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Improvement of Functional Properties of Soybean Incorporated Meat Beef Burger

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Abstract: Soy and soy foods are common nutritional solutions for vegetarians, due to their high protein content and versatility in the production of meat analogues and milk substitutes. The aim of this investigation is to evaluate the effect of utilizing germinated soybean flour (GSF) as a potential ingredient in producing functional beef burgers anti-oxidative and improvement of functional properties of soybean on quality of meat beef burger. Physio-chemical properties and antioxidant activity of germinated and non-germinated soy bean were investigated. Raw and germinated soy bean for24, 48 and 72 hrs incorporated beef burger formulas. Replacing SF with concentration (10 %) of meat on the quality characteristics of beef burger was studied. Germination for 48 hrs increased protein digestibility to 86.80% with increment 55.55%, against stachyose and raffinose reduction. Total flavonoids 12.5 (mg Quercetin/g DW), total phenolic compounds 43.76 (mg GAE/g DW) and antioxidant activity 71.83 % compared to dry seeds. The incorporation of GSF into beef burger processing resulted in enhancing both moisture and protein contents of cooked beef burger. Also, treatments containing GSF had producing beef burger with a good cooking and sensory properties strengthened more than both of control treatments or treatments containing of raw soybean. Our results concluded that germinated soybean flour 48hr and 72hr are recommended to be a high potential based-meat ingredient to be utilized in production of functional beef burger in addition to its health and nutritional benefits.

Key words: Beef burger · Soybean · Germination · Digestibility · Cooking quality · Sensory Evaluation

INTRODUCTION

Legume seeds are important sources of energy and protein in many parts of the world, both in animal and human nutrition [1]. However, their nutritional value may be compromised in part by the presence of undesirable components, known as antinutritional factors (ANFs) [2]. antinutritional factors (ANFs) compounds interfere with metabolic processes and nutrient availability thereby, leading to the low acceptance and utilization of soybean products [3, 4].

Globally, soybean (Glycine max) seed is one of the largest sources of vegetable seed oil and protein in the feed and food industry and contains about 40% crude protein and 20% oil. It is also a source of calcium, iron, carotene and ascorbic acid. Soybean oilcake meal has become the principal protein supplement for livestock in many countries and is one of the legumes that is most frequently used in the poultry industry [5]. However, soybeans contain undesirable components such as lipoxygenase and trypsin inhibitors that limit their utilisation. Trypsin inhibitor is an ANF that affects protein digestibility [6]. A high level of trypsin inhibitors in a diet stimulates pancreatic juice secretion and causes pancreatic hypertrophy and poor growth in animals [7].

Processing legumes with heat is a quick technique for decreasing or destroying ANFs, but strict guidelines must be applied for the treatment to be effective. An optimal processing temperature must be applied, as overheating can cause protein and amino acid damage. On the other hand, under processing does not completely destroy ANFs. Heating causes considerable losses in soluble solids, especially vitamins and minerals. Increasing the time and temperature of processing has been reported to reduce the nutritive value and available lysine of legumes [8]. These processes are however affected by many and varied reports on the influence temperature-time combinations on the ANFs and amino acids profile of

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soybean among other constraints. The lack of standardisation of cooking time and temperature regimes and high technology required for either autoclaving, extrusion, micronization, infrared and other thermal based processing methods as well as the energy demand for these processes and the effect of heat on the nutrient content of the full fat soybeans posed serious challenge to average feed processors and small scale poultry farmers. Some deleterious effects of heat treatment on the nutrient and amino acid composition of soybean have also been reported by Sadiku and Jauncy [9], as nutritional losses are often associated with thermal treatments of soybean with 10°C increase in temperature [10].

Expensive facilities and equipment are required for the proper heat treatment of legumes, putting this technique out of reach of the average small-scale farmer in remote areas.

Germination and Traditional processing methods of soybeans such as, soaking and dehulling are used to reduce or eliminate the ANFs that affect protein utilization, also enhance the nutritive value of legumes by inducing the formation of enzymes that eliminate or reduce the antinutritional and indigestible factors in legumes [11].

soy bean are added to raw or cooked meat products to improve its functional properties, minimize the product cost and improving or at least maintaining nutritional and sensory qualities of end products that consumers expect [12-15]. Soy protein is one of the most widely used vegetable proteins in meat industry due to its various technological benefits, whereas it plays a significant role in the modification of the functional characteristics of meat products. It can also be used to replace part of the animal fat. With its hydrating capacity, soy protein can considerably decrease the final cost of the meat products Despite the many advantages of soybean, its use has been limited because of the characteristic beany flavor [16]. Green vegetables occupy an important role in human nutrition as they provide essential minerals and vitamins [17]. Vegetables could also serve as fillers, binders, fat replacers and sources of dietary fiber and natural antioxidants in a meat system [18]. Moreover, extension of meat products with green vegetables could reduce production costs and improve the nutritional qualities of the products [19].

For these reasons, the aim of this study use germination technique to reduce anti nutritional factors of soybeans and studying effect replaced it with germinated soybeans in meat products.

MATERIALS AND METHODS

Raw Materials: Beef meat and camel fat were obtained from local market, Egypt. Immediately after purchasing, samples were transported using ice box to the laboratory of Meat and Fish Technology, Food Technology Research Institute, Agriculture Research Center, Giza, Egypt. Other ingredients such as salt, onion, spices, garlic and starch were purchased from local market at Giza.

Preparation the Germinated Soy Bean: Germination technique was performed according to Kayembe and Jansen [20] with some modifications where germination process was achieved by soaking the seeds for 12 h. afterwards, they were spreaded on trays and covered with piece of wet cotton to exclude light. Three groups of seeds were kept at room temperature and allowed to germinated for 24 h,48 h and 72 h, respectively. Water was applied once daily to provide moisture during germination. Thereafter, the germinated soybean were dried for 72 h (at 45±2°C) in drying oven, grind and then kept at room temperature pending further analysis.

Processing the Meat Burger: Meat was washed, cut, minced (in mincer) and mixed with other ingredients (minced meat, fat, water, sodium tri-ploy phosphate, salt, dried garlic, minced onion, cumin, starch and spices), then divided into five groups (G1, G2, G3 G4 and G5) as in Table (1).

All groups were good mixed then put into burger manufacturing machine then packaged in foam dishes then wrapped by polyethylene bags, kept frozen at -18°C until analysis.

Chemical Analysis: Proximate analysis including moisture, total protein, fat and ash were carried out according to the methods of AOAC [21]. Carbohydrates content was calculated by difference.

Stachuose, raffinose and sucrose were determined according to the method of Acacia *et al*. [22].

In-vitro protein digestibility of soy bean treatments was determined according to Saunders *et al*. [23] and Alberta and Joyce [24]. Where, Hydrolysates of the raw and GSF were prepared using the pH-drop/multi-enzyme (trypsin-α-chymotrypsin-peptidase), sequential (pepsin-pancreatin) and simulated gastric and intestinal (pepsin-trypsin-α-chymotrypsin) digestion techniques.

Physical Properties: Water Holding Capacity (WHC) and plasticity were measured during storage period according to the filter press method of Soloviev [25].

Table 1: Ingredients (%) used in the preparation of different meat burger formulas

%	G1	G2	G3	G4	G5
Soy bean	10.0% of non soybean germinated	10.0% of soybean germinated for 24 h	10.0% of soybean germinated for 48 h	10.0% of soybean germinated for 72 h	10.0% of commercial soybean flour
Minced meat	62.5	62.5	62.5	62.5	62.5
Fat	10.0	10.0	10.0	10.0	10.0
Water	6.0	6.0	6.0	6.0	6.0
Sodium tri-poly phosphate	0.3	0.3	0.3	0.3	0.3
Salt	1.5	1.5	1.5	1.5	1.5
Dried garlic	1.0	1.0	1.0	1.0	1.0
Minced onion	4.7	4.7	4.7	4.7	4.7
Cumin	1.0	1.0	1.0	1.0	1.0
Starch	1.0	1.0	1.0	1.0	1.0
Spices	2.0	2.0	2.0	2.0	2.0

The cooking loss and Shrinkage were determined as the method described by AMSA [26].

Total phenols were estimated by the Folin-Ciocalteu method reported by Elfalleh *et. al*. [27]. The amount of total flavonoids was measured spectrophotometrically according to Nasri *et. al.* [28]. The DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity of methanolic extracts was determined following the method reported by Okonogi *et. al.* [29].

Organoleptic Evaluation: Organoleptic evaluation of meat burger was carried out according to Watts *et al.* [30].

Texture Profile Analysis: Texture Profile Analysis was determined according to Bourne [31].

Statistical Analysis: Data were subjected to Analysis of Variance (ANOVA). Means comparison was performed using Duncan's test at the 5% level of probability as reported by Snedecor and Cochran [32].

RESULTS AND DISCUSSION

The presented data in Table (2) pointed the chemical composition and protein digestibility of non soybean germinated and germinated soybean for 24h to 72 h, it could be concluded that seeds content from protein and moisture increase as percentage with increasing the germination period, it could be noticed that no significant differences of moisture content between D1, D2, D3 and C samples, while found significant differences of moisture content between R samples and D1, D2, D3 and C samples. On the other hand, noticed significant differences of protein content between all samples, it was also found that, treatment D3 has the highest content of protein and moisture when compared with treatments D2, D1 and R respectively. This results comply with the obtained results by Kayembe and Jansen [20] who found that crude protein increased as the number of days of germination of soybeans increased and was higher than the crude protein content of raw beans, Kaushik *et al*. [1] also reported an increase in crude protein with germination. Similar increases in protein have been reported for other legumes such as lablab beans Osman [33], mung beans Mubarak [34], faba beans and kidney beans Alonso *et al*. [35]. The apparent increase in protein can be attributed to the utilization of carbohydrates as an energy source for developing sprouts [20, 2].

From the same Table (2), it could noticed that fat, carbohydrate and ash content decreased in all treatments (D1, D2 and D3) and the rate loss increased with increasing the germination period, where significant

Table 2: Chemical composition of raw and germinated soybean (on wet weight) and protein digestibility.

	Moisture %	Protein %	Fat %	Ash %	Carbohydrate%	Digestibility (%)
R	$4.95^{b}\pm0.70$	$36.40^{e}\pm0.35$	$17.11^{a}\pm0.45$	$5.98^{b}\pm0.56$	$35.56^{a}\pm0.95$	$55.8^{e}\pm0.63$
D1	$6.30^{a}\pm0.55$	$39.61^{d}\pm0.21$	$16.44^{ab}\pm0.43$	$5.84^{b}\pm0.38$	$31.81^{bc}\pm0.80$	$60.75^{d}\pm0.7$
D2	$6.48^{a}\pm0.57$	$41.50^{c}\pm0.20$	$15.72^{bc}\pm0.41$	$5.70^{b}\pm0.25$	$30.60^{cd}\pm0.75$	$72.30^{c}\pm0.65$
D3	$6.98^{a}\pm0.55$	$42.26^{b}\pm0.30$	$15.39^{c}\pm0.35$	$5.56^{b}\pm0.22$	$29.81^{d}\pm0.73$	$79.0^{b}\pm0.8$
C	$7.43^{a}\pm0.9$	$47.85^{a}\pm0.50$	$5.93^{d}\pm0.44$	$6.35^{a}\pm0.5$	$32.44^{b}\pm0.98$	$86.80^{a}\pm0.75$
LSD	1.117	0.601	0.759	0.321	1.545	1.289

The values in same a column followed by the same letter are not-significantly different ($P\leq 0.05$).

R= non soybean germinated;

D1= soybean germinated for 24 h;

D2= soybean germinated for 48 h

D3=soybean germinated for 72 h;

C= commercial flour of soybean defatted

Table 3: Total phenolic, total flavonoids, antioxidant activity and DPPH of dry, soaked and germinated seeds

Treatments	Total phenolic (mg GAE/g DW)	Total flavonoids (mg quercetin/g DW)	DPPH (%)
R	$34.6^{d}\pm0.48$	$7.12^{e}\pm0.41$	$55.3^{e}\pm0.75$
S	$30.15^{e}\pm0.53$	$8.05^{d}\pm0.32$	$59.5^{d}\pm0.63$
D 1	$36.7^{c}\pm0.45$	$10.45^{c}\pm0.28$	$67.8^{c}\pm0.7$
D 2	$39.51^{b}\pm0.46$	$11.75^{b}\pm0.35$	$75.77^{a}\pm0.58$
D 3	$43.76^{a}\pm0.5$	$12.5^{a}\pm0.45$	$71.83^{b}\pm0.65$
LSD	0.882	0.602	1.209

The values in same a column followed by the same letter are not-significantly different ($P\leq 0.05$).
R= non soybean germinated
S = soybean Soaking for 24 h D1= soybean germinated for 24 h
D2= soybean germinated for 48 h D3=soybean germinated for 72 h

Table 4: Anti- nutritional factors of dry and germinated soy bean

(% Dry matter)	R	S	D 1	D 2	D 3
Stachyose	4.05	4.00	3.20	2.40	1.52
Raffinose	0.89	0.80	0.71	0.50	0.33
Sucrose	4.50	4.23	3.95	3.54	3.06

R= non soybean germinated
S = soybean Soaking for 24 h D1= soybean germinated for 24 h
D2= soybean germinated for 48 h D3=soybean germinated for 72 h

differences in fat and carbohydrate contents were found between all samples, while no significant differences in ash content were observed between R, D1, D2 and D3 samples, while significant differences in ash content were found between of C samples and R, D1, D2 And D3 sample from the other side. From results, it could be noticed that fat, carbohydrate and ash content of raw seeds were higher than D1, D2 andD3 respectively. May be attributed this the apparent decrease of fat, carbohydrate content to the utilization of fat and carbohydrates as an energy source for developing sprouts. These results are in agreement with those reported by Kayembe and Jansen [20] for germinated soybean and Mubarak [34] and El-Beltagy [36] for germinated mung bean and El-Adawy [37] for germinated chickpea. On the other hand, soy protein digestibility value significantly increased from 55.8 ±0.63 of the dry bean to the highest value 86.8±0.75at the end of germination 72 hr. Our study indicated that, increase of protein digestibility of soy bean, this may be due to loss of anti-nutritional factors such as stachyose and raffinose contents as in Table (4). Negi *et al*. [38] stated that germination improved enzymes protolytic activities such as proteases and phytases by decrease in Anti-nutritional factors which led to increase in protein digestibility.

Total phenols, total flavonoides and DPPH radicals scavenging activity for soybean germination are tabulated in Table (3). Total phenolic compounds of dry soybean was 34.60±0.48 mg gallic acid. This value changed significantly during germination to reach to 30.15±0.53 after 12 hr soaking. At the same trend, significant gradually increase in total phenols to reach to the maximum value 43.76±0.5after 72 hr germination. Also, total flavonoides (mg quercetin □ g DW) content were significantly increased from 7.12±0.41 of dry soybean to reach the maximum value of 12.5±0.45 after72 hr of germination. At the same line, DPPH radicals scavenging activity was 55.3±0.75 of dry soybean and significantly gradually increased to reach the highest value (71.83±0.63) at the end of germination time (72 hr). Phenolic compounds have the vital role inside food to prevent the oxidation process of food and in human body as protection role against oxidative damage. Total phenolic and flavonoide compounds increased during germination times. This effects may be due to new phenolic and flavonoide compounds that were found as a result of break-down process and synthesis during germination [39].

Antioxidant activity of soybean increased during germination periods compared to dry bean, this may be due to the increased quantities of phenolic, flavonoide compounds and the other biological compounds such as small peptides resulted in degradation of protein which act as a reducing agent, hydrogen donors and chelating potential [40].

Anti-nutritional factors and protein digestibility are in shown in Table (4). From these results, it could be observed that, stachyose significantly decreased from 4.05 %of dry bean to reach the lowest value 1.5 at the end of germination time (72 hr). Also, raffinose content significantly gradually decreased from 0.89% of dry bean to the lowest value after 72 hr of germination.

Table 5: Chemical composition of processed meat burger with adding germinated soybean (on dry weight)

	Moisture	Protein	Fat	Ash	Carbohydrate
G1	61.52^{a}±0.31	15.30^{d}±0.7	14.30^{a}±0.18	3.53^{a}±0.5	5.35^{a}±0.7
G2	61.68^{a}±0.27	15.62^{d}±0.65	13.98^{ab}±0.20	3.43^{a}±0.4	5.29^{a}±0.71
G3	61.61^{a}±0.30	15.95^{c}±0.70	13.90^{b}±0.15	3.31^{a}±0.4	5.23^{a}±0.63
G4	61.58^{a}±0.23	16.28^{b}±0.60	13.83^{b}±0.17	3.20^{a}±0.45	5.11^{a}±0.65
G5	62.1^{a}±0.50	16.66^{a}±0.80	12.14^{c}±0.25	3.4^{a}±0.55	5.7^{a}±0.77
LSD	0.666	0.318	0.351	0.843	1.262

The values in same a column followed by the same letter are not-significantly different ($P \leq 0.05$).

G1= processed burger meat with adding seeds of non soybean germinated

G2= processed burger meat with adding soybean germinated for 24 h

G3= processed burger meat with adding soybean germinated for 48 h

G4= processed burger meat with adding soybean germinated for 72 h

G5= processed burger meat with adding commercial flour soybean defatted

Table 6: Physical properties of processed meat burger with adding germinated soybean

Treatments	W.H.C (cm^2/0.3 gm)	Plasticity (cm^2/0.3 gm)	Cooking loss %	Shrinkage (cm)
G1	2.1^{b}±0.15	3.1^{c}±0.18	26.14^{a}±0.12	29.85^{a}±0.95
G2	1.8^{c}±0.13	3.3^{c}±0.16	26.11^{a}±0.9	29.85^{a}±0.85
G3	1.65^{c}±0.15	3.6^{b}±0.11	25.01^{b}±1.0	26.83^{b}±.80
G4	1.35^{d}±0.12	3.9^{a}±0.14	25.85^{b}±1.0	27.41^{b}±0.7
G5	2.45^{a}±0.20	2.8^{d}±0.15	26.62^{a}±1.35	29.95^{a}±1.2
LSD	0.277	0.272	1.1753	1.662

The values in same a column followed by the same letter are not-significantly different ($P \leq 0.05$).

G1= processed burger meat with adding non soybean germinated

G2= processed burger meat with adding soybean germinated for 24 h

G3= processed burger meat with adding soybean germinated for 48 h

G4= processed burger meat with adding soybean germinated for 72 h

G5= processed burger meat with adding commercial flour soybean defatted

W.H.C. : Water Holding capacity

Anti-nutritional factors play a vital role in the soy bean during germination, that anti-nutritional factors inhibits soy protein protolytic [41].

Chemical composition results of processed meat burger with adding germinated seeds of soybean are shown in Table (5). it could be noticed that there was no significant differences of moisture, ash and carbohydrates content between all samples, while significant differences in protein and fat content were found between all samples. The data showed that G5 was the highest in protein content followed by G4,G3,G2 and G1 respectively, while moisture content of G5 was the highest when compared with the other blends followed by G2,G3, G4and G1 respectively. It could be noticed that fat content was the lowest in blend (G5) followed by G4,G3,G2 and G1. Ash content of G1 was the highest when compared with the other blends followed by G2,G5,G3 and G4. Also, carbohydrate content of G5 was the highest when compared with the other blends followed by G1,G2,G3 and G4 respectively. This may be attributed to protein, moisture, fat, ash and carbohydrate content in added flour soybean in each blend as Table (2) which affected chemical composition of processed meat burger with adding germinated seeds of soybean in each blend.

The obtained results shown in Table (6), pointed that the WHC, plasticity, shrinkage and cooking of processed meat burger with adding germinated seeds of soybean. WHC is defined as water holding capacity and not easily lost and it is an indicator of the quality of the meat products. There is a direct relationship between WHC, plasticity and shrinkage and an inverse relationship with the cooking loss.

From this results it could be noticed that G4 was the best significant value of WHC, plasticity, Shrinkage and Cooking loss (as quality properties) followed by G3, G2, G1 and G5. This may be due to G4 is higher of protein and lower of lipid content when compared with G3, G2, G1. It is known that, fat is a hydrophobic substance (water repelling and do not blend with water with emulsifying, while the protein work as binder for water).It could be noticed that sample G5 had the highest content of protein also had the lowest value of plasticity, shrinkage and cooking loss (as quality properties).This may be attributed to the development of protein denaturation during processing of the commercial flour soybean defatted by heating which affected protein capacity as binder for the water while protein quality do not affected during germination process which improved functional

Table 7: Texture profile analysis of processed meat burger with adding germinated soybean

Treatments	Hardness (N)	Springiness (mm)	Gumminess (N/mm2)	Chewing (N/mm)	Cohesiveness (N)	Adhesiveness (MJ)
G1	12.64	0.191	6.79	14.968	0.40	29.51
G2	12.26	0.188	6.71	13.821	0.40	29.48
G3	10.93	0.179	6.66	13.003	0.43	29.45
G4	9.99	0.173	6.55	12.850	0.44	29.43
G5	13.15	0.218	7.05	14.983	0.40	29.82

G1= processed burger meat with adding non soybean germinated

G2= processed burger meat with adding soybean germinated for 24 h

G3= processed burger meat with adding soybean germinated for 48 h

G4= processed burger meat with adding soybean germinated for 72 h

G5= processed burger meat with adding commercial flour soybean.

Table 8: Organoleptic evaluation of processed meat burger with adding germinated soybean

Treatments	Color	Taste	Aroma	Texture	Overall Acceptability
G1	$8.0^{a}\pm0.20$	$7.75^{ab}\pm0.25$	$8.0^{a}\pm0.24$	$8.0^{c}\pm0.25$	$7.94^{b}\pm0.20$
G2	$8.0^{a}\pm0.10$	$7.85^{ab}\pm0.25$	$8.0^{a}\pm0.22$	$8.0^{c}\pm0.23$	$7.96^{b}\pm0.15$
G3	$8.0^{a}\pm0.215$	$8.0^{a}\pm0.20$	$8.0^{a}\pm0.15$	$8.15^{b}\pm0.15$	$8.04^{a}\pm0.15$
G4	$8.0^{a}\pm0.215$	$8.0^{a}\pm0.10$	$8.25^{a}\pm0.15$	$8.35^{a}\pm0.15$	$8.15^{a}\pm0.15$
G5	$8.0^{a}\pm0.20$	$7.5^{b}\pm0.35$	$8.0^{a}\pm0.20$	$8.0^{c}\pm0.25$	$7.875^{b}\pm0.25$
LSD	0.3475	0.4437	0.3555	0.193	0.162

The values in same a column followed by the same letter are not-significantly different ($P\leq 0.05$).

G1= processed burger meat with adding non soybean germinated

G2= processed burger meat with adding soybean germinated for 24 h

G3= processed burger meat with adding soybean germinated for 48 h

G4= processed burger meat with adding soybean germinated for 72 h

G5= processed burger meat with adding commercial flour soybean

properties of protein and protein capacity as binder for the water. These results are in agreement with those reported by Mahmoud *et al*. [42]; Shahin *et al*. [43] and Mahmoud *et al*. [44].

Texture profile analysis (TPA) for meat burger samples were determined as hardness, cohesiveness, gumminess, chewing, springiness and adhesiveness values (Table 6), where hardness (N) = maximum force require to compress the sample, springiness (mm) = ability of sample to recover its original form after a deforming force was removed (S), cohesiveness (N) = extent to which sample could be deformed prior to rupture (E1/E2, E1 being the total energy require for the first compression and E2 the total energy require for the second compression, gumminess (N/mm2) = force necessary to disintegrate a semisolid sample for swallowing (Firmness × cohesiveness), chewing (N/mm) = work to masticate the sample for swallowing (springiness × gumminess) Lukman *et al*. [45].

Results of Texture profile analysis indicated that G4 samples were softer and tender followed by G3, G2, G1 and G5 samples. Whereas, G4 samples had lowest values of hardness, gumminess, springiness and chewing, also G4 samples had highest values of cohesiveness and adhesiveness, this may be due to G4 samples had the highest values of plasticity (Table 5). These results were in agreement with the scores given for texture of (G4 samples) which had the higher scores then the others samples (Table 8).

From the obtained results in Tables (8) for Organoleptic evaluation attributes, it could be noticed that, there was no significant differences between all samples for color and aroma properties, while G4 samples was the significantly highest for taste, texture and overall acceptability properties followed by G3,G2,G1 and G5 respectively.

CONCLUSION

In conclusion, the results suggested that germinated soybean flour 48hr and 72hr are recommended to be a high potential based-meat ingredient for utilize in production of functional beef burger in addition to its health and nutritional benefits with acceptable physical and sensory quality.

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