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Quality Characterization of Burger Formulated with Tempeh

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The objective of this work was to utilize tempeh (soybean, chickpea) for preparation of healthy beef burger and improving the quality characteristics and storage stability of beef burger. Quality characteristics, water holding capacity (WHC) and cooking measurements (cooking loss,cooking yield shrinkage and moisture retention), of beef burger substitutes (soy, chickpea individually), at different levels (50, 70,100%) were studied. The results showed that tempeh beef burger contain 54.70 and 46.98% of the essential amino acids for soy tempeh and chickpea tempeh, respectively. Also, it was found that the burger formulated 50% of soy tempeh recorded the highest protein content (50.90%) and calories (417.78 kcal/100g) compared to the other groups of tempeh. Burger formulated with soy and chickpea tempeh showed increasing in crude fiber and carbohydrate whilecontained similar protein and ash content. Mineral contents (Fe and Ca) of tempeh beef burger were significantly higher than that of found in control sample. The results showed a significant increase in the moisture retention, and cooking yield and there was a significant decrease in the cooking loss and shrinkage due to the addition of soy tempeh and chickpea tempeh compared with the control. The sensory evaluation also showed that the burger 50% Tempeh, (both types), had the highest degree of sensory evaluation, receptivity. Microbial quality criteria of all burger samples were within permissible counts reported by Egyptian Standards. Generally, the formulated tempeh beef burger like seemed to be more preferable by consumer with respect to all organoleptic properties. Finally, it is recommended to utilize Tempeh as meat replacer to prepare a beef burgers like at lower cost with improving health, cooking properties and sensory parameters.

Keywords: Soybeans, Chickpeas, Fermentation, Chemical composition, Tempeh and burgers.

Introduction

The new functional foods that have positive effects on health have been increased every passing day due to increasing of the awareness of consumers.The foodindustry has targeted healthy and diversified food for the development of new products in the market all over the world. The fermented food is one example of recent products demanded by a considerablepopulation group whose interest in variability and new foods with functional, nutritional, and tasty attributes has increased lately (Kaur & Das, 2011; Cruz, 2014). Fermentation is based on using of microorganisms that can exhibit its beneficial role under specific conditions (Siddik et al., 2018; Sugiharto & Ranjitkar, 2019)

The primary objective of the fermentation of cereals and seeds is not as much their preservation, but rather the modification of their organoleptic and nutritional properties. Fermentation is one of the processes that decrease the level of anti-nutrients in food grains and increase minerals extractability (Badau et al., 2005). Fermentation is helping in improved availability of vitamins and protein solubility and amino acid patterns as well as increasing the feedstuff palatability (Zhang et al., 2017; Dawood & Koshio, 2018).

Tempeh (Indonesian spelling) also referred to as tempeh, is a collective name for a sliceable mass of precooked fungal fermented beans, cereals or some other food processing by-products bound together by the mycelium of a living mold (mostly

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Rhizopus sp.). Yellow-seeded soya beans are the most common and preferred raw material to make tempeh (Nout & Kiers, 2005). Tempeh can be favorable food source due to its fitness advantages, affordability, and sustainability. Tempeh is anindigenous food from Indonesia, where it has been consumed as a staple source of protein for more than 300 years (Shurtleff & Aoyagi, 2020). Tempeh fermentation as plant-based protein source and technology may be considered and studied as key parts of feeding the world in a sustainable way environmentally, economically, and public health-wise. Supporting tempeh fermentation as a low-cost, health-promoting, and sustainable food processing technology to produce protein-rich foods using of various beans, legumes, and grains (Amadeus etal., 2021). Due to its good nutritional value, tempeh is used as a daily diet and a meat substitute. In addition, the presence of phenolic compounds in tempeh may help improve its functional properties. Currently, soybeans are being imported from other countries due to the insufficient production of soybean in Indonesia (Mursalina & Silalahi, 2012).

As consumption of meat and production have been considered unsustainable in terms of public health and environment (Godfray et al., 2018), the use of legumes instead of animal protein in new food products and traditional foods is remarkable. Nuts, soy, and lentils, which are consumed widely, especially in the Asia-Pacific location. They are well known for positive health values because of their favorable fatty acid composition, low glycemic indices, high contents of dietary fiber, foliate and vitamin B12, and, specifically for soy, high stages of isoflavones (Lukito, 2001).

Soybean (*Glycine max*), additionally known as soja bean or soya bean, Soybeans are also a significant source of polysaccharides, soluble fibers, phytosterols, lecithins, saponins, and phytochemicals mainly isoflavones which either individually or collaboratively help in promoting health byreducing the incidence of debilitating diseases like hyperglycemia, high blood pressure, dyslipidemia, obesity, inflammation soybean seeds have been primarily used in Asia to prepare a variety of fresh, fermented, and dried foods (RajniModgil et al., 2020).

High soy diet along with low animal protein consumption is considered as a functional food to decrease risks of malignancies such as prostate and breast cancers. Whole soy protein intake was suggested to reduce levels of total cholesterol, low-density lipoproteins, and triglycerides. Moreover, soybean ingestion relief's menopausal hot flashes, preserves bone density and reduce fractures in postmenopausal women (Cassidy et al., 2000; Michelfelder, 2009). Besides containing 40% protein of high biological value and essential amino acids particularly glycine, tryptophan and lysine, it also possesses 23% carbohydrate, 20% fat and reasonable amount of minerals, vitamins and dietary fiber, and is high in antioxidants, omega-3 fatty acids and other beneficial compounds like phytosterols, lecithin and phenolic acids (Kulkarni, 2007).

Chickpea (*Cicer arietinum* L.) is one of the oldest and most widely consumed legumes in the world. It is a staple food crop in some tropical and subtropical countries and it's extensively cultivated in the Northwest of México, being a good source of proteins (180 – 290 g kg^{-1} of sample, DM) and essential amino acids consisting of Lys,Leu, Ile, and Trp. However, chickpea proteins are deficient in total sulphur-containing (Met + Cys) essential amino acids (Reyes-Moreno etal., 2004). Chickpea *(Cicer arietinum* L.) is a cool season legume crop grown world-wide as a food crop. The seed is the main edible part of the plant. It is also called garbanzo gram or Bengal gram (FAO, 2008). Chickpea is acknowledged as the poor man's meat because of its high nutritional value, especially its protein content (Saiema et al., 2015).

The objective of this work was to partial substitution of meat with the high protein tempeh (prepared from fermented soybean and chickpeas) for the production of economical burgers high in nutritional value and Suitable for vegetarians and non-vegetarians who spread so much.

Material and Methods

Material

Soybean (*Glycine max*) variety Giza 111 and chickpea (*Cicer aritinum,* L.) were obtained from Agriculture Research Center, Giza, Egypt. *Rhizopus oligosporus* strain ATCC 22959 was obtained from the Egyptian Microbial Culture Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt. The beef meat changed into imported from Brazil and changed into acquired from the neighborhood butcher keep inside the day before each test. The meat stored in a refrigerator at 5±1°C in a single day. Other Ingredients: Spices (black pepper), onion and salt (Sodium chloride) were obtained from the local market. Media: the following media were used to maintain the microbial growth and are given in gram/L of distilled water with the following composition Tryptic Glucose Yeast Agar medium APHA(1992), MacConkey agar medium (Difco-Manual, 1984),and Nutrient agar medium.

Preparation of inoculums and tempeh

The method of Aderibigbe & Kolade (2003) was used to prepare the inoculums (spore'ssuspension). The mold was grown on Tryptic Glucose Yeast Agar medium.Tempeh was organized the usage of one of the Indonesian techniques as described through Aderibigbe & Adebayo (2002).

The following diagram showed the production process of tempeh:

Soybean or chickpea seeds are washed with tap water

↓

Boiled for 30 min in vinegar solution (0.1%, pH 4) (1: 3 w/v)

↓

Soaked in previously vinegar solution for 24 h

↓

Dehulled by hand under running tap water

↓

Cooked for 60 min in vinegar solution (1:3 w/v)

↓

Discard vinegar solution and spread seeds onto a perforated aluminum tray to drain excess solution and to cool till 25°C

↓

Add inoculum (4 $\times 10^7$ spores suspension (10ml) /100g seeds) and mix well with the whole mass of seeds

↓

Place the inoculated seeds in sterilized foil dishes and cover them by muslin

↓

Incubate in dark places at 28°C for 20 hr

↓

Raw tempeh cake

Fig. 1. Production process of soybean and chickpea tempeh

Preparation of tempeh burger

Seven formulas of burger samples were prepared with 0 % (control), 50, 70 and 100 % of soy and chickpea Tempeh individually by replacing the beef meat. The element chances of burger formulations are shown in Table 1. The components of each formulated burger have been homogenized in Braun Cutter Machine (CombiMax seven hundred,USA), then the homogenized mixtures were processed into burger of about 60g weight, 8 cm diameter and1 cm in thickness. The samples burger was frozen at-18 ± 2°C prior to analysis.

Cooking of beef burger samples

Samples of red meat burger cooked in a preheated electrical grill for a total of 5 min, 2.5 min for each side (70ºC core temperature), then cooking yield, moisture retention and shrinkage have been determined according to El-Magoli et al. (1996), while cooking loss become calculated consistent with the method mentioned by using Jamaet al. (2008) using the following equation:

Cooking loss = [(weight of raw sample – weight of cooked sample) ÷ weight of raw sample] × 100→ (1)

$$\text{Cooking yield (\%)} = \frac{\text{Cooked weight}}{\text{Raw weight}} \times 100 \rightarrow (2)$$

Moisture retention (%) = percent yield*moisture in cooked patties/100→ (3)

Water holding capacity (WHC)

Water holding capacity (WHC) of burger samples was measured according to the method described by Honikel (1998). The burger samples (0.3g) become carefully flattened in a tumbler plate and protected with shells clear out paper (whatman No. 41) then pressed for 10min the use of a mass of 1 kg weight. Two zones had been shaped on clear out paper, their floor location become measured the use of plan meter. The WHC was calculated as cm2/0.3g by way of subtracting the vicinity of the inner region from that of the outer.

Sensory evaluation

The Sensory characteristics of the cooked burger samples were carried out by well-trained 20 panelists of Food Technology Research Institute (FTRI). Panelists were asked to evaluate, odor, texture, taste, tenderness and appearance of cooked samples according to the method described by American Meat Science Association (1995).

TABLE 1. Ingredient of burger formulations (g⁄100g)

Treatments	Beef meat	Soy Tempeh	Chickpea Tempeh	Spices (Black pepper)	Onion	Garlic	Starch	Salt
Control	76	--	-	1	10	1	10	2
Soy tempeh (100%)	--	76	-	1	10	1	10	2
Soy tempeh (70%)	22.8	53.2	-	1	10	1	10	2
Soy tempeh (50%)	38.0	38.0	-	1	10	1	10	2
Chickpea tempeh(100%)	--	-	76	1	10	1	10	2
Chickpea tempeh (70%)	22.8		53.2	1	10	1	10	2
Chickpea tempeh (50%)	38.0	---	38.0	1	10	1	10	2

Moisture, protein, fat, fiber and ash

Contents of raw and cooked samples were determined as described inAOAC (2018). Total carbohydrates were calculated by the differences. All proximate composition experiments were performed in triplicate and expressed as g/100g of burger.

Amino acids and chemical composition

Amino acids compositions were determined in soy and chickpea tempeh using HPLC-Pico-Tag method according to Millipore Cooperative AOAC(2018).

Mineral contents

Zinc, iron, calcium, potassium, sodium, magnesium, manganese and cupper were determined using a PyeUnicum SP1900 Atomic Absorption Spectroscopy instrument (Perkin Elmer model 4100ZL) as described by AOAC(2018).

The caloric value

It was calculated using the following conversion factors: 9 Kcal/g of lipid,4 Kcal/g of carbohydrate and 4 Kcal/g of protein (Frary & Johnson, 2005).

Total phenolic content (TPC)

Total phenolic contents were determined using the Folin-Ciocalteau (FC) method according to Singleton et al. (1999).

Thiobarbituric acid reactive substance (TBARS)

Thiobarbituric acid (TBA) values were determined by the method described by Schmedes & Holmer (1989). The samples (5 g) were blended with 25 mL of 20% trichloroacetic acid solution (200 g/L of trichloroacetic acid in 135mL/L phosphoric acid solution) in a homogenizer for 30 s. The homogenized samples were filtered through Whatman filter paper number 4 to remove beef particles from the filtrate. Then 2 mL of 0.02 M aqueous TBA solution (3 g/L) was added to 2 mL of filtrate in a test tube. After that, test tubes were incubated at100℃ for 30 min and cooled with running tap water. The absorbance of solutions was measured at 532 nm using a UV-VIS spectrophotometer (UV-1200, Shimadzu, Japan). The TBA were calculated from a standard curve and expressed as mg malonaldehyde perkilogram of burger sample.

Microbiological analysis

Ten grams representing beef burger were mixed with 90ml of sterile saline solution (8gNaCl/L distilled water) in a blender, under aseptic conditions, to give 1/10 dilution. Serial dilutions were prepared to be used for counting total aerobic bacteria, coliform group, proteolytic bacteria, lipolytic bacteria, psychrophilic bacteria and yeast & mold counts (APHA, 1992) and (Difco–Manual, 1984)

Statistical analysis

Data for sensory evaluation, physical and chemical evaluation was subjected to analysis of variance (ANOVA) followed by Duncan's multiple range tests (P≤0.05) and carried out using SPSS computer program (SPSS, 1999).

Results and Discussion

Chemical composition of raw materials

Data in Table 2 show the chemical composition of soy and chickpea raw seeds and after producing tempeh from them. The data show soybean exceeds chickpea seeds in protein, fiber, fat and ash while chickpea exceeds at carbohydrate which was estimated by difference. In the processed tempeh, the protein content was increased by 20.15 and 18.88% in soy and chickpea tempeh,respectively. Other components took the opposite direction which showed significant reduction in both types of tempeh.

The increase in protein contents in tempeh may be due to the fermentation process and this has been reported in several studies. Ojokoh and Yimin (2011) in a study on the effect of fermentation on soy products refer that a decrease of carbon ratio in thetotal mass, resulting in redistribution of nutrient percentages. Microorganisms utilize carbohydrates as an energy source and produce carbon dioxide as a by-product. This causes the nitrogen in the fermented samples to be concentrated and thus the proportion of protein in the total mass increases. Also, these results agree with those of Abu-Salem & Abou-Arab (2011) on chickpea tempeh where theyfound that,the protein content was increased from 24.63 to 28.85, while fat content was decreased from 5.62 to 2.84 g/100g in raw seed and tempeh product, respectively.

Amino acids content of tempeh

Amino acid composition is an important factorin determination of protein nutritional values. Table 3 Shows the amino acids composition of soybeans and chickpeas tempeh. It could be noticed that, the percentage ratios of essential amino acids to the total amino acids was 54.85 and 56.83% for soy bean and chick pea tempeh, respectively. This value was well above the 39% considered to be adequate for ideal protein food for adults and comparable to that of egg (50%). Ten essential amino acids were determined, while the tryptophan could not be determined may be because of the acid hydrolysis method of determination that was employed which destroyed the tryptophan (Sumbo and Victor, 2014).

TABLE 2. Chemical analysis of raw soy bean and chickpea and its tempeh product (g/100g) dry basis

Treatments	Crude Protein	Crude fiber	Fat	Ash	Carbohydrates
Soybean seeds	37.70±0.10^{b}	11.70±0.10^{a}	18.21±0.10^{a}	5.40±0.10^{a}	26.99±0.057^{c}
Chickpea seeds	24.73±0.57^{d}	2.86±0.57^{c}	5.70±0.10^{c}	3.30±0.10^{c}	63.41±0.10^{a}
Soybean Tempeh	45.30±0.10^{a}	8.30±0.10^{b}	17.50±0.10^{b}	4.20±0.10^{b}	24.70±0.10^{d}
Chickpea Tempeh	29.40±0.10^{c}	2.40±0.10^{d}	2.86±0.57^{d}	2.60±0.10^{d}	62.74±0.10^{b}
LSD 5%	0.1355	0.2112	0.1632	0.0969	0.1841

LSD, least significant difference (at the letters on the above numbers showed that significantly difference between them.0.05).

The total amounts of essential amino acid were almost equal 54.7 and 46.98 g/100g protein for soybeans and chickpeas tempeh respectively. These values are higher than those recommended by the FAO/WHO for essential amino acids (37.8 g/100g protein) as shown in the same Table 3. From the same table it could be noticed that also Methionine + Cysteine recorded the lowest value for soy beans and chick pea tempeh (3.50 and 3.20g/100g) respectively, which agree with Wan Saidatul Syida et al. (2018) who reported that legumes are deficient in sulphur containing amino acids.

It's clear that amino acids content (essential and non-essential) of soy tempeh are higher than those of chickpea tempeh, may be due to the high quality protein of soy beans. Wan SaidatulSyida et al. (2018) reported that soy proteins isolate should contain all the essential amino acids required for human nutrition (growth, maintenance, and stress). These results agree with other similar studies by Angulo-Bejarano et al. (2008) and Abu-Salem & Abou-Arab (2011).

Physicochemical properties of burger blends

Water holding capacity (WHC) of meat is described because the capacity of meat to maintain fast to its very own or delivered water during processing. It is taken into consideration as an critical component impacts on ingesting satisfactory, tenderness, juiciness, thawing drip and cooking loss offrom the obtained data in Table 4. It could be noticed that, moisture retention of burger formulated with soy and chickpea tempeh were increased compared to the control group.This may be due to the increment of fiber content in burger formulations. Cooking yield showed similar trendof moisture retention which was increased in burger incorporated with soy tempeh by 100 and 70 %which recorded the highest cooking yield (94.6 and 94.1%, respectively).

TABLE 3. Amino acids composition of soybeans and chickpeas tempeh (g/100g protein)

Amino acids	Soybeans tempeh	Chickpea tempeh	FAO / WHO /UNU (1985)
Essential amino acids			
Histidine	3.20	3.00	2.00
Isoleucine	5.70	5.34	4.00
Leucine	8.80	7.80	7.00
Threonine	5.20	4.50	4.00
Lysine	7.60	6.50	5.50
Methionine + Cysteine	3.50	3.20	3.50
Phenylalanine + Tyrosine	12.20	10.74	6.80
Valine	8.50	5.90	5.00
Total	54.70	46.98	37.80
Non-essential amino acids			
Aspartic acid	10.05	9.14	
Serine	5.83	3.29	
Alanine	5.18	3.08	
Arginine	10.04	9.22	
Proline	7.85	6.05	
Glycine	6.07	4.90	
Total	45.02	35.68	

Alakali et al. (2010) reported that, the improvement in cooking yield after tempeh addition could be linked to the fat and water retention capability to retain moisture in the matrix of formulated patties. However, the enhancement of moisture and fat retention of such patties may be accredited to rises in the water absorption capacity of denatured protein and the thermal dissociation of proteins or the gelatinization and swelling of starch and fiber (Alakali et al., 2010 and Modi et al., 2004).

WHC considered as an important factor effects on eating quality, tenderness, juiciness, thawing drip and cooking loss of meat (Morsi, 1988). WHC values showed increasing in some burger blends compared to the control group, the highest value were found in blends contained 50% soy tempeh and 50% chickpea tempeh (2.7 and 2.4 cm2/0.3g), respectively.

Also, data in Table 4 showed the cooking shrinkage percentage of burger treatments, it could be noticed that cooking shrinkage percentage was decreased in all treatments except chickpea tempeh (50%). The lowest of cooking shrinkage (4.4%) was noticed in 100% soy tempeh followed by (7.21%) for 100% chickpea tempeh compared to (15.65%) for control group.

The decrease shrinkage of tempeh formulated patties compared to the controlcould be due to the binding and stabilizing property of tempeh, which held the meat particles together, and prevented changes in product moisture, juice losses and consequently the shape and texture of the product (Naveena et al., 2008). Cooking loss refers to the reduction in weight of red meat in the course of the cooking technique (Drummond & Sun, 2005). From the results in Table 4, it could be observed that cooking loss was affected by the water retention degree. In this concern the best samples (lowest cooking loss) were chickpea tempeh andsoy tempeh (100%) which recorded 3.40 and 5.32 %, respectively.

pH values is one of very important tastes for meat products freshness, where, pH value due to accumulation of alkaline compounds outcome degradation of protein and this consider indicating for meat spoilage. The pH value of various groups of tempeh burger ranged between 5.6 to 6.4 for alltreatments compared to 5.97 for control group. According to Abd-El-Aziz et al. (2018), these values were within the normal limits of the products of this kind. This means that the pH values of these products did not influence neither by type nor by the ratio of added tempeh meat replacer.

Chemical composition and total calories of burger blends

Data in Table 5 shows the chemical composition and the total calories of burger prepared from soy and chickpea tempeh by different ratio compared to the control group (100%beef meat). The results showed that, protein, fat crude fiberand ash content ranged from 29.36 to 50.90, 2.86 to 17.50, 2.22 to 8.33 and from 2.56 to 9.83 compared to 56.70, 15.16, 0.86 and 12.07% for control group, respectively.On the other hand the total calories ranged from 388.71 to 417.78 compared to 424.10 kcal/100g. Burger with 50% soy tempeh seems the best blend and almost close to the control group, which recorded the highest content of protein (50.90%), ash (9.83%) and total calorie (417.78 kcal/100g). Also it contains moderate values of fat (16.37%) and crude fiber (6.19%), while showed the lowest content of carbohydrates (16.69%).

TABLE 4. Some physicochemical properties of burger blends

Treatments	%Moisture retention	%Shrinkage	WHC $cm^2/0.3g$	%Cooking loss	%Cooking yield	pH
Control	29.83	15.65	1.3	9.58	90.4	5.97
Soy tempeh (100%)	53.63	4.40	0.6	5.32	94.6	5.80
Soy tempeh (70%)	52.62	10.78	1.5	5.48	94.1	6.00
Soy tempeh (50%)	45.44	13.46	2.7	8.10	91.8	6.40
Chickpea tempeh(100%)	39.72	7.21	0.7	3.40	90.9	5.70
Chickpea tempeh(70%)	32.63	12.42	1.7	8.90	89.4	5.90
Chickpea tempeh(50%)	29.98	16.83	2.4	9.05	88.2	5.60

On the contrary, blend of burger made from 100% chickpea tempeh recorded the lowest content of protein (29.36%), fat (2.86%) and ash (2.56%).Replacing of beef meat with soy and chickpea tempeh showed increasing in crude fiber and carbohydrates while showed reduction in protein and ash content. Fat content showed slight increase in soy tempeh groups, while showed more decreasing in chickpea tempeh groups. These results reflect the levels of these ingredients in the two types of tempeh, as shown in Table 2. In similar studies Abd-El-Aziz et al. (2018) reported that, replacing meat with Bulgur flour and soybean concentrate resulted in increase in carbohydrates content and reduction in other proximate composition components. Amadeus et al. (2021) mentioned that the comparison of the six pork entries at the USDA Food Data Central database, the U.S. Commercial tempeh contained similar protein content material, less total and saturated fat, greater carbohydrates, and higher fiber content material. Tempeh generally contained more calcium, extra iron, much less sodium than red meat.

Minerals content

Although cereals and legumes are the major sources of minerals but minerals from plant sources have very low bioavailability because they are found complexed with non-digestible material such as cell wall polysaccharides as well as phytate. Fermentation is one of the processing methods that are applied to free these complexed minerals and make them tooreadily bioavailable. There are different mechanisms by which fermentation increases the mineral bioavailability. Firstly, fermentation reduces phytic acid that binds minerals making them free and more available. Secondly, fermentation loosens the complex matrix that embeds minerals. Both phytase and α-amylase make loose by degrading phytate and starch, respectively. Moreover, some fermenting microorganisms have the ability to degrade fiber which loosens the food matrix further (Smith et al., 2018).

TABLE 5. Chemical composition and total calories of soybean andchickpea Tempeh and its burgerblends (g/100g) dry basis.

Treatments	Crude protein	Fat	Crude fiber	Ash	Carbohydrates	kcal/100g
Control	56.70±0.1000[a]	15.16±0.15[c]	0.86±0.0551[f]	12.07±.06[a]	15.20±0.10[g]	424.10±0.61[a]
Soy tempeh (100%)	45.33±0.0577[d]	17.50±0.10[a]	8.33±0.0577[a]	9.23±0.05[c]	19.60±0.10[d]	417.23±0.93[bc]
Soy tempeh (70%)	49.80±0.1000[c]	16.36±0.11[b]	7.47±0.0985[b]	8.89±0.08[d]	17.46±0.05[e]	416.37±0.90[c]
Soy tempeh (50%)	50.90±0.0436[b]	16.37±0.06[b]	6.19±0.285[c]	9.83±0.02[b]	16.69±0.19[f]	417.78±1.46 [b]
Chickpea tempeh (100%)	29.36±0.0577[g]	2.86±0.05[f]	2.43±00577[d]	2.56±0.05[g]	62.76±0.11[a]	394.33±0.29[d]
Chickpea tempeh (70%)	44.80±0.100[e]	5.56±0.05[e]	2.51±0.0289[d]	7.26±0.05[e]	39.85±0.05[c]	388.71±0.08[f]
Chickpea tempeh (50%)	37.23±0.0577[f]	6.76±0.05[d]	2.22±0.010[e]	5.35±0.01[f]	48.43±0.10[b]	403.55±0.29[e]
LSD5%	0.1355	0.1632	0.2112	0.0965	0.1841	1.3823

LSD, least significant difference(atthe letters on the above numbers showed that significantly difference between them 0.05).

As shown in Table 6. The replacement of the soy and chickpea tempeh in beef burger manufacture decreased all tested minerals except Ca and Fe which showed highest compared to control beef burger group.According to(Food &Drug Administration, 2019; National Institute of Health, 2020; United States Department of Agriculture, 2019). Tempeh contained 81.6 to 94.35 mg of calcium

The highest increase of Ca and Fe (95.43 and 7.72) was showed in burger soy tempeh (100%), while the highest decrease of Fe (3.60) and Ca (50.90) was showed in chickpea tempeh (50%). The highest increases in minerals in tempeh burger may be due the fermentation process. Damanik et al. (2018) mentioned that the processing method of making tempeh such as soaking, steaming, and fermentation caused decreasing or increasing proximate in mineral contents. They attributed the decreasing of a some mineral content materials to leaching process that resulted in food cell wall broken and occurs discharging of component composing material. Some mineral will be damaged in most processing because it's sensitive to pH, oxygen, light, and heat.

Total phenolic content

The total polyphenols in raw soy and raw chickpea and its tempeh burger blends were presented in Table 7. From these data it could be noticed that, polyphenols content was significantly increased in all burger blends compared to control group (0.831), raw chickpea (61.00) and raw soy (91.40) mg GAE/100g, respectively. This increase was gradual and consistent with the ratio of replacing the beef meat with the tempeh burger blends. These results agree with those of Kuligowski et al. (2017), who found that most significant increase of polyphenol content and the highest amount was noticed between second and fourth day of fermentation which was about 10 times more comparing to dehulled and cooked soybeans. The increased content of polyphenols can be a result of enzymatic degradation of seed cells and better extraction after destroyed connections with other compounds. AyodejiAdebo & Medina-Meza (2020) attributed the increase in total phenolic content during the fermentation to the activities of β-glucosidase, which is capable of hydrolyzing phenolic phucosides to release free phenolic.

TABLE 6. Minerals content of raw soy and chickpea and its tempeh burger (mg/100g) blends

Treatments	Na	K	Ca	Mg	P	Fe	Zn	Mn	Cu
Raw soy	150.20	854.5	295.5	280	704	13.7	4.90	3.80	2.00
Chickpea raw	108.5	785.5	160.7	155.2	398	6.90	3.85	1.72	1.25
Control	275.20	380.00	8.47	320.10	295.70	1.93	3.88	11.50	2.10
Soy tempeh (100%)	90.65	345.60	95.43	120.25	245.60	7.72	3.20	2.26	0.82
Soy tempeh (70%)	145.56	355.00	69.35	180.50	260.65	5.20	3.90	2.75	1.50
Soy tempeh (50%)	185.90	362.50	51.95	220.25	270.65	6.10	4.50	3.05	1.70
Chickpea tempeh(100%)	72.80	250.57	85.30	105.10	109.73	5.20	2.10	5.28	0.52
Chickpea tempeh(70%)	133.60	289.5	62.45	170.60	162.60	4.52	3.20	4.85	1.02
Chickpea tempeh(50%)	174.20	315.50	50.90	212.90	202.75	3.60	3.60	4.60	2.05

TABLE 7. Total phenolic content in raw soy and chickpea and its tempeh burger blends

Treatments	Total phenolmg GAE/100g
Rawsoy	91.40
Chickpearaw	61.00
Control	0.831
Soy tempeh (100%)	700.4
Soy tempeh (70%)	493.6
Soy tempeh (50%)	350.6
Chickpea tempeh (100%)	202.5
Chickpea tempeh (70%)	141.8
Chickpea tempeh (50%)	101.6

Thiobarbituric acid value (TBA) of tempeh burger

Data in Table 8 showed the Thiobarbituric acid value (TBA) determined as mg malonaldehyde of the prepared tempeh burger at zero time and during six month storage period at -18 ± 2°C. It could be noticed that there was reduction in TBA value for all treatments compared to control group during the storage period. Chickpea tempeh (100%) recorded the lowest value of TBA over the period of storage followed by Chickpea tempeh 70%.

It was noticed that all treatment have continued to maintain the value of TBA within the acceptable limits, which ranged from 0.10 to 0.32 mg malonaldehyde per 1 kg samples compared to 0.42mg for control group after storage for 6 months at -18°C. This may be due to the fermentation results of soy and chickpea tempeh which may be contains lactic acid bacteria .The results of TBA value (which considerable indicator for lipid oxidation) suggest that lactic acid bacteria in the manufacture of tempeh led to lower TBA values during storage periods (Yousif & Muhssen, 2019) .

The TBA values of the different burger treatments at zero-time and after storage for 6 months at -18°C were below the level of incipient rancidity (≥ 1) as reported by Abd-El-Aziz et al. (2018).

Microbiological evaluation

Fresh tempeh desserts must be consumed within 1 or 2 days. This is due to clean tempeh carries 60% moisture or the mold proteolytic enzymes will cause ammonia to from which results in an undesirable taste. Storage stability of tempeh can be prolonged by drying, frying, dehydration, freezing, and other preservation methods (Tahiret al., 2018). After the production of the tempeh burger, the microbial investigation of the samples was carried out over the period of storage for total count bacteria, Coliforms, Psychrophilic bacteria Proteolytic, lipolytic bacteria.

The data in Table 9 showed that the total counts of bacterial in burger blends immediately after processing (zero time) ranged between 3×10^3 and 6×10^3 compared to 7×10^3 cfu/g for control group. During frozen storage at-18ºC up to 6 months total counts were gradually increased with increasing the storage time, but the control group had the highest values at the end of storage period $9x10^4$. Although all treatments remained without spoilage through the storage period, chickpea (100%) group seem the best treatments,where it recorded 2.5 $x10^3$ and 4 $x10^4$ cfu/g, after 4 and 6 months of storage,respectively. Total bacterial counts had been used to assess sanitary quality, safety. The microbial activity leads to certain changes in flavor, color and accumulation of toxins in meat (Fliss et al.,1991).The bacterial population in tempeh has also been reported to affect the functionality of the product as immune stimulants (Soka et al., 2015).

Tempeh a fermented soybean-primarily based food is a remarkably nutritious useful meal with health blessings. Unfortunately, tempeh is relatively perishable, with a shelf existence of 24–48 hr (Kustyawati et al., 2020). From the obtained data in Table10, It was observed that psychrophilic bacterial countsin all treatments ranged from 20 to 40 compared to 60cfu/g for control group at zero time. After 6 month of storage at-18 ± 2°C it ranged from 1.5 $x10^2$ to 4.5 $x10^2$ compared to $8x10^2$ cfu /g.

TABLE 8. TBA value (mg/kg) of burger treatments during frozen storage at-18 ±2°Cfor 6 months

Treatment	Zero- time	2 month	4 month	6 month
Control	0.450±0.010[a]	0.450±0.010[a]	0.420±0.010[ab]	0.400±0.010[b]
Soy tempeh (100%)	0.150±0.010[hgi]	0.150±0.010[hgi]	0.160±0.010[g]	0.153±0.0058[gh]
Soy tempeh (70%)	0.300±0.10[d]	0.210±0.100[f]	0.220±0.100[ef]	0.200±0.100[ef]
Soy tempeh (50%)	0.350±0.010[c]	0.3300±0.010[cd]	0.3000±0.010[d]	0.3200±0.010[cd]
Chickpea tempeh (100%)	0.110±0.01[j]	0.110±0.01 [j]	0.110±0.01[j]	0.100±0.01[j]
Chickpea tempeh (70%)	0.120±0.010[ji]	0.116±0.0153[ji]	0.110±0.010[j]	0.110±0.010[j]
Chickpea tempeh (50%)	0.250±0.010[e]	0.250±0.010[e]	0.230±0.010[ef]	0.220±0.010[ef]

LSD; least significant difference. (at the letters on the above numbers showed that significantly difference between them 0.05).

TABLE 9.Total bacterial count (cfu/g) of burger treatments during frozen storage at - 18 ± 2°C for 6 months

Storage period (months)	Treatments						
	Control	Soy tempeh100%	Soytempeh 70%	Soytempeh 50%	Chickpea tempeh 100%	Chickpea tempeh 70%	Chickpea tempeh 50%
Zero	$7x10^3$	$3\ x10^3$	$5\ x10^3$	$6\ x10^3$	$5\ x\ 10^3$	$4\ x10^3$	$5\ x10^3$
2	$5x10^3$	$2.5\ x10^3$	$2.5\ x10^3$	$5.5\ x10^3$	$3\ x10^3$	$8.5\ x10^3$	$3.5\ x\ 10^3$
4	$4\ x10^4$	$2\ x10^4$	$5\ x10^4$	$1\ .5x10^4$	$2.5\ x10^3$	$3\ x10^2$	$4x104^4$
6	$9x10^4$	$7\ x10^4$	$7\ x10^4$	$8\ x10^4$	$4\ x10^4$	$6\ x10^4$	$5\ x10^4$

The spoilage of tempeh is without difficulty detectable with the aid of looking on the coloration, texture and aroma. Therefore, an expiration date isn't always necessary (Kustyawati et al., 2020). Also, all burger treatments were free from proteolytic, lipolytic bacteria and coliforms (0.0 cfu/g) immediately after the processing and at all storage time. No significant growth of typical colonies of bacteria that could affect the microbiological quality of the product, making tempeh safe for consumption.

The bacterial population in soybean tempeh was negligible especially compared to other parameters such as soybeans cooking method and hygiene of the facility (Pramudito etal., 2021).Generally, microbial quality criteria of all burger samples had been within permissible counts reported by Egyptian Standards (2005), which recommend that the total bacterial counts had been no longer exceed 5X10 CFU/g, so good production practices also contribute to the absence of pathogenic bacteria, as well as storage conditions.

Sensory evaluation of tempeh burger

Data in Table 11 show the average scores of sensory evaluation of tempeh burger groups after preparation. It could be noticed that, no significant different was found between control group and soy tempeh 50% in color, flavor, odor, and overall acceptability. Also, chickpea tempeh 50% showed no significant different in flavor, odor and overall acceptability compared to control group. Lakshmy et al. (2015) reported that high palatability and acceptability of tempeh products is mainly due to the development of improved flavors and texture due to fermentation. The cultures used in food fermentations are also contributing secondary reactions to the formation of precise flavor and texture. During fermentation, several volatile compounds are formed which to a complex blend of flavors in products. Although all tempeh burger treatments were acceptable but that soy tempeh 50% appears to be the best in sensory evaluation followed by chickpea tempeh 50% which recorded the highest scores in all attributes properties. This means that beef can be replaced by up to 50% of chickpea or soy tempeh without affecting the taste or general acceptance of the product. Fibri & Frost (2020) also reported that the change in sensory evaluation scores when product information was provided, according to raw materials (bean type) included, origin (local or imported), and production methods.

TABLE 10. Psychrophilic bacteria count (cfu/g) of burger treatments during frozen storage at-18 ±2°C for 6 months

Storage period (months)	Treatments						
	Control	Soy tempeh 100%	Soy tempeh 70%	Soy tempeh 50%	Chickpea tempeh 100%	Chickpea tempeh 70%	Chickpea tempeh 50%
Zero	$6x10^1$	$2 x10^1$	$2 x10^1$	$3 x10^1$	$3.5 x 10^1$	$3.5 x10^1$	$4x10^1$
2	$9x10^1$	$2 x10^1$	$2.5 x10^1$	$2.5 x10^1$	$3 x10^1$	$4 x10^1$	$4.5 x 10^1$
4	$4x10^2$	$1 x10^2$	$2 x10^2$	$2 x10^2$	$1 x10^2$	$2 x10^2$	$4x10^2$
6	$8x10^2$	$1.5 x10^2$	$2.5 x10^2$	$3 x10^2$	$2.5 x10^2$	$3.5 x10^2$	$4.5 x10^2$

TABLE 11. Sensory evaluation of tempeh burger blends

Treatments	Color	Flavor	Odor	Softness	Texture	Appearance	Overall acceptability
Control	$8.9±0.57^a$	$8.70±0.48^a$	$8.90±0.57^a$	$9.00±0.47^a$	$9.00±0.47^a$	$9.00±0.47^a$	$8.90±0.57^a$
Soy tempeh (100%)	$6.00±1.05^e$	$6.10±1.10^c$	$5.90±1.10^c$	$5.90±0.99^d$	$5.70±1.16^e$	$5.70±1.06^e$	$6.00±1.05^d$
Soy tempeh (70%)	$7.60±0.52^{dc}$	$7.50±0.53^b$	$7.60±0.52^b$	$7.40±0.70^c$	$7.50±0.53^c$	$7.60±0.52^c$	$8.00±0.67^b$
Soy tempeh (50%)	$8.40±0.52^{ab}$	$8.40±0.52^a$	$8.30±0.67^a$	$8.20±0.63^b$	$8.30±0.67^b$	$8.40±0.52^b$	$8.30±0.67^{ab}$
Chickpea tempeh(100%)	$5.70±0.67^e$	$5.50±0.53^c$	$5.70±0.67^c$	$5.70±0.67^d$	$5.50±0.53^e$	$5.50±0.53^e$	$5.60±0.52^d$
Chickpea tempeh(70%)	$7.30±0.67^d$	$7.00±0.67^b$	$7.20±0.63^b$	$7.20±0.63^c$	$6.90±0.57^d$	$6.80±0.63^d$	$7.30±0.67^c$
Chickpea tempeh(50%)	$8.20±0.63^{bc}$	$8.30±0.67^a$	$8.30±0.67^a$	$8.30±0.67^b$	$8.00±0.47^b$	$8.30±0.67^b$	$8.30±0.67^{ab}$
LSD 5%	0.6115	0.6011	0.6379	0.6239	0.5969	0.5861	0.6329

LSD, least significant difference (at the letters on the above numbers showed that non significantly difference between them.0.05).

Conclusion

The production of legume-based tempeh can promote products made from common beans, giving an opportunity to the traditional food. Fermentation process by *R. oligosporus* affected physical and functional properties of legumes on essential components, and can be consumed by Vegetarians and sympathizers. Generally, it could be concluded that tempeh beef burger like, As a very good functional and dietary properties meat replacer, 50,70 and 100% of meat weight used in beef burger like formulations resulted in producing burger. It has a high content of carbohydrates, calories, protein and a good source of fibers. It is also a good source of many bioactive components. The soybean tempeh burger 50%and chickpea tempeh burger 50% confirmed better rankings in all attributes evaluated sensory evaluation.

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جودة البرجر المصنوع من التيمبا

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الهدف من هذه الدراسة هو استخدام التيمبا (فول الصويا والحمص) لإعداد برجر لحم صحي وتحسين خصائص الجودة وثبات التخزين للبرجر . تمت دراسة صفات الجودة ، وإمكانية الحفاظ على الماء (WHC) وقياسات الطهي (الفقد اثناء الطهي ، وإنتاجية الطهي,الانكماش والاحتفاظ بالرطوبة) لبدائل برجر اللحم (تيمبا فول الصويا ، والحمص) ، بمستويات مختلفة (٥٠ ، ٧٠ ، ١٠٠٪) كلا على حدا وأظهرت النتائج أن برجر لحم تيمبا يحتوي على ٥٤,٧٠ و ٤٦,٩٨٪ من الأحماض الأمينية الأساسية لفول الصويا والحمص للتيمبا على التوالي ، كما وجد أن البرجر المكون من ٥٠٪ من تيمبا الصويا يحتوى على أعلى نسبة بروتين (٥٠,٩٠٪) وسعرات حرارية (٤١٧,٧٨ كيلو كالورى / ١٠٠ جم). مقارنة بمجموعات التيمبا الأخرى. أظهر البرجر المصنوع من تيمبا فول الصويا والحمص زيادة في محتوى الألياف والكربوهيدرات بينما البروتين والرماد نفس النسبة . كانت نسبة (الحديد والكالسيوم) فى برجر التيمبا أعلى بكثير من تلك الموجودة بعينة الكنترول : أظهرت النتائج زيادة معنوية في قيم الاحتفاظ بالرطوبة وإنتاجية الطهي و انخفاض معنوي في قيم الفقد و الانكماش اثناء الطهي ويرجع ذلك لإضافة التيميا (فول الصويا والحمص مقارنة بالكنترول . كما أظهر التقييم الحسي أن برجر التيمبا بنسبة ٥٠٪ (كلا النوعين) كان له أعلى درجة من القبول الحسي للتقييم. كانت معايير الجودة الميكروبية لجميع عينات البرجر ضمن الأعداد المسموح بها طبقا للمواصفات القياسية المصرية. بشكل عام ، وجد ان برجر التيمبا مفضل لدى المستهلك فيما يتعلق بجميع الخصائص الحسية. وأخيرًا ، يوصى باستخدام التيمبا كبديل للحوم لإعداد برجر لحم صحي بتكلفة أقل مع تحسين خصائص الطهي والمعايير الحسية