and LAC TIV (12.09±0.19; $p < 0.05$) had the least in all fortified products in comparison with tofu (control). These values generally compared well with those reported by Karadbhajne and Bhoyarkar, (2010), who reported the effects of different coagulants on the antioxidant activities of tofu. The control tofu also lacks starter or probiotic bacteria as well as other beneficial microbes that are necessary for non-dairy products like soy yoghurt and soy cheese to ripen, increase in bioactivity, or limit the growth of harmful microbes. As a result, the tofu matrix provides a favorable environment for the growth of bacteria that cause spoilage. Additionally, no preservative was applied to extend the shelf-life of the control paneer, whereas fortified tofu contains preservatives including oryzanol in RBO and lignan in SO. Therefore, it may be concluded that spoilage-causing bacteria cause bioactive chemicals in the tofu matrix to degrade, which results in a reduction in antioxidant capacity (Sengupta *et al.,* 2016).

3.4.2. Antioxidant activity determination by 2,2–Diphenyl–1–Picrylhydrazyl (DPPH) radical scavenging activity of control and fortified tofu

Table 4 shows that the DPPH radical inhibition assay of control tofu revealed lower values than those of fortified tofu. Among the fortified tofu, calcium lactate-coagulated fortified tofu had the highest DPPH radical scavenging activity. Shokunbi *et al.* (2011) showed a significant increase in antioxidant activity when different coagulants were used in the production of tofu. According to the current research, tartaric acid and calcium lactate maintained the antioxidant content of enriched tofu due to their synergistic interactions with oryzanol and lignan. LAC TII revealed the lowest DPPH values among freshly-made fortified tofu (30.26±0.48 w/w% of tofu), which may be because tofu contains RBO and SO in a 50:50 ratio. According to certain investigations, sesame oil and rice bran oil were in charge of the radical scavenging function. In some studies, it has been reported that rice bran oil and sesame oil are responsible for radical scavenging activity (Sapwarobol, *et al.,* 2021). Phenolic chemicals can shield the biomolecules by swiftly decreasing reactive oxygen species, such as free radicals. According to Loganayaki *et al.* (2013), the hydroxy groups of flavonoids have the capacity to give hydrogen or electrons to DPPH free radicals, which results in the termination reaction of those free radicals. In this way, the radical scavenging activity that was observed in our results for the tofu that had been supplemented with RBO and SO may be increased by the addition of more hydroxy groups from phenolic and flavonoid components.

3.4.3. Ferric-reducing antioxidant power assay of control and fortified tofu

Table 4 presents the results of the ferric-reducing antioxidant power (FRAP) of control tofu and fortified tofu. It was observed that with an increase in RBO content in blended oil-incorporated tofu, there was an increase in the FRAP values of fortified tofu.

Table 4. Antioxidant properties of control and fortified tofu on day 0 of storage at 4 °C in a refrigerator

Sample	Polyphenol (μg.100 g^{-1} of tofu)	DPPH Radical Scavenging Activity (w/w % of tofu)	FRAP (μm. g^{-1} of tofu)
		Tofu (control)	
CIT TI	11.25±0.25	33.48±0.36	0.35±0.05
LAC TI	12.25±0.41	32.15±0.45	0.32±0.02
TAR TI	11.56±0.34	33.96±0.49	0.34±0.06
CAL TI	11.24±0.45	34.29±0.28	0.34±0.04
		Vegetable oils fortified tofu	
CIT TII	12.64±0.34[a]	33.47±0.58[a]	0.41±0.04[b]
LAC TII	12.41±0.64[a]	30.26±0.48[a]	0.40±0.08[c]
TAR TII	12.25±0.74[b]	34.52±0.64[b]	0.46±0.09[b]
CAL TII	13.26±0.48[a]	38.59±0.84[a]	0.49±0.07[b]
CIT TIII	13.09±0.26[a]	34.26±0.67[a]	0.42±0.05[a]
LAC TIII	12.64±0.41[a]	36.49±0.57[a]	0.47±0.06[a]
TAR TIII	15.06±0.48[c]	38.41±0.84[a]	0.52±0.03[a]
CAL TIII	14.60±0.39[a]	39.48±0.58[a]	0.48±0.06[a]
CIT TIV	13.01±0.41[b]	35.22±0.64[a]	0.40±0.03[a]
LAC TIV	12.09±0.19[a]	33.29±0.95[a]	0.34±0.05[a]
TAR TIV	12.43±0.18[a]	37.95±0.45[a]	0.38±0.04[b]
CAL TIV	12.68±0.69[a]	38.42±0.94[a]	0.38±0.06[a]

Results are expressed as mean ±SD (n=3). Statistically significant differences were determined by One Way ANOVA and Tukey's Post-hoc test. Mean values having different superscript letter in columns are significantly different [a]$p < 0.05$, [b]$p < 0.01$ and [c]$p < 0.001$ vs. tofu (control).

CIT TI: citric acid coagulated tofu; **LAC TI:** lactic acid coagulated tofu; **TAR TI**: tartaric acid coagulated tofu; **CAL TI**: calcium lactate coagulated tofu; **CIT TII:** citric acid coagulated tofu with RBO and SO (50:50); **LAC TII**: lactic acid coagulated tofu with RBO and SO (50:50); **TAR TII**: tartaric acid coagulated tofu with RBO and SO (50:50); **CAL TII**: calcium lactate coagulated tofu with RBO and SO (50:50); **CIT TIII**: citric acid coagulated tofu with RBO and SO (80:20); **LAC TIII**: lactic acid coagulated tofu with RBO and SO (80:20); **TAR TIII**: tartaric acid coagulated tofu with RBO and SO (80:20); **CAL TIII**: calcium lactate coagulated tofu with RBO and SO (80:20); **CIT TIV**: citric acid coagulated tofu with RBO and SO (20:80); **LAC TIV**: lactic acid coagulated tofu with RBO and SO (20:80); **TAR TIV**: tartaric acid coagulated tofu with RBO and SO (20:80); **CAL TIV**: calcium lactate coagulated tofu with RBO and SO (20:80) ; RBO: rice bran oil; SO: Sesame oil; DPPH:2,2–diphenyl–1– Picrylhydrazyls; FRAP: ferric reducing antioxidant power

This may be due to the increase in antioxidants. Among the fortified tofu, tartaric acid-coagulated fortified tofu had the highest FRAP values, and lactic acid-coagulated tofu showed the lowest FRAP values. Among the tartaric acid-coagulated fortified tofu, TAR TIII had the highest FRAP value (0.52±0.03; $p < 0.05$) and among the lactic acid-coagulated tofu, LAC TIV showed the lowest FRAP value (0.34±0.05; $p < 0.05$). The combination of RBO and SO's potential antioxidant activity can be determined by measuring the antioxidant capacity of ferric-reducing compounds. The ability to reduce ferricyanide complex to ferrocyanide complex, which then reacts with ferric chloride to form a ferric-ferrous complex, may indicate the presence of antioxidant compounds (phenolic and flavonoid compounds) in the blend of RBO and SO containing fortified tofu (Sengupta *et al.,* 2016). This reaction occurs when ferric chloride is reduced to the ferrocyanide complex. Based on our findings, it can be assumed that tofu supplemented with RBO and SO in an 80:20 ratio would contain strong antioxidant chemicals that promote a decrease of iron**.**

3.5. Microbiological properties of control and fortified tofu

The microbiological qualities of control tofu and fortified tofu are presented in Table 5**.** Control and fortified tofu's microbiological quality may be influenced by the soy milk's quality, heat treatment, unsanitary manufacturing practices, and conditions after production, such as handling, packaging, and storage. The results concerning TPC (log CFU. g^{-1}) of all treat-

Table 5. Microbiological properties of control and fortified tofu during storage at 4 °C in a refrigerator

Sample	**TPC (log CFU·g^{-1})**			
Tofu (control)	0 day	3 days	6 days	9 days
CIT TI	4.2×10^{3}	4.4×10^{3}	4.5×10^{5}	4.8×10^{7}
LAC TI	4.5×10^{3}	4.6×10^{3}	4.6×10^{5}	4.7×10^{7}
TAR TI	4.5×10^{3}	4.5×10^{3}	4.6×10^{5}	4.6×10^{5}
CAL TI	5.4×10^{3}	5.6×10^{4}	5.4×10^{5}	5.8×10^{5}
	Vegetable oils fortified tofu			
CIT TII	4.1×10^{2}	4.1×10^{2}	4.2×10^{4}	4.2×10^{4}
LAC TII	4.2×10^{1}	4.2×10^{2}	4.2×10^{2}	4.2×10^{3}
TAR TII	4.1×10^{3}	4.2×10^{4}	4.3×10^{4}	4.3×10^{4}
CAL TII	4.4×10^{2}	5.6×10^{4}	5.4×10^{5}	5.8×10^{5}
CIT TIII	4.2×10^{2}	4.4×10^{2}	4.5×10^{3}	4.8×10^{4}
LAC TIII	4.3×10^{2}	4.4×10^{2}	4.2×10^{2}	4.4×10^{4}
TAR TIII	4.3×10^{2}	4.5×10^{3}	4.6×10^{5}	4.6×10^{5}
CAL TIII	4.2×10^{2}	4.6×10^{2}	4.5×10^{3}	4.8×10^{4}
CIT TIV	4.2×10^{2}	4.4×10^{3}	4.5×10^{4}	4.8×10^{4}
LAC TIV	4.1×10^{2}	4.6×10^{3}	4.6×10^{3}	4.7×10^{5}
TAR TIV	3.6×10^{3}	3.5×10^{3}	4.3×10^{4}	4.6×10^{4}
CAL TIV	4.1×10^{2}	4.1×10^{2}	4.4×10^{3}	4.8×10^{4}
Sample	**YEAST AND MOLD (log CFU·g^{-1})**			
Tofu (control)	0 day	3 days	6 days	9 days
CIT TI	–	2×10^{2}	24×10^{3}	3×10^{6}
LAC TI	–	2×10^{2}	2×10^{3}	3×10^{6}
TAR TI	–	1×10^{2}	2×10^{3}	3×10^{6}
CAL TI	–	3×10^{2}	2×10^{3}	3×10^{6}
	Vegetable oils fortified tofu			
CIT TII	–	1×10^{2}	1×10^{3}	2×10^{6}
LAC TII	–	1×10^{2}	1×10^{3}	2×10^{6}
TAR TII	–	2×10^{2}	2×10^{3}	2×10^{6}
CAL TII	–	1×10^{2}	2×10^{4}	24×10^{6}
CIT TIII	–	–	1×10^{2}	2×10^{2}
LAC TIII	–	–	1×10^{2}	2×10^{2}
TAR TIII	–	–	–	2×10^{2}
CAL TIII	–	–	2×10^{3}	2×10^{4}
CIT TIV	–	–	–	2×10^{2}
LAC TIV	–	–	1×10^{2}	2×10^{3}
TAR TIV	–	–	1×10^{2}	2×10^{3}
CAL TIV	–	–	1×10^{2}	3×10^{3}

Results are expressed as mean.

CIT TI: citric acid coagulated tofu; **LAC TI:** lactic acid coagulated tofu; **TAR TI**: tartaric acid coagulated tofu; **CAL TI**: calcium lactate coagulated tofu; **CIT TII:** citric acid coagulated tofu with RBO and SO (50:50); **LAC TII**: lactic acid coagulated tofu with RBO and SO (50:50); **TAR TII**: tartaric acid coagulated tofu with RBO and SO (50:50); **CAL TII**: calcium lactate coagulated tofu with RBO and SO (50:50); **CIT TIII**: citric acid coagulated tofu with RBO and SO (80:20); **LAC TIII**: lactic acid coagulated tofu with RBO and SO (80:20); **TAR TIII**: tartaric acid coagulated tofu with RBO and SO (80:20); **CAL TIII**: calcium lactate coagulated tofu with RBO and SO (80:20); **CIT TIV**: citric acid coagulated tofu with RBO and SO (20:80); **LAC TIV**: lactic acid coagulated tofu with RBO and SO (20:80); **TAR TIV**: tartaric acid coagulated tofu with RBO and SO (20:80); **CAL TIV**: calcium lactate coagulated tofu with RBO and SO (20:80) ; RBO: rice bran oil; SO: Sesame oil; TPC: total plate count

ments (tofu) were not significantly ($p > 0.05$) different. After 9 days of storage, there were no appreciable changes in the TPC values of any treatment during the course of storage, which saw counts gradually rise in all treatments. Yeast and mold count levels grew throughout storage in all treatments; however, they did not differ significantly from one another. Additionally, the results showed that neither the control nor the fortified tofu had any coliform, *Salmonella*, or *E. coli* during storage (data not shown). It has been found that whereas boiling milk at 80 °C for 15 minutes normally totally destroys coliforms, yeast, and mold, these organisms may reappear in a sample of tofu under post-manufacturing conditions. The increasing trend of TPC and yeast and mold count during the storage of tofu has also been reported in some studies (Das *et al.,* 2018). The TPC, yeast, and mold results revealed that oryzanol and lignan, which are retained in the enriched tofu matrix, had an inhibitory effect on microorganisms. In contrast to the control tofu, microorganisms multiplied less after storage in fortified tofu that had RBO and SO. After 12 days of storage, it was noticed that fungus had begun to grow on the fortified tofu's surface. Our findings were in line with those made by Das *et al.* (2018), who observed that paneer or tofu surfaces showed obvious sliminess and reddish-brown or yellowish-brown degradation after 12 days of storage due to microbial buildup, damaged paneer or the fact that the tofu was discarded.

3.6. Sensory evaluations of control and fortified tofu on day 0 at 4 °C in refrigerator

The sensory evaluation of the control and fortified tofu produced using various coagulants is given in Table 6**,** showing that fortified tofu showed significantly ($p < 0.05$) higher acceptability than control

Table 6. Sensory evaluations of control and fortified tofu on day 0 at 4 °C in a refrigerator (n=20) by 9-point hedonic rating

Sample	Color	Taste	Aroma	Acceptability
		Tofu (control)		
CIT TI	6.02±0.25	5.14±0.14	4.19±0.37	6.19±0.25
LAC TI	6.02±0.47	4.49±0.67	4.18±0.51	6.18±0.65
TAR TI	7.30±0.95	6.45±0.69	6.95±0.62	6.14±0.34
CAL TI	7.14±0.64	6.14±0.25	6.94±0.19	6.01±0.29
		Vegetable oils fortified tofu		
CIT TII	8.95±0.74^{a}	7.66±0.18^{b}	8.34±0.54^{a}	8.01±0.18^{c}
LAC TII	7.29±0.58^{a}	7.14±0.64^{a}	8.19±0.37^{a}	8.01±0.18^{c}
TAR TII	7.31±0.34^{a}	6.45±0.17^{b}	6.66±0.85^{a}	7.02±0.47^{b}
CAL TII	6.98±0.19^{a}	6.32±0.59^{c}	6.25±0.26^{a}	7.01±0.19^{c}
CIT TIII	7.39± 0.34^{c}	7.30±0.14^{a}	7.79± 0.39^{b}	6.99± 0.39^{a}
LAC TIII	7.89± 0.18^{c}	7.48± 0.32^{a}	7.88± 0.25^{a}	7.92± 0.18^{b}
TAR TIII	8.04±0.37^{c}	8.25±0.36^{c}	8.03±0.48^{a}	8.14±0.39^{a}
CAL TIII	7.85±0.64^{a}	7.64±0.19^{a}	7.67±0.29^{a}	7.69±0.19^{b}
CIT TIV	7.35±0.98^{a}	6.95±0.18^{a}	6.19±0.18^{a}	6.45±0.19^{c}
LAC TIV	7.05±0.93^{a}	6.46±0.29^{a}	6.12±0.45^{a}	6.37±0.29^{c}
TAR TIV	7.75±0.59^{c}	7.01±0.17^{a}	7.14±0.33^{a}	7.27±0.18^{c}
CAL TIV	7.84±0.37^{c}	7.32±0.26^{a}	7.29±0.22^{a}	7.39±0.43^{a}

Results are expressed as mean ±SD (n=3). Statistically significant differences were determined by One Way ANOVA and Tukey's Post-hoc test. Mean values having different superscript letter in columns are significantly different $^{a}p < 0.05$, $^{b}p < 0.01$ and $^{c}p < 0.001$ vs. tofu (control).

CIT TI: citric acid coagulated tofu; **LAC TI:** lactic acid coagulated tofu; **TAR TI**: tartaric acid coagulated tofu; **CAL TI**: calcium lactate coagulated tofu; **CIT TII:** citric acid coagulated tofu with RBO and SO (50:50); **LAC TII**: lactic acid coagulated tofu with RBO and SO (50:50); **TAR TII**: tartaric acid coagulated tofu with RBO and SO (50:50); **CAL TII**: calcium lactate coagulated tofu with RBO and SO (50:50); **CIT TIII**: citric acid coagulated tofu with RBO and SO (80:20); **LAC TIII**: lactic acid coagulated tofu with RBO and SO (80:20); **TAR TIII**: tartaric acid coagulated tofu with RBO and SO (80:20); **CAL TIII**: calcium lactate coagulated tofu with RBO and SO (80:20); **CIT TIV**: citric acid coagulated tofu with RBO and SO (20:80); **LAC TIV**: lactic acid coagulated tofu with RBO and SO (20:80); **TAR TIV**: tartaric acid coagulated tofu with RBO and SO (20:80); **CAL TIV**: calcium lactate coagulated tofu with RBO and SO (20:80) ; RBO: rice bran oil; SO: Sesame oil

tofu as typified by the color, taste, and aroma. Overall acceptability was highest in TAR TIII (8.14±0.39; $p < 0.05$) and lowest in LAC TIV (6.37±0.29; $p < 0.001$). This result was in accordance with the results of the sensory evaluation of tofu by Mitra *et al.* (2013) and Bandyopadhyay *et al.* (2005), who showed that tofu coagulated with calcium salt and tartaric acid was rated superior in terms of color, taste, aroma, and overall acceptability to the tofu obtained from citrus juices. The organoleptic properties of tofu are significantly influenced by the combination of RBO and SO. Therefore, the quality of the milk and vegetable oil used to make tofu depend on each other. According to certain research, cheese made with palm oil was substantially softer than cheese made with whole milk fat when milk fat was replaced at 100% (Bandyopadhyay *et al.,* 2005; Mitra *et al.,* 2013). We noticed the same pattern with a fresh soft-fortified tofu called TAR TIII that contained a 100% blend of RBO and SO (80:20). It was explained that this was because the presence of fat causes the protein bonds to break down, reducing rigidity and bringing about smoothness and a softer texture, which led to a higher level of acceptability overall than with the control tofu.

3.7. Oxidative stabilities of control and fortified tofu

The effect of citric acid, lactic acid, tartaric acid, and calcium lactate as coagulants on FFA, PV, TBA, PAV, and TV of the control and fortified tofu over 9 days of storage is given in Table 7 (A, B, C, and D). It was observed that FFA, PV, TBA, PAV, and TV of both the control and fortified tofu increased during the total storage period of 9 days. The results revealed that the above-mentioned values for fortified tofu were lower than those of the control tofu due to the presence of antioxidants. The results were in agreement with Kumar and Bector, (1991), who studied the effect of synthetic antioxidants on the shelf-life of tofu. The control and fortified tofu's peroxide values both steadily rose throughout storage, whereas the control tofu's value significantly dropped at the end of the storage period. Thiobar-

Table 7. Oxidative stabilities of control and fortified tofu prepared by citric (A)/ lactic (B)/ tartaric (C)/ calcium lactate (D) during storage at 4 °C in a refrigerator

7.A: Oxidative stabilities of control and fortified tofu prepared by citric acid during storage at 4 °C in a refrigerator

Sample	Days	FFA	PO	TBA	PAV	TV
			Tofu (control)			
CIT TI	0	0.21±0.11	0.04±0.01	0.001±.002	1.04±0.01	1.12±0.09
	3	0.22±0.05	0.05±0.02	0.005±.002	1.05±0.01	1.15±0.06
	5	0.35±0.30	0.07±0.02	0.007±.004	1.07±0.02	1.21±0.02
	9	0.36±0.22	0.07±0.03	0.009±.005	1.14±0.03	1.28±0.09
			Vegetable oils fortified tofu			
CIT TII	0	0.03±0.10^{a}	0.00±00	0.007±0.005 ^{a}	1.14±0.01^{a}	1.14±0.04^{a}
	3	0.05±0.02^{a}	0.00	0.005±0.003^{a}	1.15±0.02^{a}	1.15±0.06^{a}
	5	0.04±0.02^{a}	0.02±0.01 ^{a}	0.006±0.003^{a}	1.16±0.02^{a}	1.20±0.09^{a}
	9	0.09±0.12^{a}	0.02±0.01^{a}	0.012±0.01^{a}	1.15±0.01^{a}	1.19±0.07^{a}
CIT TIII	0	0.01±0.01^{a}	0.00	0.005±0.004 ^{a}	1.10±0.01^{b}	1.10±0.09^{c}
	3	0.01±0.01^{a}	0.00	0.006±0.03^{b}	1.10±0.02 ^{a}	1.10±0.09 ^{a}
	5	0.02±0.02^{a}	0.00	0.006±0.02^{c}	1.11±0.02 ^{a}	1.11±0.02^{b}
	9	0.02±0.02^{a}	0.02±.001 ^{a}	0.007±0.0^{2}b	1.11±0.02^{b}	1.15±0.06^{a}
CIT TIV	0	0.02±0.02^{a}	0.00	0.008±0.004 ^{a}	2.66±0.02 ^{a}	2.66±0.06^{a}
	3	0.03±0.22^{a}	0.00	0.004±0.002^{a}	2.67±0.03^{b}	2.67±.0.06^{a}
	5	0.03±0.03^{a}	0.01±0.01 ^{a}	0.008±.0.08^{a}	2.67±0.02^{b}	2.69±0.08 ^{a}
	9	0.03±0.03^{a}	0.02±0.01 ^{a}	0.02±.0.01 ^{a}	2.67±0.02^{b}	2.71±0.01^{b}

Results are expressed as mean ±SD (n=3). Statistically significant differences were determined by One Way ANOVA and Tukey's Post-hoc test. Mean values having different superscript letter in columns are significantly different $^{a}p < 0.05$, $^{b}p < 0.01$ and $^{c}p < 0.001$ vs **CIT TI**

CIT TI: citric acid coagulated tofu; **CIT TII**: citric acid coagulated tofu with RBO and SO (50:50); **CIT TIII**: citric acid coagulated tofu with RBO and SO (80:20); **CIT TIV**: citric acid coagulated tofu with RBO and SO (20:80); FFA: Free fatty acids (%) of oil extracted from tofu (control) and fortified tofu; PV: Peroxide value, Meq kg^{-1} of oil extracted from tofu (control) and fortified tofu**;** TBA: Thio barbituric acid, mol of MDA /oil extracted from tofu (control) and fortified tofu; PAV: ρ– anisidine value, TV: Totox value; MDA: Malonaldialdehyde; RBO: rice bran oil; SO: Sesame oil

7.B: Oxidative stabilities of control and fortified tofu prepared by lactic acid during storage at 4 °C in a refrigerator						
Sample	**Days**	**FFA**	**PO**	**TBA**	**PAV**	**TV**
			Tofu (control)			
LAC TI	**0**	0.22±0.21	0.03±0.01	0.002±0.001	1.04±0.01	1.10±0.012
	3	0.25±0.21	0.06±0.02	0.005±0.002	1.05±0.01	1.17±0.06
	5	0.34±0.3	0.07±0.02	0.007±0.003	1.07±0.02	1.21±0.04
	9	0.39±0.32	0.07±0.03	0.009±0.004	1.12±0.03	1.26±0.06
			Vegetable oils fortified tofu			
LAC TII	**0**	0.03±0.1 [a]	0.00±00	0.005±0.004 [a]	1.13±0.01 [a]	1.13±0.04[a]
	3	0.06±0.02 [a]	0.00±00	0.003±0.002 [a]	1.13±0.02 [a]	1.13±0.02 [a]
	5	0.06±0.02 [a]	0.02±0.01[a]	0.006±0.003 [a]	1.14±0.02 [a]	1.18±0.05 [a]
	9	0.10±0.1 [a]	0.02±0.01[a]	0.01±0.01[b]	1.15±0.01[b]	1.19±0.06 [a]
LAC TIII	**0**	0.01±0.01 [a]	0.00	0.005±.004 [a]	1.17±0.01 [a]	1.17±0.07 [a]
	3	0.01±0.01[a]	0.00	0.03±0.03 [a]	1.16±0.02 [a]	1.16±0.04[b]
	5	0.02±0.02 [a]	0.00	0.04±0.02[a]	1.16±0.02[a]	1.16±0.02[a]
	9	0.02±0.02 [a]	0.02±0.01[a]	0.05±0.02 [a]	1.18±0.02 [a]	1.22±0.04 [a]
LAC TIV	**0**	0.02±0.02[a]	0.00	0.008±0.004 [a]	2.65±0.02[a]	2.65±0.08 [a]
	3	0.03±0.29 [a]	0.00	0.004±0.002 [a]	2.65±0.03 [a]	2.65±0.01 [a]
	5	0.03±0.03 [a]	0.01±0.01[a]	0.007±0.008	2.65±0.02 [a]	2.67±0.03 [a]
	9	0.04±0.03 [a]	0.02±0.01[a]	0.03±.0.01	2.69±0.02 [a]	2.71±0.08[c]

Results are expressed as mean ±SD (n=3). Statistically significant differences were determined by One Way ANOVA and Tukey's Post-hoc test. Mean values having different superscript letter in columns are significantly different [a]$p < 0.05$, [b]$p < 0.01$ and [c]$p < 0.001$ vs. **LAC TI**.

LAC TI: lactic acid coagulated tofu; **LAC TII**: lactic acid coagulated tofu with RBO and SO (50:50); **LAC TIII**: citric acid coagulated tofu with RBO and SO (80:20); **LAC TIV**: lactic acid coagulated tofu with RBO and SO (20:80); FFA: Free fatty acids (%) of oil extracted from tofu (control) and fortified tofu**;** PV: Peroxide value, Meq kg^{-1} of oil extracted from tofu (control) and fortified tofu**;** TBA : Thiobarbituric acid, mol of MDA /oil extracted from tofu (control) and fortified tofu**;** PAV : ρ– anisidine value, TV : Totox value; MDA : Malonaldialdehyde RBO: rice bran oil; SO: Sesame oil

7.C: Oxidative stabilities of control and fortified tofu prepared by tartaric acid during storage at 4 °C in a refrigerator						
Sample	**Days**	**FFA**	**PO**	**TBA**	**PAV**	**TV**
			Tofu (control)			
TAR TI	**0**	0.23±0.21	0.04±0.01	0.002±0.001	1.03±0.01	1.11±0.06
	3	0.29±0.21	0.06±0.02	0.005±0.002	1.05±0.01	1.17±0.07
	5	0.34±0.3	0.07±0.02	0.007±0.003	1.08±0.02	1.22±0.09
	9	0.36±0.32	0.06±0.03	0.010±0.004	1.12±0.03	1.24±0.06
			Vegetable oils fortified tofu			
TAR TII	**0**	0.02±0.1 [a]	0.00	0.008±0.004 [a]	1.13±0.01 [a]	1.13±0.02[a]
	3	0.06±0.02 [a]	0.00	0.004±0.002 [a]	1.13±0.02 [a]	1.13±0.06[a]
	5	0.06±0.02 [a]	0.02±0.01 [a]	0.007±0.003 [a]	1.14±0.02[b]	1.18±0.07[a]
	9	0.10±0.1 [a]	0.02±0.01[a]	0.02±0.01 [a]	1.15±0.01[a]	1.19±0.08[a]
TAR TIII	**0**	0.01±0.01 [a]	0.00	0.005±0.004 [a]	1.16±0.01 [a]	1.16±0.04[a]
	3	0.01±0.01[a]	0.00	0.06±0.03[b]	1.16±0.02[a]	1.16±0.06[a]
	5	0.02±0.02[a]	0.00	0.04±0.02 [a]	1.16±0.02 [a]	1.16±0.06[b]
	9	0.02±0.02 [a]	0.02±0.01[a]	0.05±0.02[a]	1.18±0.02[a]	1.22±0.02 [a]
TAR TIV	**0**	0.02±0.02 [a]	0.00	0.006±0.004 [a]	2.65±0.02 [a]	2.65±0.03 [a]
	3	0.03±0.29 [a]	0.00	0.007±0.002 [a]	2.65±0.03 [a]	2.65±0.04 [a]
	5	0.03±0.03 [a]	0.01±0.01 [a]	0.008±0.008 [a]	2.65±0.02 [a]	2.67±0.01 [a]
	9	0.04±0.03 [a]	0.02±0.01 [a]	0.009±.01	2.65±0.02 [a]	2.73±0.09[c]

Results are expressed as mean ±SD (n=3). Statistically significant differences were determined by One Way ANOVA and Tukey's Post-hoc test. Mean values having different superscript letter in columns are significantly different [a]$p < 0.05$, [b]$p < 0.01$ and [c]$p < 0.001$ vs **TAR TI**.

TAR TI: tartaric acid coagulated tofu; **TAR TII**: tartaric acid coagulated tofu with RBO and SO (50:50); **TAR TIII**: tartaric acid coagulated tofu with RBO and SO (80:20); **TAR TIV**: tartaric acid coagulated tofu with RBO and SO (20:80); FFA: Free fatty acids (%) of oil extracted from tofu (control) and fortified tofu**;** PV: Peroxide value, meq·kg^{-1} of oil extracted from tofu (control) and fortified tofu**;** TBA: Thio barbituric acid, mol of MDA /oil extracted from tofu (control) and fortified tofu**;** PAV : ρ–anisidine value, TV: Totox value; MDA: Malonaldialdehyde ; RBO: rice bran oil; SO: Sesame oil

7. D: Oxidative stabilities of control and fortified tofu prepared by calcium lactate acid during storage at 4 °C in a refrigerator

Sample	Days	FFA	PO	TBA	PAV	TV
			Tofu (control)			
CAL TI	**0**	0.21±0.21	0.04±0.01	0.001±.001	1.04±0.01	1.12±0.06
	3	0.25±0.21	0.06±0.02	0.005±.002	1.06±0.01	1.18±0.04
	5	0.34±0.3	0.07±0.02	0.007±.003	1.07±0.02	1.21±0.03
	9	0.39±0.32	0.07±0.03	0.009±.004	1.12±0.03	2.52±0.05
			Vegetable oils fortified tofu			
CAL TII	**0**	0.03±0.10^{a}	0.00	0.002±.004^{a}	1.12±0.01^{a}	1.12±0.06^{a}
	3	0.06±0.02^{a}	0.00	0.004±.002^{a}	1.13±0.02	1.12±0.04^{b}
	5	0.06±0.02^{a}	0.02±0.01	0.006±.003^{a}	1.13±0.02^{a}	1.17±0.08^{b}
	9	0.10±0.1^{a}	0.02±0.01^{a}	0.01±0.01^{a}	1.15±0.01^{a}	1.19±0.04^{b}
CAL TIII	**0**	0.01±0.01^{a}	0.00	0.005±.004^{a}	1.16±0.01^{b}	1.16±0.06^{a}
	3	0.01±0.01^{a}	0.00	0.01±0.03^{a}	1.17±0.02^{b}	1.17±0.07^{a}
	5	0.02±0.02^{a}	0.00	0.04±0.02^{a}	1.18±0.02^{b}	1.18±0.09^{a}
	9	0.02±0.02^{a}	0.02±0.01^{a}	0.05±0.02^{a}	1.18±0.02^{a}	1.22±0.04^{a}
CAL TIV	**0**	0.02±0.02^{a}	0.00	0.008±.004^{a}	2.65±0.02^{a}	2.65±0.06^{a}
	3	0.03±0.29^{a}	0.00	0.009±.002^{a}	2.65±0.03^{a}	2.65±0.06^{a}
	5	0.03±0.03^{a}	0.01±0.01^{a}	0.01±.008^{b}	2.65±0.02^{a}	2.65±0.04^{a}
	9	0.04±0.03^{a}	0.02±0.01^{a}	0.02±.010^{a}	2.67±0.02^{a}	2.71±0.02^{a}

Results are expressed as mean ±SD (n=3). Statistically significant differences were determined by One Way ANOVA and Tukey's Post-hoc test. Mean values having different superscript letter in columns are significantly different $^{a}p < 0.05$, $^{b}p < 0.01$ and $^{c}p < 0.001$ vs **CAL TI**.

CAL TI: calcium lactate coagulated tofu; **CAL TII**: calcium lactate coagulated tofu with RBO and SO (50:50); **CAL TIII**: calcium lactate coagulated tofu with RBO and SO (80:20); **CAL TIV**: calcium lactate coagulated tofu with RBO and SO (20:80); FFA: Free fatty acids (%) of oil extracted from tofu (control) and fortified tofu**;** PV: Peroxide value, Meq kg^{-1} of oil extracted from tofu (control) and fortified tofu**;** TBA : Thio barbituric acid, mol of MDA /oil extracted from tofu (control) and fortified tofu**;** PAV : ρ– anisidine value, TV : Totox value; MDA : Malonaldehyde; RBO: rice bran oil; SO: Sesame oil

bituric acid (TBA) values indicated significant increasing trends after storage for 9 days in the generation of secondary lipid peroxidation products (Table 7: A, B, C, and D). A fast rate of increase in AV or TBA values was observed after up to 9 days of storage. This would have been feasible as a result of the breakdown or volatilization of secondary oxidation products. In comparison to the control tofu, RBO and SO-enriched tofu exhibited better oxidative stability, as seen by the decreased concentration of degradative compounds found in these products. Since the PAV monitors secondary oxidation products, which are more stable during the heating process, it is a more accurate and useful test than the PV. The significantly lower concentration of secondary reaction products as indicated by PAV in RBO and SO-fortified tofu compared to control tofu might be due to the increase in SFA or MUFA with the decrease in PUFA in control tofu, which are the primary targets of thermal oxidative reactions. The TV in the control and fortified tofu increased during storage. However, the levels of TV in the current investigation did not rise continuously with longer storage times. This improved oxidative stability may have been brought about by the nutritional value of minor components such as tocopherols, tocotrienols, and oryzanol in RBO and sesamin and sesamolin in SO (Gulla and Waghray, 2011).

4. CONCLUSION

The research aimed to create antioxidant-rich vegetable oils (rice bran oil and sesame oil) with tofu to combat protein-energy malnutrition in developing countries like India. The fortified tofu was found to be superior to control soy paneer in terms of protein,

softness, antioxidant properties, and microbiological quality. The study found that tartaric acid was the most suitable coagulant for producing functional tofu, which is the richest source of vegetarian protein. This could help Indian tofu manufacturers produce nutritionally better-quality functional tofu.

CONFLICT OF INTEREST

The authors are unanimous in publishing this paper. There is also nobody to contradict this manuscript.

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DECLARATION OF COMPETING INTEREST

The authors of this article declare that they have no financial, professional or personal conflicts of interest that could have inappropriately influenced this work.

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AUTHORSHIP CONTRIBUTION STATEMENT

S. Sengupta: Conceptualization, Formal analysis, Investigation, Methodology, Writing –original draft.

J. Bhowal: Conceptualization, Methodology, Writing –review & editing.

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