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Investigation of protein level, amino acid profile and cytotoxic effects of plant-based proteins and meat analogues

Bitkisel-bazlı proteinler ve et analog ürünlerin protein seviyesi, amino asit profili ve sitotoksik etkilerinin incelenmesi

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Abstract

According to the Nova food classification system, plant proteins containing vegan analogues are classified into processed and ultra-processed. The recent sectoral developments highlight the importance of nutritional assessments of plant proteins and vegan analogues. This study investigated the protein level, essential (EAA)- and non-essential (non-EAA) amino acid profiles, and cytotoxic effects of plant-based proteins and meat analogues. Therefore, four meat products (burger meatball, pastırma, stuffed meatball, and sausage), soy and pea proteins, and four meat analogues were purchased from retail markets. All samples were subjected to the Kjeldahl test for protein content (%), LC-MS/MS test for EAA- and non-EAA profiles, and MTS assay for their cytotoxic effects. The results showed that the protein contents of soy protein, pea protein, meat analogues, and animal-origin meat products were determined to be 60.9%, 81.8%, 18.5 ± 9.3%, and 18.1 ± 9.7%, respectively. The EAA to non-EAA ratio in the meat analogues and meat products was 29.2/70.8 and 27.9/72.1, respectively. Besides, the MTS test indicated that the cell viability of HCT-116 cells at 24th and 48th h in the sausage analogues was significantly reduced by 59.84 ± 1.84%. In contrast, in pastırma and beef stuffed meatball analogues at 48th h, it was significantly decreased by 57.34 ± 0.52% and 62.70 ± 0.79%, respectively ($p<0.05$). Overall, we concluded that the health effects of processed and ultra-processed plant-based proteins and meat analogues on human health need further investigation through bioavailability and molecular-based techniques.

Keywords: Amino acid, Cytotoxicity, Nutrition, Plant-based Meat Analogue, Plant-based Protein, Protein

Öz

Bitkisel protein içeren vegan ürünler, Nova gıda sınıflama sistemi'nce işlenmiş ve ultra-işlenmiş sınıflarında değerlendirilmektedir. Sektörel gelişmeler, bitkisel kaynaklı vegan ürünlerin nütrisyonel değerlendirmelerini öne çıkarmaktadır. Bu çalışmada, bitkisel bazlı proteinler ve et analogların, protein, esansiyel (EAA)- ve non-esansiyel (non-EAA) amino asit kompozisyonları ve ürünlerin sitotoksik etkilerinin incelenmesi amaçlanmıştır. Bu bağlamda, zincir marketlerden dört adet hayvansal et ürünü (burger köftesi, pastırma, içli köfte ve sucuk), soya ve bezelye proteinleri ve dört adet bitkisel bazlı et analog ürünleri alınmıştır. Örneklerin protein içeriği Kjeldahl, amino asit profili LC-MS/MS ve sitotoksik etkileri MTS yöntemleri ile analiz edilmiştir. Bulgulara göre, protein içerikleri sırasıyla, soya proteini tozu %60,9, bezelye proteini tozu %81,8, et analog ürünleri %18,5 ± 9,3 ve hayvansal et ürünleri ise %18,1 ± 9,7 olarak bulunmuştur. EAA/non-EAA oranı et analog ürünlerinde 29,2/70,8 ve hayvansal et ürünlerinde 27,9/72,1 olarak tespit edilmiştir. MTS testi, HCT-116 hücre canlılığında, sucuk analog ürünü için 24. saatte %59,84 ± 1,84; pastırma ve dana içli köfte analog ürünleri içinse 48. saatte %57,34 ± 0,52 ve %62,70 ± 0,79 oranlarında anlamlı azaldığını göstermiştir (p<0.05). Özetle, işlenmiş ve ultra işlenmiş bitkisel proteinler ve et analog ürünlerinin insan sağlığı üzerindeki etkilerinin biyoyararlanım ve moleküler tabanlı yöntemler ile de araştırılması gerektiği sonucuna varılmıştır.

Anahtar kelimeler: *Amino asit, Sitotoksisite, Nütrisyon, Bitkisel-bazlı Et Analog, Bitkisel-bazlı Protein, Protein*

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1. Introduction

The protein demand for 7.5 billion people worldwide is more than 200 million tonnes annually. However, a surplus of 2.3 billion more people by 2050 will seriously affect the demand for protein. Almost 60% of the protein supplied globally is met by plant sources, with animal products making up 40% (Wu et al., 2014; Toujgani et al., 2023). Globally, the livestock sector responsible for human nutrition contributes 15% of greenhouse gas emissions and about 30% of biodiversity loss. Therefore, the livestock sector is becoming increasingly critical to finding modern dietary needs with sustainable protein sources (Cheng et al., 2022; GWR, 2023).

Recently, plant-based vegan products as alternatives to meat and dairy were valued at about $5 billion, with projections indicating an increase to 85 billion dollars by 2030, representing 6% of global traditional meat production (25 billion tons/year) (Siegrist & Hartmann, 2023). Pea and soy are the most widely preferred sources of plant-based proteins, which are not only utilized in meat and dairy analogues but also in beverages, protein and vitamin supplements, and snacks (Munialo & Vriesekoop, 2023). Vegan products, increasingly like animal-based counterparts in sensory attributes (taste and texture), have launched over 4400 new products since 2015 (Curtain & Grafenauer, 2019; Andreani et al., 2023).

The food industry is transforming as consumer demand grows for ethical, nutritionally, and sustainable balanced vegan products. The focus is shifting toward plant-based proteins that replicate the texture and composition of animal-derived meat and dairy (McClements & Grossmann, 2021a). Textured plant-based proteins, which are processed to achieve a fibrous texture like meat, are predominantly produced using extrusion technology, a high-temperature (150-170 °C) and high-pressure (7.5-10 MPa) method (Boukid, 2021; Kazir & Livney, 2021). The process induces physicochemical changes in the ingredients (Zhang et al., 2019; Pismag et al., 2024).

Plant-based meat analogues, first developed in the 1960s, usually contain water (50-80%), textured plant-based protein (15-20%), non-textured plant protein (10-25%), flavor enhancers (3-10%), fat (0-15%), binders (1-5%), colorants (0-0.5%), and other specific ingredients to enhance texture (Gómez-Luciano et al., 2019; Lima et al., 2022). Compared to animal-based meats, plant-based meat analogues usually have higher carbohydrate content, lower fat (i.e., saturated fat), and similar protein levels. Moreover, these products are rich in dietary fibre and can provide essential vitamins and minerals (Romão et al., 2022).

While research on alternative proteins and plant-based meat analogues has mainly focused on production and functionality methods, studies on their nutritional properties and health effects are still very early. Given the growing market share and increasing consumer requests, further research in these areas is crucial (Banach et al., 2022; Lin et al., 2023; Flint et al., 2023).

Specifically, the primary objectives of this study are to (1) highlight the nutritional aspects (i.e. protein and amino acids' profile) associated with plant-based proteins (soy and pea) and meat analogues (kibbeh, sausage, pastırma, and burger of beef meat) compared to those of animal origin; (2) investigate their cytotoxic effects compared to their identicals of animal origin, and (3) provide pre-clinical data for further research. By addressing these objectives, this study aims to contribute to the nutritional and cytotoxic gaps of some sustainable plant-based protein sources and meat analogues for the future needs of a growing population. This work, therefore, contributes to further insight into alternative proteins' nutritional benefits and health impacts, which are increasingly gaining importance in sustainable food sources.

2. Material and methods

2.1. Material

The soy and pea proteins, animal-based meat products (kibbeh, sausage, pastırma, and burger of beef meat) and meat analogues (kibbeh, sausage, pastırma, and burger), a total number of 10 samples, were collected from chain supermarket outlets in Istanbul, Türkiye, and were stored at +4 °C in the laboratory for further analyses (Table 1).

Table 1. Nutritional contents of plant-based proteins, animal-based products and meat analogues (per 100 g serving)

Material/value		Energy (Kcal)	Fat (g)	Saturated fat (g)	Carbohydrate (g)	Glucose (g)	Protein (g)	Salt (g)	Fibre (g)
Plant-based protein	Soy	331	1.2	0.3	30.9	0.5	70	-	23.6
	Pea	435	8.5	2	4.5	1.2	85	-	4
Meat analogue	Kibbeh	296	17	8	30	6	8.5	1.1	-
	Sausage	400	35	20	4	-	14	1.7	-
	Pastırma	270	16	3.9	0.5	-	31	4.5	-
	Burger	270	22	13	4	-	14	1.3	-
Animal-based meat products (beef)	Kibbeh	302	16	2.4	29	6.4	6.9	4.8	-
	Sausage	196	6	-	7	-	27	-	-
	Pastırma	227	10	-	5	-	27	-	-
	Burger	185	8	3	6	0.5	18	1.2	-

**All nutritional values are based on the information provided on the packaging of the products and are standardized per 100 g serving. Energy values are expressed in kilocalories (Kcal), and macronutrients are given in grams (g).*

2.2. Method(s)

2.2.1. Total protein analysis

Protein analysis followed TS 1620 and AOAC 981.10 protocols (Tabak et al., 2021). Approximately 1-2 g of the sample was weighed on nitrogen-free filter paper and transferred to a Kjeldahl flask. A Kjeldahl catalyst tablet (Merck 1.15348, Germany) and 25 mL concentrated sulfuric acid (H_2SO_4) (Merck 112080) were added. The flask was placed in a digestion apparatus (InKJel M, Germany) and heated until the mixture turned green. After cooling, the flask was transferred to a Kjeldahl distillation unit (Velp Scientifica UDK139, Italy). During the distillation process, 3-4 drops of indicator and 100 mL of 3.5% boric acid solution (H_3BO_3) (Tekkim TK 40000073, Türkiye) were added to the receiving Erlenmeyer flask. After distillation, the collected solution was titrated with 0.1 N hydrochloric acid (HCl) (Sigma Aldrich 07102, Germany) until it turned pink. The protein content (%) was calculated using the equations 1 & 2:

$$\text{Azot (N) (\%)} = V_{asit} \times N_{asit} \times 1.4 / M \quad (1)$$

$$\text{Protein (\%)} = \text{N (\%)} \times 6.25 \quad (2)$$

2.2.2. Determination of amino acid profile

The samples (0.5 g) were placed into a screw-capped glass tube, followed by the addition of 4 mL hydrolysis reagent (JASEM JSM-CL-508, Türkiye). The sample was hydrolyzed at 110 °C for 24 h. After hydrolysis, the hydrolysate was allowed to cool to room temperature and centrifuged at 4000 rpm for 5 min. A 40 µL aliquot of the supernatant was pipetted and diluted to 1 mL with distilled water. From the diluted solution, 50 µL was transferred into a vial, mixed with 50 µL of an internal standard solution, and vortexed for 5 s. Subsequently, 700 µL of additional reagent was added, and the mixture was vortexed for another 5 s. Finally, the supernatant was transferred to an HPLC vial, and 3 µL was injected into a Jasem amino acid column (JASEM JSM-CL-575). The amino acid profile of the sample was analyzed using an Agilent 1290 Infinity coupled with an Agilent 6470 Triple Quadrupole System. Amino acid concentrations were quantified with a limit of detection (LOD) of 0.001 mg/100 g and a limit of quantification (LOQ) ranging from 0.007 to 0.161 mg/100 g (Tabak et al., 2021). The statistical analysis results are shown in tables as mean values and standard deviations.

2.2.3. *In-vitro* cytotoxicity (MTS) assay

The samples were prepared according to ISO 10993-12 Sample Preparation and Reference Materials standard (AOAC International., 1990). According to the table of standard surface areas and extract liquid volumes, the sample was prepared by incubating it in Dulbecco's Modified Eagle Medium (DMEM1x) (Gibco ™ 11965092, USA) without serum and antibiotics at 37 °C for 24 h. ISO 10993-5 standards performed MTS test. The medium was removed from the cultured cells (HCT-116), and the flask was washed with 1 mL phosphate buffer saline (PBS) (HyClone, USA). Then, 1 mL of Trypsin-EDTA (Multicell 325-043-EL, Canada) was added to the flask and incubated for 3-4 min (Nüve EC160, Türkiye). Following incubation, the detachment of cells from the surface was observed under a microscope. The tubes were centrifuged at 2000 rpm for 2 min (Nüve NF 800, Türkiye). The supernatant was discarded, and the cell pellet was resuspended in 1 mL of fresh growth medium. The plates were incubated at 37 °C with 5% CO_2 for 24 h (Nüve EC160). After incubation, extracts were applied to the cells for 24 h. At the end of the treatment, the medium in the wells was removed, and MTS solution was added to each well. The plates were incubated for 2 h, and absorbance was measured at 570 nm using a BioTek 800 TS ELISA microplate reader (Agilent, USA) (Abdik, 2022).

2.2.4. Statistical evaluation

The mean and standard deviation (±) values of EAA and non-EAA levels for different sample groups (animal-origin meat products and plant-based meat analogues) were calculated using Microsoft Excel. The differences in EAA and non-EAA levels between sample groups were analyzed with a t-test method using the SPSS 20 software package (IBM Corporation, NY, USA). $p<0.05$ was accepted as statistically significant.

3. Results

This study investigated protein levels, EAA and non-EAA profiles and cytotoxic effects of some plant-based proteins (i.e., soy and pea) and meat analogues. To do this, four meat products of animal origin (burger meatball, pastırma, stuffed meatball, and sausage), soy and pea proteins, and four meat analogues were purchased from the retail markets. The results showed that the effects of processed and ultra-processed plant-based proteins and meat analogues on human health need further investigation through bioavailability and molecular-based techniques.

3.1. Total protein contents

The samples' protein contents (%) were determined using the Kjeldahl method. The protein contents of soy and pea proteins were 60.9% and 81.8%, respectively. Among the meat analogues, the highest protein content was measured in sausage with 25.9%, followed by pastırma (25.5%), burger (16.5%), and stuffed kibbeh (6.3%). The average protein content of the meat analogues was 18.5 ± 9.3%. The protein content of animal meat products was determined to be close to those of meat analogues. Specifically, beef pastırma contained 30.9%, beef sausage 18.0%, beef burger 16.3%, and beef stuffed kibbeh 7.3% protein. The average protein content of animal-based meat products was calculated to be 18.1 ± 9.7%. These results showed that the average protein content of meat analogues was 2.2% higher than that of animal-origin meat products (Table 2).

Table 2. The protein contents of the samples analyzed (per 100 g serving)

Plant proteins and meat analogue	Protein content (%)	Protein content (%)	Animal-origin meat product
Soy Protein	60.9	-	n/a
Pea Protein	81.8	-	n/a
Burger analogue	16.5	16.3	Beef Burger
Pastırma analogue	25.5	30.9	Beef Pastırma
Stuffed analogue	6.3	7.3	Beef Stuffed
Sausage analogue	25.9	18.0	Beef Sausage
Mean ± Ss.	**18.5 ± 9.3**	**18.1 ± 9.7**	**Mean ± Ss.**

Mean ± Ss (±) values represent the average protein content in the samples.

3.2. Amino acid profiles

The levels of EAA and non-EAA in soy and pea proteins were measured using the LC-MS/MS method, with a limit of detection (LOD) of 0.001 mg/100 g and a limit of quantification (LOQ) range between 0.007 and

0.161 mg/100 g. The amino acid content of regular beef was retrieved from https://fitaudit.com/food/137541/amino (FitAudit, 2025). According to the results, the EAA content in soy protein was measured at 17640 ± 1179 mg/100 g, while pea protein contained 26780 ± 1622 mg/100 g, while the EAA content of the regular beef was declared as 13104 ± 826 mg/100 g. The data revealed that the total EAA levels in plant-based protein sources were 34.2% in soy and 104.3% in pea, which were higher than the EAA content in animal-based beef. On the other hand, regular beef was found to contain higher levels of methionine (857 mg/100 g) and tryptophan (325 mg/100 g) compared to the plant-based meat analogues (Table 3).

Table 3. EAA contents in plant-based proteins (soy & pea) and animal-origin regular beef (mg/100 g) (per 100 g serving)

EAA (mg/100 g)	Plant-based protein				Animal-origin regular beef	
	Soy	%	Pea	%	Beef	%
Histidine	1740	9.9	3580	13.4	1105	8.4
Isoleucine	2750	15.6	4750	17.7	1448	11.1
Leucine	3460	19.6	4980	18.6	2652	20.2
Lysine	3080	17.5	3030	11.3	2911	22.2
Methionine	510	2.9	580	2.2	857	6.5
Phenylalanine	1140	6.5	3750	14.0	1253	9.6
Threonine	1670	9.5	2800	10.5	1425	10.9
Tryptophan	250	1.4	300	1.1	325	2.5
Valine	3040	17.2	3010	11.2	1128	8.6
Total	**17640 ± 1179**	**100.0**	**26780 ± 1622**	**100.0**	**13104 ± 826**	**100.0**

The table shows EAA content (mg/100g) and their relative percentages (%) for plant-based proteins (soy & pea) and animal products.

The non-EAA content in soy protein was 28980 ± 1857 mg/100 g, while it was 39300 ± 2047 mg/100 g in pea protein. In raw beef, the non-EAA content was reported as 17630 ± 1468 mg/100 g. These values show that the non-EAA levels in plant-based protein sources are 134.6% in soy and 204.4% in pea, higher than in animal-origin beef. On the other hand, regular beef contained higher cystine (342 mg/100 g), while cysteine, hydroxyproline, and taurine could not be detected (Table 4).

Table 4. Non-EAA contents in plant-based proteins (soy and pea) and animal-origin regular beef (mg/100 g) (per 100 g serving)

Non-EAA (mg/100 g)	Plant-based protein				Animal-origin regular beef	
	Soy	%	Pea	%	Beef	%
Alanine	2880	9.9	3750	9.5	1882	10.7
Arginine	3050	10.5	4540	11.6	2105	11.9
Aspartic Acid	4540	15.7	5070	12.9	2984	16.9
Cystine	250	0.9	300	0.8	342	1.9
Cysteine	250	0.9	300	0.8	n.a.	0.0
Glutamic Acid	5870	20.3	6550	16.7	5063	28.7
Glycine	1220	4.2	3740	9.5	1500	8.5
Hydroxyproline	3950	13.6	4200	10.7	n.a.	0.0
Proline	3080	10.6	4030	10.3	1361	7.7
Serine	1240	4.3	3750	9.5	1265	7.2
Taurine	n.d.	0.0	n.d.	0.0	n.a.	0.0
Tyrosine	2650	9.1	3070	7.8	1128	6.4
Toplam	**28980 ± 1857**	**100.0**	**39300 ± 2047**	**100.0**	**17630 ± 1468**	**100.0**

The table shows non-EAA content (mg/100g) and their relative percentages (%) for plant-based proteins (soy & pea) and animal products.

The EAA level in the burger analogue was 4650 ± 224 mg/100 g, while in the beef burger, it was 4140 ± 196 mg/100 g, indicating that the vegan burger contains 12.3% more EAA. Conversely, the non-EAA level in the burger analogue was 11050 ± 616 mg/100 g, which is 16.5% lower than the 9480 ± 458 mg/100 g found in the beef burger. The EAA level in pastırma analogue was 6130 ± 278 mg/100 g, while beef pastırma was 9540 ± 381 mg/100 g, which is 55.6% higher. On the other hand, the non-EAA level in the pastırma analogue was 13960 ± 1235 mg/100 g, while in the beef pastırma, it was 16970 ± 807 mg/100 g, a 21.5% increase. The EAA level in the stuffed meatball analogue was 2310 ± 153 mg/100 g, while the beef stuffed meatball was 2750 ± 196 mg/100 g, 19% higher. In contrast, the non-EAA level in the stuffed meatball analogue was 3720 ± 137 mg/100 g, 16% lower than the 4340 ± 194 mg/100 g found in the beef stuffed meatballs. The EAA level in the sausage analogue was 7930 ± 645 mg/100 g, 62.1% higher than the 4890 ± 209 mg/100 g in the beef sausage. Conversely, the non-EAA level in the sausage analogue was 15090 ± 905 mg/100 g, 19.6% lower than the 12610 ± 803 mg/100 g found in the beef sausage (Table 5).

Table 5. Comparison of EAA and non-EAA in meat analogues and animal-origin meat products

EAA	**Meat analogue (mg/100 g)**				**Animal-origin meat product (mg/100 g)**			
	Pastırma	**Stuffed Meatballs**	**Burger**	**Sausage**	**Pastırma**	**Stuffed Meatballs**	**Burger**	**Sausage**
Histidine	840	200	450	590	1280	350	420	500
Isoleucine	930	600	540	1730	1260	770	500	590
Leucine	980	340	860	1940	1500	320	770	780
Lysine	940	330	810	510	1330	240	710	860
Methionine	450	110	380	220	690	110	330	440
Phenylalanine	350	140	350	500	1230	310	300	440
Threonine	610	270	430	700	750	330	400	400
Tryptophan	240	140	150	300	330	120	160	190
Valine	790	180	590	1440	1170	200	550	690
Total EAA	**6130 ± 278**	**2310 ± 153**	**4560 ± 224**	**7930 ± 645**	**9540 ± 381**	**2750 ± 196**	**4140 ± 196**	**4890 ± 209**
non-EAA	**Meat analogue (mg/100 g)**				**Animal origin meat product (mg/100 g)**			
	Pastırma	**Stuffed Meatballs**	**Burger**	**Sausage**	**Pastırma**	**Stuffed Meatballs**	**Burger**	**Sausage**
Alanine	890	300	780	870	1750	500	710	820
Arginine	980	230	640	910	1500	170	680	660
Aspartic Acid	950	310	1350	1100	1920	320	1290	1550
Asparagine	950	310	1350	1100	1920	320	1290	1550
Cystine	220	140	220	220	250	220	220	230
Cysteine	220	140	220	220	250	220	220	230
Glutamic Acid	5000	480	1990	3190	2960	500	1400	2100
Glycine	800	270	650	1060	1170	410	560	640
Hydroxyproline	1240	500	1840	800	1500	770	1220	2760
Proline	940	350	510	1200	1020	240	450	550
Serine	850	330	890	1990	1350	450	860	920
Taurine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Tyrosine	920	360	610	2430	1380	220	580	600
Total non-EAA	**13960 ± 1235**	**3720 ± 137**	**11050 ± 616**	**15090 ± 905**	**16970 ± 807**	**4340 ± 194**	**9480 ± 458**	**12610 ± 803**
Total amino acid content	**20090 ± 5537**	**6030 ± 997**	**15610 ± 4589**	**23020 ± 5063**	**26510 ± 5254**	**7090 ± 1124**	**13620 ± 3776**	**17500 ± 5459**

EAA are those that the body cannot synthesize and must be obtained through the diet, while the body can produce non-essential amino acids (non-EAAs). The values represent the content of each amino acid in milligrams per 100 grams of the respective meat product. Percentages indicate the relative proportion of each amino acid to the total amino acid content in the product.

3.3. *In-vitro* cytotoxicity (MTS) results

The time-dependent cytotoxic effects of soy and pea proteins, meat analogues, and animal-origin meat products on HCT-116 colorectal cancer cells were assessed using MTS assay. As described in the Methods section, cells were seeded into 96-well plates and treated with media containing the respective products at specific concentrations. After 24 and 48 h incubations, the effects on cell viability (%) were evaluated using MTS assay. A significant decrease in cell viability was observed in the sausage analogues, with a value of 59.84 ± 1.84%, after 24 h of incubation with HCT-116 cells. No significant difference was found between the other groups. After 48 h of incubation, cell viability significantly decreased in the pastırma analogue, beef and analogue stuffed meatballs, and sausage analogues, with values of 57.34 ± 0.52%, 54.95 ± 2.20%, 63.03 ± 1.16%, and 62.70 ± 0.79%, respectively. Cell viability decreases were also observed in the other groups (Table 6).

Table 6. Effects of soy and pea proteins, meat analogues and animal origin meat products on the cell viability of HCT-116 cell line after 24 h and 48 h of incubation.

24 h	Control		Sample		48 h	Control		Sample	
	HCT-116 (%)	Ss. (±)	HCT-116 (%)	Ss. (±)		HCT-116 (%)	Ss. (±)	HCT-116 (%)	Ss. (±)
Soy Protein	100	-	70.01	0.87	Soy Protein	100	-	74.34	0.99
Pea Protein	100	-	77.45	0.59	Pea Protein	100	-	73.95	0.75
Vegan Burger	100	-	74.55	1.17	Vegan Burger	100	-	72.11	0.37
Beef Burger	100	-	88.88	5.64	Beef Burger	100	-	66.83	0.49
Pastırma analogue	100	-	80.68	1.6	Pastırma analogue	100	-	57.34	0.52
Beef Pastırma	100	-	72.33	0.57	Beef Pastırma	100	-	75.74	1.13
Meatballs analogue	100	-	73.56	0.68	Meatballs analogue	100	-	63.03	1.16
Beef Meatballs	100	-	74.09	0.57	Vegan Meatballs	100	-	54.95	2.20
Sausage analogue	100	-	59.84	1.84	Sausage analogue	100	-	62.70	0.79
Beef Sausage	100	-	81.99	0.80	Beef Sausage	100	-	68.06	1.96

The values are presented as %, with Ss. (±) for each sample.

3.4. Statistical results

The differences in EAA and non-EAA profiles between soy and pea proteins, meat analogues and animal-origin meat products were analyzed using a t-test ($p<0.05$). The statistical results indicated no significant differences between EAA and non-EAA levels of meat analogues and animal-origin meat products, suggesting they possess analogous amino acid levels and characteristics ($p=0.81> 0.05$).

4. Discussion and conclusions

The protein demanded by the 7.3 billion people in the world is currently met by plant sources (57%) and animal sources (43%). The increasing demand for protein has gained significant interest from researchers because of the growing global population, climate and environmental changes, policies aimed at reducing the demand for animal-based protein products, healthy eating trends, and modern dietary preferences (Toujgani et al., 2023; Monica et al., 2025). Additionally, the livestock sector serves meat production purposes and is responsible for 18% of anthropogenic greenhouse gas emissions. In other words, 100 g of animal-based protein produces 50 kg of greenhouse gas emissions (Hertzler et al., 2020; Munialo & Vriesekoop, 2023). Consequently, the current study has focused on a pressing issue by examining plant-based proteins and meat analogues regarding their nutritional content and cytotoxic effects on human colon health.

Plant species are limited in the global food system for humans (Knez et al., 2023). Plant-based proteins are rich sources of amino acids, and mainly depend on soy, pea, wheat, oats, chickpeas, almonds, hazelnuts, and seeds (Lin et al., 2023). Therefore, exploiting a broad range of plant protein sources is paramount to integrating food safety and sustainability, in line with the United Nations (UN) Decade of Action framework and the Second International Conference on Nutrition in 2014 (FAO, 2024; IPSUS, 2025). Soy and pea proteins are particularly famous for their availability, superior emulsification activity, and stability compared to other plant-based protein sources. These features justify the use of soy and pea proteins in meat analogues. Our study determined the total protein contents as 60.9% for soy protein powder and 81.8% for pea protein powder. Soy protein isolate contains 90%, soy protein concentrate contains 65–90%, and soy flour contains 50–65% of total protein (Messina et al., 2022). Similarly, the protein content of pea protein isolate is reported as 80% (Trindade et al., 2023). In the current work, the total protein results obtained for soy and pea protein powders collected in Istanbul align with the findings of the international literature.

This current study indicates that meat analogues' average total protein content is comparable to that of animal-based meat products. In the literature, the protein content of burger analogues is reported to range from 15.4–17.3% (Bakhsh et al., 2021), while sausage analogues range from 2.33–8.79% (Corrêa et al., 2023). For their animal-based counterparts, a study conducted in Australia found the protein content of beef sausages to range between 13.8–15.1% (Cunningham et al., 2015). Thus, this study has revealed that the protein contents of meat analogues are consistent with those previously reported in the literature. The differences in protein content between meat analogues and animal-origin meat products were analyzed using a t-test ($p<0.05$). Statistical results showed no significant differences between the two groups ($p=0.81>0.05$). However, the statistical findings suggest that plant-based meat analogues are analogues at the protein level to animal-origin meat products.

Nutritionally, meat-eaters are reported to consume 47.1 g of meat and 11.5 g of plant-based food daily, whereas vegans consume 73 g of plant-based food and 63 g of soy protein (Mariotti & Gardner, 2019). Though an analogue product, meat replacements are expected to recreate processed meat's visual aspect and mouthfeel. Besides, they face the complex task of reproducing the nutritional profile (amino acids). Additionally, various meat analogues require different plant-based components. Since processed meat production has not been optimized for plant-based ingredients, the current solution is the mixture of two or more plant-based protein sources (Da Silva et al., 2024). Because plant-based proteins determine meat analogues' nutritional and structural characteristics (Ishaq et al., 2022). In this study, the ratio of EAA to non-EAA for soy and pea proteins, which are primary ingredients in meat analogues, was found to be 37.8/62.2 and 40.5/59.5, respectively. These ratios indicate a higher proportion of non-EAA than that of beef, which has a ratio of 42.6/57.4 (Fit Audit, 2025). Beef presents a more balanced EAA/non-EAA ratio than the plant-based protein powders and products analyzed. Rizzolo-Brime et al. (2023) demonstrated that the total EAA content and lysine amino acid in plant-based meat analogues are higher than in their animal counterparts. Amongst the non-EAA in the regular beef, the level of alanine was reported as 1882 mg/100 g in the literature, as presented in Table 4. The current work detected it as 890 mg/100 g in the vegan pastırma sample and 1750 mg/100 g in the regular pastırma sample. On the other hand, Erdemir and Aksu (2017) reported that the level of alanine was determined as 866.6 ± 264.4 mg/100 in raw beef meat and 42.3 ± 73.7 mg/100 g in pastırma. Similarly, Deniz et al. (2016) reported that the level of alanine in pastırma on the 21st day was 133.1 ± 18.9 mg/100 g. Interestingly, this study detected alanine in soy (2880 mg/100 g) and pea (3750 mg/100 g) protein samples, respectively. Alanine was also found in plant-based meat analogues (300 to 890 mg/100 g), which are lower than soy and pea proteins. Interestingly, our results also revealed that cystine and cysteine levels of the plant-based meat analogues were similar to each other (140 to 220 mg/100 g), and in animal-origin meat products (220 to 250 mg/100 g). Some studies demonstrate that meat analogues can have relatively better nutritional composition, but there is insufficient proof to consider them healthier than their meat counterparts (Rizzolo-Brime et al., 2023). However, in this current study, the EAA level was lower than the non-EAA content. Lysine was identified as the highest among the amino acids, consistent with Rizzolo-Brime's (2023) findings. Aaslyng et al. (2023) reported that meat analogues meet only 50% of the recommended daily lysine intake, while leucine and valine meet 70%. Another study by Van Vliet et al. (2020) identified the highest methionine levels in beef, followed by pea and soy proteins, which align with our results. Therefore, fortification of protein could be an option to mimic the amino acid profile of the meat alternatives.

Research on the cytotoxic effects of meat analogues is still in its early stages (Crimarco et al., 2022). There are many unknown variables and open questions regarding potential risks possibly present in meat analogues, including processing-related compounds such as n-nitrosamines, acrylamide, and heterocyclic aromatic amino acids (Gräfenhahn & Beyrer, 2024). Many plant-based alternatives are considered ultra-processed food, and a study by the World Cancer Research Fund showed that people who consumed more ultra-processed food had an increased risk of developing and dying from cancer (World Cancer Research Fund, 2023). In this work, an MTS assay was conducted to evaluate the cell viability of animal-based meat analogues. Overall, meat analogues significantly reduced the viability of HCT-116 colorectal cancer cells at 48 h ($p<0.05$). This finding suggests that meat analogues may reduce cell viability due to senescence-related cell proliferation or the cytotoxic effects of their metabolites. Previous studies on meat analogues and their health effects report no significant differences in some inflammatory markers (interleukin-12, interleukin-12B, interleukin-10, and transforming growth factor-beta) between vegan and animal-product consumers.

This recent research identified the nutritional (protein, amino acid) and cytotoxic knowledge gaps associated with plant-based proteins (soy, pea) and meat analogues compared to animal-origin meat products to guide future research directions. The primary outcome, exhibiting the novelty of the research, is to offer a new experimental data-based perspective on the protein content and cytotoxic effects of plant-based proteins and their meat analogues. The key findings in this recent study highlight that (1) there is a need for regulatory oversight, improved nutritional and cytotoxic monitoring, and further research on plant-based proteins and meat replacements, (2) ensuring the nutritional and health aspects of plant-based proteins and meat analogues need collaboration and cooperation among food scientists, industry leaders, and policymakers, and (3) research on plant-based proteins and meat analogues should focus on nutritional and health aspects because health is a non-negotiable requirement. Overall, we concluded that the health effects of processed and ultra-processed plant-based proteins and meat analogues on human health need further investigation through bioavailability and molecular-based techniques.

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Author contribution

Yasemin Yılmazer: Method, Writing- First draft and Editing, and Visualization. İsmail Hakkı Tekiner: Method, Supervision, Writing - Reviewing and Editing. Aleyna Çavdar: Investigation and Writing – First draft. Sermin Durak: Writing – Reviewing and Editing. Rula Abdülhamitoğlu: Investigation.

Declaration of ethical code

The authors of this article declare that the materials and methods used in this study do not require ethics committee approval and/or special legal permission.

Conflicts of interest

There is nothing to declare.

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