

Replacement of meat by mycoproteins in cooked sausages: Effects on oxidative stability, texture,

and color

Narges Shahbazpour¹, Kianoush Khosravi-Darani^{2*}, Anousheh Sharifan¹, Hedayat Hosseini²

¹Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran; ²Department of Food Science and Technology, Faculty of Nutrition Science and Food Technology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran

***Corresponding Author:** Kianoush Khosravi-Darani, Department of Food Science and Technology, Faculty of Nutrition Science and Food Technology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email: k.khosravi@sbmu.ac.ir, kiankh@yahoo.com

Received: 23 June 2021; Accepted: 16 November 2021; Published: 12 December 2021 © 2021 Codon Publications



PAPER

Abstract

Processed meat is one of the most consumed products worldwide. Naturally, production of proteins with animal origins includes limitations such as costs, energy, time, and environmental problems. Thus, replacement of meats by alternative biomaterials such as mycoproteins can be promising. Mycoproteins with hyphal morphologies, including branches and lengths, have close structures to meat and can be a potential alternative for meat products. Therefore, the major objectives of this study included complete replacement of sausage meats by mycoproteins and comparing characteristics of the novel formula with those of meat. In general, physicochemical, microbial, nutritional, and mechanical characteristics of the formulas were assessed. Results showed that the mycoprotein substitution improved the nutritional and health effects due to the higher valuable protein and lower lipid contents. Besides, it had a high content of essential amino acid and unsaturated fatty acid, compared to meat sausage. Absence of yeasts, molds, Salmonella spp., Eshrichia (E.) coli, and Staphiloccocus (S.) aureus verified the effectiveness of the heat treatment and also the effectiveness of the hygienic procedures in both samples. With regard to phycicochemical properties, more contents of moisture and lipids in sausages containing mycoprotein were linked to further water binding capacity (WBC) (P < 0.05) and oil binding capacity (OBC) in them, compared to beef samples. Besides, the mycoprotein sample had lower (P < 0.05) values of carbohydrates, ash, and pH, compared to the beef sample. In contrast, beef sausages had better textural characteristics, such as hardness, cohesiveness, gumminess, and springiness indexes, compared to mycoprotein sausages. Higher water and OBC values of the mycoproteins led to the filling of the protein interstitial spaces as well as decreasing of the textural attributes. Thus, it resulted in the use of less oil and water in mycoprotein formulations. In conclusion, mycoproteins can be addressed as appropriate replacements for meats in sausages.

Keywords: meat alternatives; mycoproteins; nutritional values; sausages; textural properties

Introduction

In recent decades, the world population has significantly increased from 2.6 to 8 billion individuals (Gabriel *et al.*, 2014). If the global population grows at the recent rate, it may reach 9 billion individuals by 2042, which could pose

serious problems in providing food to all (Upadhyaya *et al.*, 2016). Approximately, 1 billion people globally will not be able to properly access food sources with sufficient energy and proteins (Godfray *et al.*, 2010). This can result in serious medical problems such as defective immune system and stunted growth. In contrast, high

consumption of meat products can pose serious health problems as well (Qian et al., 2020). Research and development of meat replacements majorly focuses on the production of products that imitate the physical characteristics of meat such as appearance, taste, and texture, as well as providing its nutritional values. Muscle products, such as chicken and steaks, minced products, such as burgers and nuggets, and emulsion products, such as Frankfurter and Mortadella sausages, are the major meat replacements (Kyriakopoulou et al., 2021). However, excessive meat consumption can significantly affect the global climate change (Hashempour-Baltork et al., 2020b). Thus, the quest for novel substitutes for animal proteins requiring less financial resources, energy, and time consumption can be promising. One of the best meat substitutes includes mycoproteins with relatively similar textures to meat (Upadhyaya et al., 2016). The common source of mycoproteins is usually Fusarium (F.) venenatum, a filamentous fungus generally recognized as safe (GRAS) (Hashempour-Baltork et al., 2020b). Generally, mycoproteins contain 10 g of carbohydrates, 13 g of fats, 25 g of fibers, and 45 g of proteins, as well as various vitamins, carotenes, minerals, and essential amino acids (EAA) in 100 g of dry matter (Finnigan et al., 2019). Recent studies on human volunteers have demonstrated that biological values of proteins from mycoproteins are similar to biological values of proteins from milks.

Commonly, sensory characteristics, such as texture, taste, and overall appearance of the final products, are critical for their overall acceptance. Fusarium biomass is virtually odorless and tasteless and is appropriate to imitate the consistency and taste of regular meats. Comparing mycoproteins with textured soya and poultry muscle tissues, recent studies using microscopy techniques have shown that large cable-like fibers (especially in vegetable protein textures) result in rubbery textures, which are unfavorable when chewing. Technically, further fibrous and rubbery eating qualities occur in poultry and mycoprotein products due to their tight packaging laminations, compared to those that occur in products of vegetable protein origins (Hashempour-Baltork et al., 2020b). Ideally, mycoproteins can be good replacements for meat since their dry weights include nearly 50% of proteins, similar to grilled steaks. However, the fungi include lower fat quantities (~13%) than those steaks do. Furthermore, this is a vegetable fat with cholesterol alternative (ergosterin) and good fiber content (~25%), which are increasingly accepted by the health-conscious people (Finnigan et al., 2017). Based on the literature, mycoproteins can mitigate the problem of food unavailability worldwide. Moreover, even routine production of mycoproteins would need only lower water resources and occupy less land (Hashempour-Baltork et al., 2020b). The use of mycoproteins can limit foodborne diseases and lower blood cholesterol. Toxin analysis and allergy assays have shown no general concerns (Hashempour-Baltork *et al.*, 2020a). However, a little information is available on various formulations for the replacement of food meats by mycoproteins. Hence, the major objective of the current study was to compare physicochemical, microbial, nutritional, and mechanical characteristics of sausages containing mycoproteins with those containing meat.

Materials and Methods

Preparation of sausages

Sausage samples (40% red meat) were prepared in three replications in a famous meat production factory, Tehran, Iran. Frozen beef samples were defreezed at 4°C for 16 h before use. Then, beef samples were minced twice using laboratory mincer (Model MK-G1800, Panasonic, Japan) equipped with 6–10 mm steel plates. Mycoprotein masses were provided by Ghazabon Paya, Iran. Two sausages of mycoproteins and meats were prepared for 4 kg batters (Table 1) based on the guidelines from meat producers. Formulations of both samples were mostly similar, with differences in meat and mycoprotein contents.

To prepare sausages, minced beef/mycoprotein was transferred into a bowl chopper (Robot Coupe Model R-10, France) and mixed slowly with the dry ingredients, except spices. Ice was continuously added to the mixture in the chopping process to control the temperature. Then, oil and spices were added to the mixture, respectively. The total time of mixing was 10 min while the final temperature of the batters was set below 12°C. Then, the batters were stuffed into impermeable cellulose casings using a hydraulic piston-type stuffer. Then, the sausages were cooked at 76°C for 60 min using a steam chamber and then cooled down to a final temperature of 10°C using ice-water bath and stored at 4°C overnight (Kamani et al., 2019). In general, each sausage type was prepared in two batches. Totally, two sausages from each batch were chosen for further analysis.

Ingredient	Content (% w/w)
M 1/ 1 ·	40
Meat/mycoprotein	40
Sunflower oil	10
Ice	20
Mixed spices	3.5
Soy protein isolate	5
Gluten	10
Flour	10
Salts	1.5

Physicochemical characteristics

Proximate pH, moisture, protein, lipids, carbohydrate, ash, peroxide value, water holding capacity (WHC), oil binding capacity (OBC), and water binding capacity (WBC) of the two sample types were assessed using official methods (AOCS, 2017).

Microbial characteristics

The count of microorganisms, *Escherichia* (*E.*) *coli*, *Salmonella* spp., *Staphylococcus* (*S.*) *aureus*, *Bacillus* (*B.*) *cereus*, *Clostridium* (*C.*) *perfringens*, yeasts, and molds were enumerated based on the ISO protocols (ISO, 2013).

Nutritional characteristics

Vitamins

Briefly, 10 g of the ground samples were weighed using a 50-mL glass beaker. Then, 20 mL of fresh 5% (w/v) metaphosphoric acid solution were added to the vessel and mixed well. The mixture was then homogenized by stirring at room temperature (RT) for 2 min. The homogenate was centrifuged at 3000 rpm for 5 min; then, the upper solution was filtered using Albet no. 1305 filter papers and re-filtered using 0.45-µm Millipore filters for liquid chromatography (LC) analysis (Valls et al., 2001). Then, stock solutions of 100 μ g mL⁻¹ vitamin B₅ (pantothenic acid), vitamin B_{q} (folic acid), vitamin B_{2} (riboflavin), and vitamin B7 (biotin) (Sigma-Aldrich, St. Louis, MO, USA) were prepared and stored in 4 °C until use. The vitamin content of the sample was assessed using the standard curve, and high-performance liquid chromatography (HPLC) (Waters, USA) was used for the assessment of vitamin B according to Sasaki et al. (2020). The method included the use of Capcell Pak C18 SG120 HPLC Column (250 nm \times 4.6 mm with 5-µm particle sizes) (Osaka Soda, Japan), gradient elution of phosphate buffer-acetonitrile (pH 3), ion-pairing reagent (mobile phase) with 1.0 mL min⁻¹ flow rate and UV detection (210 nm). The vitamin B compound was separated within 60 min. The value of 0.01 μ g g⁻¹ was set as the detection limit.

Amino acids

Briefly, 10 g of dried sausage sample were hydrolyzed using 1 N hydrochloric acid (50 mL) based on an original protocol by Czauderna *et al.* (2003). Amino acid (AA) standards were purchased as a cell-free AA mixture from Sigma-Aldrich, St. Louis, Missouri, USA. The sample was then centrifuged (10,000 g) to collect the hydrolysate.

One-hundred microliters of this hydrolysate were carefully injected into the HPLC instrument (Waters, USA) at 40°C. The HPLC instrument was equipped with Zorbax Eclipse-AAA Column (4.6 mm × 150 mm, 5 µm) (Agilent Technologies, USA) as well as a fluorescence detector. Furthermore, sodium dihydrogen phosphate (NaH₂PO₄) solution (40 mmol l⁻¹) was used in the instrument as Mobile Phase A and acetonitrile:methanol:water solution (45:45:10 v/v/v) as Mobile Phase B.

Fatty acids

Generally, the Folch method (chloroform:methanol 2:1 v/v) was used for the sample lipid extraction (Folch et al., 1957). Fatty acid methyl esters (FAME) were methylated based on the European Official Methods of Analysis (Godfray et al., 2010; Hashempour-Baltork et al., 2018). The FAME were analyzed using gas chromatography (GC) (Agilent 6890, Agilent Technologies, USA) equipped with capillary column (30 m per 0.25 mm ID, 0.25-µm film thickness) and flame ionization detector (FLD) (Thermo TR-5, ThermoFisher Scientific, USA). The instrument used helium (He) as the carrier gas with 0.2 mL min⁻¹ flow rate based on a method described by Hashempour-Baltork et al. (2017). Fatty acid (FA) was identified by comparing the sample and the reference methyl ester chromatograms (Sigma-Aldrich, USA).

Mechanical characteristics

Texture profile analysis (TPA) was performed using Stable Micro Systems Texture Analyzer Model TA.XT Plus (Stable Micro Systems, UK). The analyzer was equipped with a 50-kg load cell. The sample was cut into pieces of 25 mm and axially fixed on the platform. Two-cycle compression assay was carried out with up to 50% of the strain compression of the original height using steel probes. Return speed, distance, and contact force were 2 mm s⁻¹, 50 mm, and 20 g, respectively. The various attributes of the food, including cohesiveness, hardness (N), adhesiveness (N.S), springiness (%), gumminess, chewiness, and springiness, were assessed (Kamani *et al.*, 2019).

Statistical analysis

Descriptive data of the study were recorded as mean \pm SD (standard deviation) using SPSS Software v.17 (IBM Analytics, USA). Duncan's test was used for the comparison of means and different letters represent significant statistical differences (P < 0.05). For each sample of each test, three replicates were used.

Results and Discussion

Physicochemical assessments

Proximate analyses of sausages formulated with mycoproteins and beef are shown in Table 2. Results of moisture contents demonstrated that moisture in sausages formulated with mycoproteins significantly was higher (P < 0.05) than moisture in sausages formulated with beef. Protein and lipid contents seemed to increase with the substitution of mycoproteins in the formulation of sausages. Higher contents of moisture and lipids were linked to further WBC (P < 0.05) and OBC in mycoprotein samples, compared to beef samples. Based on the results, mycoprotein formulation included lower values of carbohydrates, ash, and pH, verified by the previous studies (Hashempour-Baltork et al., 2020). The low pH in mycoprotein samples was associated with low pH of mycoproteins (4.7) in comparison to pH of meat (5.6). In another report, use of mycoproteins significantly increased nutritional values of fish sausages (e.g., ash, carbohydrate, fat, and protein) (P < 0.05) (Bahmani and Movanes, 2021). In this study, the pH of fish sausages enriched with mycoproteins increased during storage (Bahmani and Movanes, 2021). No significant difference (P < 0.05) was generally reported between the two formulations of sausages in WHC and peroxide values (P > 0.05). Characterization of these two sausage samples indicated that lesser oil and water should be used in the formulation due to the higher WBC and OBC values in mycoproteins than beef. In addition to higher OBCs, higher proteins and lower carbohydrates could be used in mycoprotein samples as appropriate meals for obese people. These findings verified previous findings, which addressed mycoproteins as healthy nutritious proteins (Finnigan et al., 2019).

Microbial assessments

Microbiological analysis was carried out on Day 1 after cooking to understand heat behaviors of mycoproteins and compare microbial patterns of the samples. Absence

 Table 2.
 Proximate analysis and pH of beef sausage.

of yeasts, molds, *Salmonella* spp., and *E. coli* verified the effectiveness of the heat treatment in both samples. Due to the absence of *S. aureus*, the hygienic procedures seemed to be effectively preventive. The number of *B. cereus* and *C. perfringens* were similarly reported to be less than 10 cfu g⁻¹ in both samples. Researchers demonstrated that the presence of NaCl and phosphate might inhibit the bacterial growth (Kim *et al.*, 2021). Moreover, a similar report has been published on increased load of *Pseudomonas* spp. in fish sausages enriched with mycoproteins during refrigerated storage (Bahmani and Movanes, 2021).

Nutritional assessments

Vitamins

Levels of vitamins B_2 , B_5 , B_7 , and B_9 were assessed in both samples (Table 3). Contents of vitamin B_9 showed no significant difference (P < 0.05) between the samples. For other vitamins, mycoprotein sausages significantly achieved higher scores (P < 0.05). According to Hashempour-Baltork *et al.* (2020), who comprehensively compared the vitamin B content in meats and mycoproteins, contents of riboflavin, niacin, pyridoxine, and pantothenic acid was 9, 14, 5, and 10 µg g⁻¹ in mycproteins and 0.018, 0.5, 0.052, and 0.35 µg g⁻¹ in meats. These differences were seen in the formulated products as well.

Table 3.	The level of vitamin B group in mycoprotein/beef
sausages	(µg/g).

Treatment	Vit B2	Vit B5	Vit B7	Vit B9
Mycoprotein sausage	3.31 ± 0.5 ^{a*}	0.02 ± 0.05^{a}	8.81 ± 1.45ª	0.48 ± 0.05ª
Beef sausage	1.51 ± 0.5 ^b	0.01 ± 0.04 ^b	2.57 ± 1.5 ^b	0.51 ± 0.03ª

*Mean \pm SD, Different letters represent significant differences (P < 0.05).

Treatment	Moisture (%)	Protein (%)	Lipid (%)	Carbohydrate (%)	Ash (%)	рН	Peroxide (mEq/kg)	WHC** (%)	WBC** (mL/g)	OBC** (mL/g)
Mycoprotein- sausage	59 ± 1.9ª*	12.5 ± 1.0ª	13.9 ± 1.2ª	9.8 ± 1.1 ^b	2 ± 0.5 ^b	5.7 ± 0.01⁵	1.2 ± 0.2ª	54 ± 2.5ª	0.98 ± 0.09ª	0.37 ± 0.08ª
Beef sausage	47.5 ± 1.5 ^b	11.6 ± 1.1 ^b	8.9 ± 1.3 ^b	10.9 ± 1.2ª	3 ± 0.4ª	6.5 ± 0.01ª	1.3 ± 0.2ª	55 ± 3.3ª	0.79 ± 0.1 ^b	0.3 ± 0.08 ^b

*Mean ± SD, Different letters represent significant differences (P < 0.05).

**WHC: water holding capacity, WBC: water binding capacity, OBC: oil binding capacity.

L-Tyrosine	4.35 ± 0.97^{a}	4.950 ± 0.907^{a}
L-Valine	5.90 ± 0.70^{a}	4.10 ± 0.07^{b}
Phenyl Alanin	9.78 ± 0.87^{a}	3.15 ± 0.50^{b}
Proline	5.30 ± 0.77^{b}	17.1 ± 0.60 ^a
Tryptophan	6.7 ± 0.60^{a}	1.5 ± 0.70 ^b
*Mean ± SD, Diffe 0.05).	erent letters represent signi	ficant differences (P <

Table 4. The amino acid profile in mycoprotein/beef sausages

Content in

mycoprotein-sausage

(%g per 100 g protein)

 $4.84 \pm 0.99^{b*}$

6.74 ± 0.07^a

5.25 ± 0.03^b

11.21 ± 0.15^b

12.92 ± 0.52b

4.15 ± 0.23^b

 3.20 ± 0.75^{a}

5.90 ± 0.63^a 7.80 ± 0.35^a

7.50 ± 0.61^a

1.90 ± 0.80^a

5.35 ± 0.23^a

13.75 ± 0.77^a

Content in beef-

sausage (mg per

100 g protein)

14.90 ± 0.59^{a*}

6.0 ± 0.18^a

96 ± 0.05^a

15.20 ± 0.10^a

29.02 ± 0.12^a

9.05 ± 0.35^a

 $1 10 + 0.85^{b}$

 3.00 ± 0.53^{b}

5.01 ± 0.33^b

 3.0 ± 0.66^{b}

1.2 ± 0.11^b

1.04 ± 0.17^b

11.95 ± 0.95^b

Amino acids

(µg/g).

Amino acid

L-Alanine

L-Arginine

L-Cystine

L-Glutamic

L-Histidine

L-Isolucine

L-Leucine

L-Lysine

L-serine

L-Methionine

L-Theronine

Glycine

Aspartic acid

Based on the nutritional and physiological roles, AAs can be differentiated as EAAs, including valine, tryptophan, threonine, phenylalanine, methionine, lysine, isoleucine, leucine, histidine (essential for infants), arginine (semi-essential), and nonessential amino acids (NEAA), including tyrosine, serine, proline, glycine, glutamine, glutamic acid, cysteine, asparagine, aspartic acid, and alanine (Damodaran and Parkin, 2017). The AA profiles showed that mycoprotein sausages included almost all EAAs, compared to beef sausages (Table 4). These findings were similar to those of other studies, reporting the presence of EAAs in single-cell proteins (SCP) of F. venenatum (Hashempour-Baltork et al., 2020). In fact, three food categories totally provide 80.9% of daily protein needs of humans (Górska-Warsewicz et al., 2018). The significance of protein nutrition includes EAA content, biological value, digestibility, net protein use, and protein efficiency ratio. Hashempour-Baltork et al. (2020) compared the quality of the mycoproteins with that of meat proteins and demonstrated that nutritional indices of these two sources were almost similar. Monteyne et al. (2020) reported that mycoproteins were good food sources enriched with EAAs.

Table 5.	Fatty acid	profile of	mycoprotein/beef	sausage.

Fatty acid	Content in mycoprotein- sausage (% w/w)	Content in beef- sausage (% w/w)
Palmitic (C16:0)	18.8 ± 0.20 ^b	32.4 ± 0.29 ^{a*}
Stearic (C18:0)	10.90 ± 0.16ª	28.3 ± 0.20 ^b
Oleic (C18:1)	24.95 ± 0.11ª	9.13 ± 0.365 ^b
Linoleic (C18:2)	25.35 ± 0.15ª	21.31 ± 0.353 ^b
α -Linolenic (C18:3)	15.14 ± 0.18ª	5.10 ± 0.748 ^b

*Mean \pm SD, Different letters represent significant differences (P < 0.05).

Fatty acids

The FA composition of the sausage samples are presented in Table 5. The total SFAs in mycoprotein and beef sausages were 29.7 and 60.7% (w/w), respectively. In fact, unsaturated fatty acid (UFA) levels in mycoprotein sausages (65.44) were significantly (P < 0.05) higher than UFA levels in beef sausages (35.54% w/w). These contents as well as previous contents highly verified the results, especially for the ratio of UFA to saturated fatty acids (SFA) of 2:1 (Reihani and Khosravi-Darani, 2018). In 2009, Hosseini et al. (2009) reported 3.2-3.5:1 ratio of UFA to SFA. Higher consumption of USFAs provides health benefits to patients with cardiovascular diseases (CVD). Naturally, the ratio of polyunsaturated fatty acid (PUFA) to SFA in beef is typically 0.1. However, the ratio decreases with an increase in meat fats (Vahmani et al., 2015). Naturally, the ratio reaches 1.44 in mycoproteins. Chicken fat naturally includes 30% of SFAs, 45% of monounsaturated fatty acids (MUFA), and 21% of PUFAs (Hashempour-Baltork et al., 2020; USDA, 2008). These values are close to those of mycoproteins (Table 5).

Mechanical assessments

Table 6 represents mechanical properties of the cooked samples. Hardness of mycoprotein sausages was significantly lower than that of meat sausages (P < 0.05). Similar decreases were reported for cohesiveness when meat was totally replaced by mycoproteins. It was reported that beef sausages needed a greater force of chewing, compared to that of nonmeat samples. This was possibly due to the occurrence of stronger networks in myofibril proteins, increasing the product resistance to compression. Mycoprotein sausages showed lower values for gumminess and springiness (P < 0.05). Lower springiness values were reported by Youssef and Barbut (2011), when soy protein extracts were used as meat alternatives in emulsified meat batters. Kamani et al. (2019) recorded lower levels of food hardness, cohesiveness, gumminess, and springiness by replacement of meats by proteins of

Treatments	Hardness (N)	Adhesiveness (N.S)	Springiness (%)	Cohesiveness	Gumminess	Chewiness
Mycoprotein-sausage	23 ± 1.1 ^{b*}	1.5 ± 0.19ª	0.49 ± 0.21^{b}	0.19 ± 0.02^{b}	20.1 ± 1.2 ^b	1.0 ± 0.01 ^b
Beef-sausage	37.12 ± 1.5 ^a	0.5 ± 0.10 ^b	0.75 ± 0.2^{a}	0.24 ± 0.01^{a}	25.4 ± 1.5 ^a	2.1 ± 0.2 ^a

Table 6. The hardness, adhesiveness, springiness, cohesiveness, chewiness and gumminess of mycoprotein/beef sausages.

*Mean ± SD, Different letters represent significant differences (P < 0.05).

plant origin in chicken sausages. Researchers concluded that proteins of nonmeat origin could include further fat and water contents, which might fill the protein interstitial spaces and decrease the product springiness (Kamani *et al.*, 2019; Youssef and Barbut, 2011). This is also addressed for mycoprotein replacement regarding WBC and OBC (Table 2). Textural analysis demonstrated association of sample meats with lower values of adhesiveness. It could be interpreted that a decrease in meat quantity might lead to a significant decrease in the consistency of the cooked emulsions.

Conclusion

This study was carried out to investigate the appropriateness of mycoproteins as complete substitutes for meat in beef sausages. The results showed that mycoprotein substitution improved nutritional and health effects due to the high-value proteins with EAAs and less lipid content (mostly UFAs). Also, it has high contents of EAA and UFA, compared to meat sausages. Absence of yeasts, molds, Salmonella spp., Eshrichia (E.) coli, and Staphiloccocus (S.) aureus verified the effectiveness of heat treatment and also hygienic procedures in samples. Phycicochemical evaluations show higher contents of moisture and lipids in sausages containing mycoprotein due to WBC and OBC, compared to beef samples. Besides, mycoprotein samples had lower values (P < 0.05) of carbohydrates, ash, and pH, compared to beef samples. However, mycoprotein sausages achieved lower scores of hardness, cohesiveness, gumminess, and springiness in mechanical assessments, compared to beef sausages. Mycoproteins could hold excessive water and fat caused by higher WBC and OBC, filling the protein interstitial spaces and decreasing the springiness. This suggested less use of oil and water in mycoprotein formulations. This study has provided valuable information for increasing public awareness on the characteristics of mycoprotein products, including nutritional, textural, and formulation characteristics.

This is the first study that substituted meat with mycoproteins in sausages. However, further studies are necessary to optimize mycoprotein sausages using texture improvement ingredients to enhance their gel-forming and textural characteristics. These are currently the major problems in the manufacturing of meat-free sausages. The production of meat alternatives seems necessary due to the preference of consumers for vegetarian diets, and the increasing nutritional awareness of the populace. Like other functional foods that were unknown in the past, after numerous studies and production of various products, today there is a unique response to these products. In the past 2 years, the COVID-19 pandemic has drawn the public attention to food security and meat supply worldwide with further global demands for meat alternatives with plant origins.

Acknowledgment

This study was supported financially by the National Nutrition and Food Technology Research Institute, School of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Conflict of interest

The authors report no conflict of interest.

References

- AOCS, 2017. Official methods and recommended practices of the aocs, reapproved 2017. Available at: https://www.aocs.org/ attain-lab-services/methods/methods/search-results?subject=B.
- Bahmani, Z. and Movanes, F., 2021. Enrichment of carp sausage with Fusarium venatum mycoprotein. Utilization and Cultivation of Aquatics 10(11–25).
- Czauderna, M., Kowalczyk, J., Niedzwiedzka, K. and Wasowska, I., 2003. A highly efficient method for determination of some amino acids and glutathione by liquid chromatography. Journal of Animal and Feed Sciences 12(1): 199–214. https://doi. org/10.22358/jafs/67697/2003
- Damodaran, S. and Parkin, K.L., 2017. Amino acids, peptides, and proteins. In: Fennema's food chemistry. CRC Press, pp. 235–356. Edited By Srinivasan Damodaran, Kirk L. Parkin. Boca Raton.
- Finnigan, T., Needham, L. and Abbott, C., 2017. Mycoprotein: a healthy new protein with a low environmental impact. In: Sustainable protein sources. Elsevier, pp. 305–325. Sudarshan R. Nadathur Givaudan Flavors, Cincinnati, OH, United States; Janitha P.D. Wanasundara Agriculture and Agri-Food Canada, Saskatoon SK, Canada; Laurie Scanlin Colorado State University, Fort Collins, CO, United States

- Finnigan, T.J., Wall, B.T., Wilde, P.J., Stephens, F.B., Taylor, S.L. and Freedman, M.R., 2019. Mycoprotein: the future of nutritious nonmeat protein, a symposium review. Current Developments in Nutrition 3(6): nzz021. https://doi.org/10.1093/cdn/ nzz021
- Folch, J., Lees, M. and Stanley, G.S., 1957. A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry 226(1): 497–509. https://doi. org/10.1016/S0021-9258(18)64849-5
- Gabriel, A., Victor, N. and du Preez James, C., 2014. Cactus pear biomass, a potential lignocellulose raw material for single cell protein production (SCP): a review. International Journal of Current Microbiology and Applied Sciences 3(7): 171–197.
- Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F. and Toulmin, C., 2010. Food security: the challenge of feeding 9 billion people. Science 327(5967): 812–818. https://doi.org/10.1126/science.1185383
- Górska-Warsewicz, H., Laskowski, W., Kulykovets, O., Kudlińska-Chylak, A., Czeczotko, M. and Rejman, K., 2018. Food products as sources of protein and amino acids; the case of Poland. Nutrients 10(12): 1977. https://doi.org/10.3390/nu10121977
- Hashempour-Baltork, F., Hosseini, S.M., Assarehzadegan, M.A., Khosravi-Darani, K. and Hosseini, H., 2020a. Safety assays and nutritional values of mycoprotein produced by Fusarium venenatum IR372C from date waste as substrate. Journal of the Science of Food and Agriculture 100(12): 4433–4441. https:// doi.org/10.1002/jsfa.10483
- Hashempour-Baltork, F., Khosravi-Darani, K., Hosseini, H., Farshi, P. and Reihani, S.F.S., 2020b. Mycoproteins as safe meat substitutes. Journal of Cleaner Production 253: 119958. https:// doi.org/10.1016/j.jclepro.2020.119958
- Hashempour-Baltork, F., Torbati, M., Azadmard-Damirchi, S. and Savage, G.P., 2017. Quality properties of sesame and olive oils incorporated with flaxseed oil. Advanced Pharmaceutical Bulletin 7(1): 97. https://doi.org/10.15171/apb.2017.012
- Hashempour-Baltork, F., Torbati, M., Azadmard-Damirchi, S. and Savage, G.P., 2018. Chemical, rheological and nutritional characteristics of sesame and olive oils blended with linseed oil. Advanced Pharmaceutical Bulletin 8(1): 107. https://doi.org/10.15171/apb.2018.013
- Hosseini, S., Khosravi-Darani, K., Mohammadifar, M. and Nikoopour, H., 2009. Production of mycoprotein by Fusarium venenatum growth on modified vogel medium. Asian Journal of Chemistry 21(5): 4017.
- ISO, 2013. making food safer according to ISO methods, culture media and associated products for pathogen detection and enumeration. Available at: http://www.oxoid.com/pdf/iso-food-safety-brochure.pdf.
- Kamani, M.H., Meera, M.S., Bhaskar, N. and Modi, V.K., 2019. Partial and total replacement of meat by plant-based proteins in chicken sausage: evaluation of mechanical, physicochemical and sensory characteristics. Journal of Food Science

and Technology 56(5): 2660–2669. https://doi.org/10.1007/ s13197-019-03754-1

- Kim, T.-K., Yong, H.-I., Jung, S., Kim, H.-W. and Choi, Y.-S., 2021. Technologies for the production of meat products with a low sodium chloride content and improved quality characteristics—a review. Foods 10(5): 957. https://doi.org/10.3390/foods10050957
- Kyriakopoulou, K., Keppler, J.K. and van der Goot, A.J., 2021. Functionality of ingredients and additives in plant-based meat analogues. Foods 10(3): 600. https://doi.org/10.3390/ foods10030600
- Monteyne, A.J., Coelho, M.O.C., Porter, C., Abdelrahman, D.R., Jameson, T.S.O., Jackman, S.R., Blackwell, J.R, Finnigan, T.J.A., Stephens, F.B., Dirks, M.L., Wall, B.T., 2020. Mycoprotein ingestion stimulates protein synthesis rates to a greater extent than milk protein in rested and exercised skeletal muscle of healthy young men: a randomized controlled trial. American Journal of Clinical Nutrition 112: 318–333. https://doi. org/10.1093/ajcn/nqaa092
- Qian, F., Riddle, M.C., Wylie-Rosett, J. and Hu, F.B., 2020. Red and processed meats and health risks: how strong is the evidence? Diabetes Care 43(2): 265–271. https://doi.org/10.2337/ dci19-0063
- Reihani, S.F.S. and Khosravi-Darani, K., 2018. Mycoprotein production from date waste using Fusarium venenatum in a submerged culture. Applied Food Biotechnology 5(4): 243–352.
- Sasaki, K., Hatate, H. and Tanaka, R., 2020. Determination of 13 Vitamin B and the related compounds using HPLC with UV detection and application to food supplements. Chromatographia 83: 839–851. https://doi.org/10.1007/s10337-020-03902-2
- Upadhyaya, S., Tiwari, S., Arora, N. and Singh, D., 2016. Microbial protein: a valuable component for future food security. In: Microbes and environmental management. Studium Press, New Delhi, p. 8. Chapter: 12 Publisher: Studium Press (USA) Editors: Jay Shankar Singh, DP Singh
- USDA, 2008. Processed, Prepared USDA National Nutrient Database for Standard Reference, Release 21. Available at: https://www.ars.usda.gov/ARSUserFiles/80400535/DATA/sr21/ sr21_doc.pdf.
- Vahmani, P., Mapiye, C., Prieto, N., Rolland, D.C., McAllister, T.A., Aalhus, J.L. and Dugan, M.E., 2015. The scope for manipulating the polyunsaturated fatty acid content of beef: a review. Journal of Animal Science and Biotechnology 6(1): 1–13. https://doi. org/10.1186/s40104-015-0026-z
- Valls, F., Sancho, M.T., Fernández-Muino, M.A. and Checa, M.A., 2001. Determination of vitamin B6 in cooked sausages. Journal of Agricultural and Food Chemistry 49(1): 38–41. https://doi. org/10.1021/jf0003202
- Youssef, M. and Barbut, S., 2011. Effects of two types of soy protein isolates, native and preheated whey protein isolates on emulsified meat batters prepared at different protein levels. Meat Science 87(1): 54–60. https://doi.org/10.1016/j. meatsci.2010.09.002