Sustainable Food Technology

PAPER

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Cite this: *Sustainable Food Technol.*, 2025, **3**, 1805

Received 25th April 2025
Accepted 5th September 2025

DOI: 10.1039/d5fb00162e

rsc.li/susfoodtech

Effects of pectin and avocado oil in the production of meat analogues obtained by high moisture extrusion and their physicochemical characterization

Darianna Ur-Mora,[a] Oscar Hernández-Meléndez[a] and Eduardo Bárzana*[b]

The growing demand for plant-based protein foods that meet nutritional needs and are also palatable to consumers has given way to meat analogues. This research studied their preparation by texturing isolated soy protein by high moisture extrusion cooking, supplemented with avocado oil and pectin as a binder. Operational conditions for the extrusion process that favored the formation of fibrous structures were determined. The extruded samples were subjected to a proximate chemical analysis that reported values of approximately 55% moisture and 27% protein. Avocado oil allowed for a more tender and juicy product, and did not interfere with the formation of fibrous structures. Scanning electron microscopy and Raman spectroscopy analyses showed that analogues with 4% pectin by weight presented a more pronounced fibrous structure and the best chewing characteristic as determined by the profile obtained in a texture analyzer.

Sustainability spotlight

Plant-based meat analogues are a more sustainable alternative to traditional meat and can mitigate the damage caused by the meat industry. This research studied a formulation of isolated soy protein, avocado oil and pectin for the sustainable production of meat analogues *via* high moisture extrusion cooking, which offered promising results that will promote a healthy lifestyle and combat hunger, thus contributing to the United Nations Sustainable Development Goals (SDGs), in particular SDG 3 (Good Health and Well-Being) and SDG 2 (Zero Hunger). In addition, meat analogues will reduce the consumption of traditional meat, which will decrease soil degradation and biodiversity loss, thus aligning with SDG 15 (Life On Land).

1 Introduction

The increase in population and socioeconomic development in the world has led to a growing demand for meat of animal origin. A reduction in its consumption will lead to a reduction in greenhouse gas emissions, as well as a decrease in the carbon footprint, water consumption and the loss of biodiversity. As a result, several people have opted for a diet based on vegetable protein influenced by concerns for the environment, animal welfare and the need for good nutrition, in addition to the restrictions of various cultures on consuming meat of animal origin.[1,2] All this has led to an increase in the demand for foods made from vegetable protein.

To satisfy this new demand, it is necessary that new alternative and analogous products to meat of animal origin meet nutritional requirements and are also pleasant to the palate. Despite important and ongoing technological advances, two challenges must be addressed: (a) the search for more sustainable production methods, and (b) the achievement of products with sensory attributes similar to those of meat.

High-moisture extrusion cooking is a widely used technique for the development of meat analogues.[3] Its versatility allows a wide range of vegetable proteins to be processed in an efficient, economical manner and with good yields. It is characterized by subjecting the raw material to a cooking temperature between 140 and 180 °C, which results in a practically sterile material. Additionally, to achieve the desired partially fibrous texture with a multilayer structure, it is necessary to include a cooling unit at the equipment outlet. The residence time ranges from 3 to 4 min to obtain a finished product with a moisture content between 40 and 80%.[4,5] Generally, the intermediate products obtained are further processed to create ready-to-eat meat analogues using conventional meat processing operations such as mincing, marinating, and mixing.[6]

The screw profile configuration is a key element in extrusion because it is modified according to the functions to be performed in each of the different sections. Temperature is one of the main operating parameters that guarantee the texturization of vegetable proteins by thermal cooking. Although high

[a]*Departamento de Ingeniería Química, Facultad de Química, Universidad Autónoma Nacional de México, Ciudad de México, Mexico*

[b]*Departamento de Alimentos y Biotecnología, Facultad de Química, Universidad Nacional Autónoma de México, Ciudad de México, Mexico*

temperatures are not required for protein denaturation to take place, temperatures between 100 and 200 °C are necessary for good texturization.[5,7] Several authors agree that to obtain a pronounced fibrous structure the maximum temperature should be in the range of 155 °C to 180 °C.[8–10]

Once the material has been cooked, cooling the hot material at the extruder outlet induces its solidification from the walls of the cooling die towards the center. This stage leads to the gradual development of flow conditions that promote the generation of fibers and overlapping layers in the final product, with an anisotropic fiber-like structure.[11–13] As the temperature decreases in the cooling die, protein–protein interactions increase and specific cross-linking occurs leading to the formation of a dense fiber product.[9,14–16] In the extrusion process, due to the effects of temperature and shear force of the screws, the three-dimensional structure of certain proteins is destroyed as a result of hydrolysis of peptide bonds, causing amino acid chains to unfold. These are realigned due to the formation of cross-links between the denatured protein chains by means of amides, disulfide bridges and hydrogen bonds and finally transformed into a fibrous structure through new isopeptide bonds. Denaturation was previously considered a decisive step in soy protein extrusion; however, it is now known that the native structure is not a prerequisite for successful extrusion. This is because concentrates and isolates whose native structure has been destroyed by heat and solvents in the previous purification and extraction steps can lead to the same results.[5]

Soy protein is among the main plant sources used to obtain meat analogues due to its excellent properties, nutritional value and a great capacity to produce fibrous structures.[17] Solid fats extracted from coconut, cocoa and oilseed oils, most commonly canola and sunflower, are often used in meat analogues. Linseed, algae, corn, safflower, olive and palm oil have also been used.[4,18] In order to improve the fatty acid profile in the analogues, avocado oil was used in the present study based on its nutritional properties. Avocado oil contains a low proportion of saturated fatty acids, which depends on the variety and the state of maturity (10–19%), a high amount of oleic acid (up to 80%), an acceptable level of polyunsaturated fatty acids (11–15%), all of high nutritional quality, and no cholesterol.[19] It is important to note that there is no known report where avocado oil has been used in the production of meat analogues based on vegetable protein.

Previous studies have reported that to obtain a fibrous structure it is necessary to have a mixture with thermodynamic incompatibility, generally caused by electrostatic repulsions, which result in phase separation.[11,12,20,21] Therefore, a mixture of thermodynamically incompatible biopolymers is a key requirement for the formation of anisotropic structures.[11,12,20,22] Two different ways are proposed: one is to use a less purified protein, such as protein concentrate instead of the isolate, since the concentrate contains carbohydrates; the other consists of using a mixture of the protein isolate with polysaccharides. Following the principle of the second route, Dekkers *et al.* (2016) recommend the use of pectin, since it constitutes the main soluble polysaccharide fraction of soy protein concentrate.[22]

The objective of the present study was to develop a fibrous product based on soy protein, avocado oil and pectin by extrusion cooking with high moisture content for use as a meat analogue, and to characterize it through its morphological, chemical and textural properties.

2. Materials and methods

2.1 Materials

ProWinner soy protein isolate (SPI) was used with a content of 85.3 g of protein, 4.6 g of lipids and 5.5 g of carbohydrates on a dry basis per 100 g of commercial product, according to the manufacturer's specifications. The avocado oil (AO) used was from El Real, Mexico. Citrus pectin was purchased from Droguería Cosmopolita. All materials were obtained from commercial suppliers in Mexico City.

2.2 Methods

2.2.1 Preparation of pectin/SPI mixtures. To maintain a constant level of moisture in the feed to the extruder, all formulations were prepared using ingredients measured on a dry weight basis. Demineralized water was added to each mixture to achieve a final moisture content of 55%. The proportion of pectin and SPI was varied by replacing part of SPI with pectin between 2 and 6% by weight.

2.2.2 High moisture extrusion. The extrusion process was carried out using a laboratory-scale co-rotating twin-screw extruder (model Evolum 25, Clextral brand, Firminy, France), shown in Fig. 1, located in the Department of Chemical Engineering at the Faculty of Chemistry. The extruder screw has a length of 100 cm, and its diameter is 2.5 cm for a L/D ratio of 40, which guarantees a correct thermomechanical treatment.

Based on preliminary results the screw speed was set at 230 rpm, with water flows of 2.6 and 3.5 L h^{-1} and an AO flow of 0.36 L h^{-1}, which were pumped using a peristaltic pump in modules 2 and 3 respectively. The hopper fed the raw material at a rate of 0.7 kg h^{-1}. A maximum barrel temperature of 165 °C was used and, as an example, Table 1 shows the temperature profile used per module. An elongated nozzle (*i.e.* module 10) was placed at the extruder outlet and covered with dry ice to promote the formation of the fibrous structure. This rectangular nozzle chamber was 30 cm long. The inner rectangle of the nozzle was 4 cm long and 0.5 cm wide. Water flow and

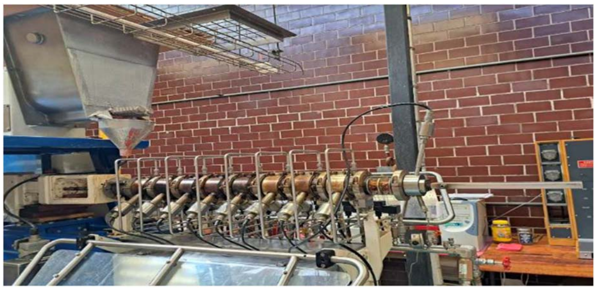

Fig. 1 Twin-screw extruder.

Table 1 Temperature profile

Module	1	2	3	4	5	6	7	8	9	10
Temperature (°C)	—	70	80	145	165	130	55	25	20	8

temperature were varied to see if these changes influenced the final texture of the extrudates. Once the best conditions were selected, they were kept constant and only the concentration of citrus pectin was varied. Dekkers *et al.* (2016) showed that between 2 and 4% of pectin is sufficient to obtain a pronounced fibrous structure.[22]

Four samples were formulated by varying the concentration of citrus pectin.

DMD 1: SPI + AO.

DMD 2: SPI + AO + 2% by weight of pectin.

DMD 3: SPI + AO + 4% by weight of pectin.

DMD 4: SPI + AO + 6% by weight of pectin.

To determine the final profile of the screw, several configurations were tested and the one with a texture similar to real meat was selected based on an empirical qualitative evaluation. The location of the components and their classification are presented in Table 2.

2.2.3 Scanning electron microscopy. Extrudates (~1 cm^2) were fixed in 2.5% glutaraldehyde for 8 h, rinsed with distilled water overnight, and dehydrated through a graded acetone series (10–100%, 2 h each). Samples were then dried using critical point drying (QUORUM K850 located at the Institute of Biology-UNAM) with CO_2, stained, and gold–palladium coated by cathodic sputtering. Micrographs at various magnifications were taken to observe the material's anisotropic structure in a JEOL 5900-LV (Japan), located at USAII-FQ.

2.2.4 Proximal chemical analysis. The content of proteins, ashes, carbohydrates, lipids and fiber was quantified. The moisture was determined by the oven drying method, proteins by the Kjeldahl method, lipids by the Soxhlet method, total carbohydrates by the phenol-sulfuric method, and the ash content by drying. For the quantification of fibers, the modified Van Soest-Wine method described by Hernández-Meléndez *et al.* (2016) was used.[23]

2.2.5 Raman spectroscopy. Raman spectroscopy of the extrudates was performed at LUCE–ICAT-UNAM. For the analysis of the samples, an excitation wavelength of 532 nm was established, and the output laser power was 300 mW. The spectra were recorded in a scanning range of 400–2000 cm^{-1}. Each sample was normalized with a 1003 cm^{-1} band of phenylalanine. The spectra were analyzed using OriginPro 9 software for baseline adjustments and the disulfide bonds were quantified from the Gaussian adjustment of the Raman vibration curves.

2.2.6 Instrumental texture profile. Instrumental texture measurements were carried out using a TA.XTplus Texture Analyzer (Stable Micro Systems, UK) equipped with a cylindrical flat-ended aluminum probe (SMSP/50; 50 mm diameter). Extrudate samples, previously cut into squares of 40 mm × 5 mm, were analyzed in duplicate at room temperature (~25 °C). Each sample underwent a double compression cycle to 50% of its original height, with a return distance of 20 mm, a test speed of 10 mm s^{-1}, and a contact force of 1 g. Force–time data were recorded, and texture parameters such as hardness, cohesiveness, springiness, and chewiness were calculated according to standard texture profile analysis procedures.

2.2.7 Statistical analysis. The nutritional composition and textural parameters of the analogues were expressed as mean ± standard error of the mean (SEM). Statistical differences among formulations were evaluated by one-way analysis of variance (ANOVA), using STATGRAPHICS Centurion, version 19. When significant differences were detected, Tukey's test was applied for multiple comparisons, using a significance level of $p < 0.05$. Additionally, Pearson's correlation coefficient (r) was calculated in Microsoft Excel to assess relationships between instrumental texture parameters and proximate composition data.

3. Results and discussion

3.1 Extrusion process

Preliminary tests were carried out to find the extrusion conditions that would provide a distinct fibrous structure. Various water flows were used and the resulting samples were evaluated. It was observed that when the flow rate was 2.64 L h^{-1}, the result was a very hard material, difficult to handle and almost petrified, as can be seen in Fig. 2. When this flow rate increased to 3.5 L h^{-1} the material was fragile and very sticky (Fig. 3), due to excessive hydration. It was concluded that an adequate water flow should be between 2.64 and 3.5 L h^{-1}, so it was decided to use a water flow of 3 L h^{-1}, with which an adequate texture was

Fig. 2 Images of the extruded analogues at a water flow of 2.64 L h^{-1}.

Table 2 Components that make up the screw according to the nomenclature used by the manufacturer[a]

Module	1			2	3	4			5	6			7	8	9	10
Type	C_2F (1)	T_2F (2)	C_2F (1)	C_2F (4)	C_2F (3)	BB 45° (1)	BB 90° (2)	C_2F (1)	C_2F (4)	BB 45° (1)	BB 90° (2)	C_2F (1)	C_2F (4)	C_2F (4)	C_2F (4)	C_2F (5)

[a] C2F: double-flight conjugate conveyor screws; T2F: double-flight trapezoidal conveyor screws; BB: 2-lobed kneading blocks. The numbers following the screw component type indicate the quantity of elements.

Fig. 3 Images of the extruded analogues at a water flow of 3.5 L h^{-1}.

obtained. The residence time of the extrusion process was approximately 4 min.

Preliminary tests were carried out with a maximum barrel temperature of 120 °C, which resulted in a smooth and homogeneous material that did not show a fibrous structure. Therefore, other maximum barrel temperatures were examined (140, 150, 160, 170 °C), with no clear differences in the resulting material. It should be noted that as the temperature increased in the cooking zone, a transition was observed that led to the formation of more pronounced fibrous structures, since high temperatures promote chemical interactions and molecular alignment that helps in the creation of fibrous structures. A high temperature leads to a better alignment of the polymer chains of the soy protein in the direction of the flow during the extrusion process. This alignment allows for a more pronounced fibrous structure. Molecular interactions such as hydrogen bonds and disulfide bridges are also favored, which give stability to the extrudates. Osen *et al.* (2014) suggest that high temperatures could cause macromolecules to unravel, thus leaving available binding sites for further cross-linking.[9] Polymer chain alignment and molecular interactions contribute to the formation of a three-dimensional network, which is related to the chewy texture of meat analogues. Therefore, the maximum barrel temperature selected for the extrusion process was 165 °C, close to the limits of the equipment. Special care was taken not to exceed the oil flow, since a higher value would lead to an overly oily product that would interfere with the formation of fibrous structures by reducing interactions between protein molecules. Another crucial parameter in the formation of fibers is the cooling matrix placed at the exit of the extruder and wrapped with dry ice to reach a minimum temperature of −12 °C.

Lipids also play a positive role in the extrusion process due to a lubricating effect in the mixing zones of the equipment.[24] This was confirmed when a test was performed that did not contain oil and a very dry and hard product was obtained as can be seen in Fig. 4. It was decided to incorporate AO at a flow rate of 0.36 L h^{-1} and visualize its effect.

Fig. 4 Images of the extruded analogues without avocado oil.

A formulation was developed with SPI and AO, but the resulting extrudate did not show solid evidence of a fibrous structure because there was no phase separation (Fig. 5a). Therefore, other formulations were developed to which 2, 4 and 6% by weight of pectin was added (Fig. 5b–d). Pectin was used as a binder with the aim of obtaining a mixture of incompatible biopolymers that remain in separate phases. A possible mechanism is that the amine group of proteins in SPI establishes electrostatic interactions with the carboxyl group of pectin, allowing the molecules to group together and stabilize each other.

They also exhibit hydrophobic interactions, since in an aqueous medium, the hydrophobic regions of both molecules tend to clump together to minimize their exposure to water. Xu *et al.* (2022) suggest that the hydrophobic effect between SPI and pectin is caused by the destruction of hydrogen bonds by nonpolar molecules.[25] Dekkers *et al.* (2016) suggest that the formation of fibers in a mixture of SPI and pectin is caused by the fact that pectin forms weaker and elongated filaments in the mixture.[22] When manually tearing the analogue obtained, the formation of fibers can be seen (Fig. 5b–d). At first glance, it is observed that the formulation containing 4% by weight of pectin has a more fibrous structure compared to the formulations containing 2 and 6% pectin.

3.2 Scanning electron microscopy

Scanning Electron Microscopy (SEM) was used to obtain information on the microstructure of the obtained extrudates. SEM images showed structural differences on the surface of the analyzed samples (Fig. 6). It was observed that the structure of the formulation that did not include pectin was practically smooth and isotropic, so no orientation was observed, reflecting that in this formulation no fibrous structures were formed. In the images of the samples that contained 2, 4 and 6% by

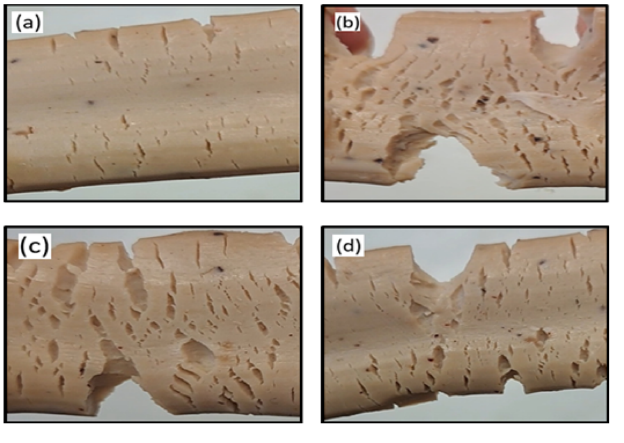

Fig. 5 Images of the extruded analogues: (a) SPI + AO; (b) SPI + AO + 2% by weight of pectin; (c) SPI + AO + 4% by weight of pectin; (d) SPI + AO + 6% by weight of pectin.

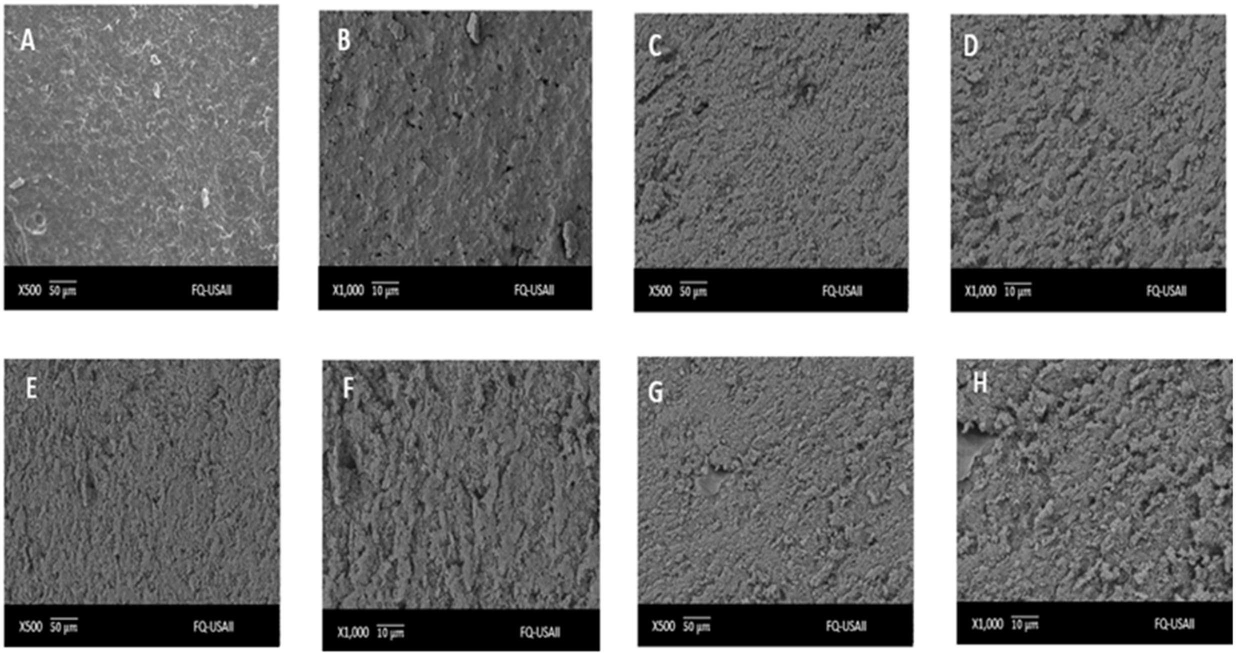

Fig. 6 Images of the extruded analogues: (A and B) SPI + AO; (C and D) SPI + AO + 2% by weight of pectin; (E and F) SPI + AO + 4% by weight of pectin; (G and H) SPI + AO + 6% by weight of pectin.

weight of pectin, a more fibrous morphology containing several elongated structures is obtained. These filaments are more imperceptible at a smaller scale; however, as the scale increases, they become more visible. A collective observation of the filaments and layers at different magnifications provides a complete description of the fibrous structure of the meat analogues extruded with high moisture. When the pectin weight concentration increased from 2 to 4%, the formation of fibers also increased. When the pectin weight concentration increased from 4 to 6%, a slight decrease in elongated structures could be observed, suggesting that low pectin concentrations may have promoted phase separation of the protein–polysaccharide mixture that contributed to fiber formation. When these concentrations were higher, they may have inhibited molecular interactions between protein molecules, resulting in a decrease in the formation of the fibrous structure. Previous studies have shown that hydrophobic interactions, hydrogen bonds, disulfide bonds and their interactions collectively maintain the structure of the protein extrudate.[14]

Considering that there was no evidence of the formation of fibrous structures in the mixture without pectin, only the samples containing 2, 4 and 6% by weight of pectin were studied for future analyses.

3.3 Proximal chemical analysis

Data from the nutritional composition of the extruded analogues were obtained on a dry basis; however, for a better understanding and analysis, the corresponding calculations are reported on a wet basis (Table 3).

Osen *et al.* (2014), Palanisamy *et al.* (2019), and Zhang *et al.* (2020) agree that a moisture content of approximately 55% is suitable for obtaining fibrous structures.[9,10,26] The moisture content obtained through high-moisture extrusion is within the range of corresponding meat analogues. Chen *et al.* (2011) suggest that an increase in moisture content leads to an increase in the interactions between disulfide bonds and hydrogen bonds and between disulfide bonds and hydrophobic interactions.[14] Other extrusion tests were also carried out where the moisture content was either too low (40%) or too high (70%) and no pronounced fibrous organization was observed, which corroborated that a moisture content of 55% was favorable for the formation of a fibrous structure. This demonstrated that moisture is directly related to the texture of the final product. Cheftel *et al.* (1992) state that when the moisture content exceeded 50% the products obtained were fibrous.[12]

It has been reported that a lipid content greater than 15% interferes with the formation of a fibrous structure[4,27] and that, to obtain a well-aligned fibrous structure, the maximum lipid content in a textured product should not exceed 10%.[13] Higher amounts act as barriers and prevent contact between the protein molecules, thus avoiding the formation of disulfide bonds and hydrogen bonds. Therefore, it can be concluded that the incorporation of AO did not interfere with the formation of fibers in the analogues obtained since the lipid content does not exceed 5% on a wet basis in any of the formulations. In addition to its nutritional contribution, AO resulted in a more tender and juicier extrudate.

Research on the appropriate nutritional profile for meat analogues has shown that an effective meat substitute should have around 30% protein content.[13,28] The analogues obtained contain a substantial protein contribution; formulations with pectin have between 25 and 32% protein on a wet basis, which

Table 3 Nutritional composition of the extruded analogues[c]

Components	DMD 2 (SPI + AO + 2% by weight of pectin)	DMD 3 (SPI + AO + 4% by weight of pectin)	DMD 4 (SPI + AO + 6% by weight of pectin)	Standard error of the mean
Moisture[a]	55.75[a]	54.95[a]	56.16[a]	0.33
Lipids[a]	3.72[a]	4.30[a]	3.68[a]	0.15
Ashes[a]	1.69[a]	1.77[b]	1.56[c]	0.03
Fibers[b]	0.73[a]	0.84[a]	0.69[a]	0.04
Proteins[a]	31.59[a]	26.44[bd]	25.51[cd]	0.96
Carbohydrates[b]	6.46[a]	11.64[b]	12.33[c]	1.17
Total	99.94	99.94	99.93	

[a] Determinations performed in triplicate. [b] Determinations performed in duplicate. [c] a–dDifferent superscript letters within the same row indicate significant difference between samples ($p < 0.05$). It can be seen that the sum of the components is close to 100%.

represents 50–65% of the daily reference value in adults. These values together with the minimum lipid content contribute to their use as an efficient nutritional substitute for traditional meat.[4]

From Table 3, the increase in carbohydrate content was found to be related to the presence of pectin in the samples, because pectin content is included in the carbohydrate content analysis and not in the one for fiber. Although a high carbohydrate content is considered undesirable, as it could modify the internal structure of the mixture in the extruder, resulting in a soft and less compact product,[29] the carbohydrate content is not significantly higher in our case.

3.4 Raman spectroscopy

Raman spectroscopy has been widely used for the study of meat of animal origin; however, it has not been frequently employed in the studies of meat analogues of plant origin. In this investigation, the data obtained by Raman spectroscopy were of great help to elucidate the main spectroscopic bands in the analogues obtained as well as for the quantification of the disulfide bridges and their relationship with the formation of fibrous structures (Fig. 7).

The band assigned to CH, CH_2 and CH_3 near 1450 cm^{-1} may represent the bending vibration of aliphatic residues. This band in the Raman spectra is proposed to monitor hydrophobic interactions between aliphatic residues. A higher intensity of this band denotes an increase in the hydrophobic interaction.[30,31] In the Raman spectra of the extruded samples, this band increases as the pectin concentration increases, which seems to indicate that the hydrophobic interaction of the aliphatic residues increased. Beattie *et al.* (2004) suggested that Raman spectroscopy could be used to predict the sensorial quality of meat, with the hydrophobicity of the myofibrillar environment being one of the most important aspects because it contributes to the shear strength, tenderness and texture of the meat.[32]

The Raman band located around 1650–1657 cm^{-1} was assigned to the amide I vibrational mode, which mainly involves C═O stretching vibrations and, in part, N–H in-plane bending of peptide groups.[33] The amide II vibration, expected between 1510 and 1560 cm^{-1}, mainly involves in-plane NH bending and CN stretching of the trans peptide bond. Due to the small change in polarizability associated with amide II, a distinct amide II Raman band cannot usually be detected in proteins. The Raman band located between 1200 and 1300 cm^{-1} corresponds to amide III and involves CN stretching and in-plane NH bending vibrations of the peptide bond, as well as contributions from Cα–C stretching and C═O in-plane bending.[34]

The intensity and localization of the phenylalanine band located between 1003 and 1004 cm^{-1} are attributed to the vibration of the benzene ring. This band is very intense and less sensitive to protein conformation or microenvironmental factors, so it is often used for normalization of Raman spectra of proteins. The intensity values of the Raman bands are determined after spectral normalization.[35,36]

The disulfide bond plays a very important role in the formation of the fibrous texture of the analogues. To form

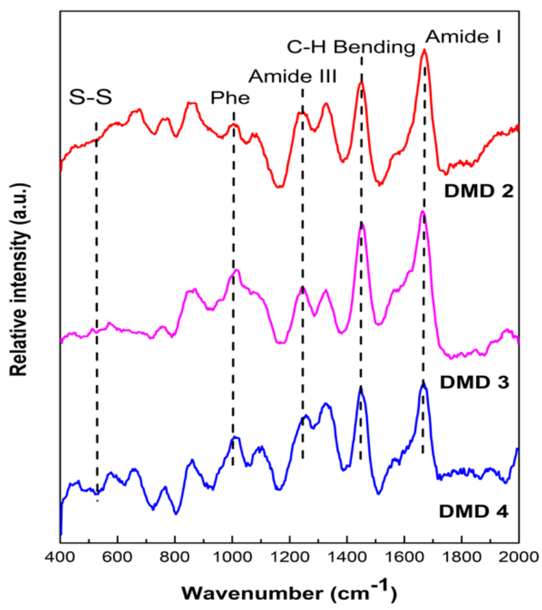

Fig. 7 Raman curves of the meat analogues with their main bands.

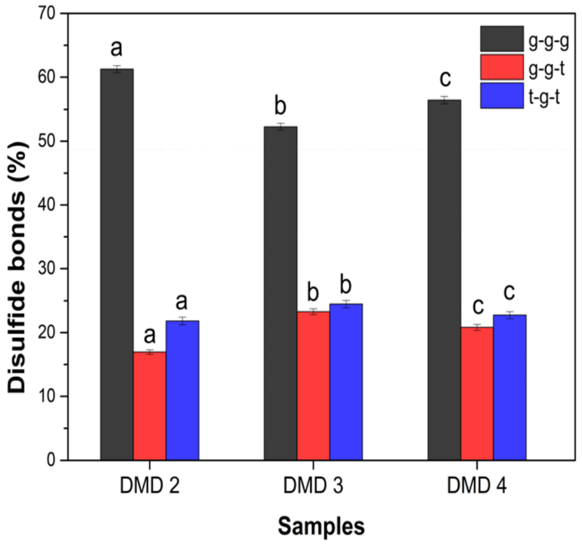

Fig. 8 Disulfide bond content of the obtained meat analogues. The t–g–t mode represents intermolecular disulfide bonds, while the g–g–g and g–g–t modes represent intramolecular disulfide bonds. Note: [a–c]different superscript letters in bars of the same color indicate significant difference between samples ($p < 0.05$).

a fibrous structure, an additional formation of disulfide bonds is necessary.[16] In order to quantify the disulfide bridges present in the extrudates, Gaussian fitting of the Raman vibration curves was performed using OriginPro 9 software. The disulfide bridges are in the characteristic band between 500 and 550 cm^{-1} and are classified according to their different vibrational spectra into 3 modes: 500–520 cm^{-1} is the gauche–gauche–gauche mode (g–g–g), 525–535 cm^{-1} is the gauche–gauche–trans (g–g–t) mode, and 535–545 cm^{-1} is the trans–gauche–trans (t–g–t) mode. The g–g–g and g–g–t modes represent intramolecular disulfide bonds, and the t–g–t mode represents intermolecular disulfide bonds. Fig. 8 shows the amount of disulfide bonds present in the samples expressed as a percentage. It can be observed that the intramolecular g–g–g mode is the predominant one in the extruded analogues obtained, which corresponds to the dominant conformation for soy proteins containing disulfide bonds. Intermolecular bonds are positively related to the formation of fibrous structures due to the stability they provide. This arrangement allows a linear and extended orientation of the polypeptide chains, which facilitates the formation of long and fibrous structures. Fig. 8 also shows an increase in these links when the pectin concentration increases from 2 to 4%; however, a slight decrease is observed when the pectin weight increases from 4 to 6%. This decrease in the formation of fibrous structures is supported by the images provided by scanning electron microscopy, where a decrease in fiber formation was observed when the pectin weight concentration increased from 4 to 6%.

3.5 Instrumental texture profile

The analysis of the texture profile allowed the quantification of the main textural mechanical parameters through the force–time curves generated from compressions performed by the texturometer. The values obtained are reported in Table 4. The hardness and chewiness values increased when the pectin weight concentration increased from 2 to 4%; however, when this concentration increased from 4 to 6% a significant decrease in these parameters was observed. This suggests that low pectin concentrations reinforced the texture of the meat analogues, while high concentrations weakened it. Dekkers *et al.* (2016) suggest that this weakening of the structure may be the result of how the incorporation of pectin inhibits the formation of a strong SPI network.[22] In addition, high pectin concentrations lead to more gelatinous products. The results obtained are supported by previous studies that suggest that a higher concentration of pectin weakens the structure.[22,37,38] As mentioned, the most important textural characteristics of meat analogues are hardness, springiness, cohesiveness, chewiness and resilience. Moisture and macronutrient content can greatly influence the textural properties of plant-based meat products.[39] For this reason, the relationship between the textural properties of the analogues and their moisture and protein content was analyzed (Fig. 9 and 10).

It was observed that the analogues in Fig. 9 that had a lower moisture content were, in turn, the hardest. Not surprisingly, chewiness, being related to hardness according to its definition,[40] followed the same trend. Hardness (r: −0.99) and chewiness (r: −0.85) showed a negative correlation with moisture content. Other research has shown how high moisture content is responsible for obtaining softer meat analogues.[14,22,29,41–43] This relationship could be explained by the need to use less force to break a structure that has a higher

Table 4 Values of textural parameters[a]

Samples	Hardness (N)	Springiness (%)	Cohesiveness (%)	Chewiness (N)	Resilience (%)
DMD 2 (SPI + AO + 2% by weight of pectin)	40.15^{a}	73.5^{a}	78.2^{a}	23.08^{ad}	35.9^{a}
DMD 3 (SPI + AO + 4% by weight of pectin)	54.55^{b}	63.5^{b}	69.8^{b}	24.18^{bd}	30.5^{bd}
DMD 4 (SPI + AO + 6% by weight of pectin)	33.10^{c}	50.4^{c}	61.9^{c}	10.33^{c}	26.9^{cd}
Standard error of the mean	3.18	3.38	2.39	2.27	1.43

[a] [a–d]Different superscript letters within the same column indicate significant difference between samples ($p < 0.05$).

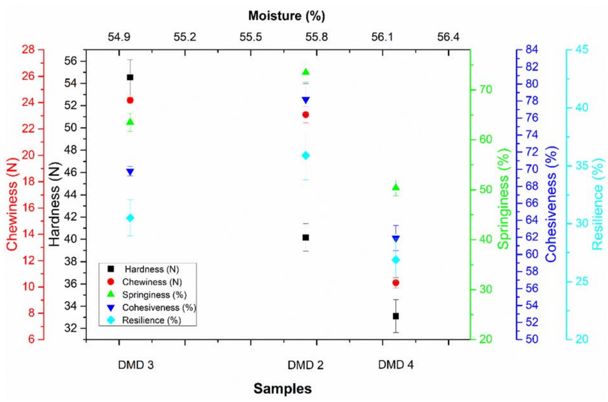

Fig. 9 Comparison between the parameters of the instrumental texture profile and the moisture content of the analogues.

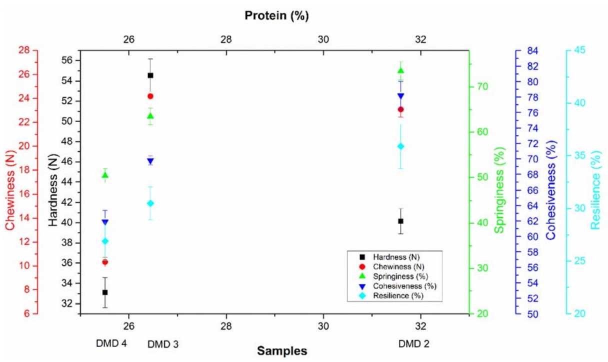

Fig. 10 Comparison between the parameters of the instrumental texture profile and the protein content of the analogues.

water content. Similarly, the springiness of the analogues obtained is negatively correlated with the moisture content (r: −0.49), that is, less springy products are obtained at higher moisture content, since a higher water content can make it difficult for the material to return to its original condition after compression. These results are supported by several authors.[8,44] Mateen *et al.* (2023) state that cohesiveness is positively related to moisture content.[42] However, these results contradict those obtained by Lin *et al.* (2000) and Wi *et al.* (2020) which, like the results obtained in this research (r: −0.40), state that an increase in water content negatively influences the cohesiveness of the analogues.[41,45] Several studies suggest that moisture content does not significantly influence the resilience of extrudates.[29,42] The results obtained show a negative correlation between moisture content and resilience (r: −0.31). From the above, it can be concluded that analogues obtained by extrusion that have a high moisture content will be less hard, less chewy, less springy, less cohesive and less resilient. This can be justified because, as has been suggested, a higher moisture content can cause incomplete texturization.[41]

Another important parameter in the analogues is their protein content and how it affects their textural properties. Several studies suggest a negative correlation between hardness and protein content.[38,46] However, this research showed a virtually non-existent correlation between both parameters (r: −0.05). Chewiness showed a positive correlation with protein content (r: 0.56). These results are supported by those obtained by Mateen *et al.* (2023);[42] in addition, Wee *et al.* (2018) suggest that foods with a higher protein content tend to be chewier.[47] In this research, protein content is positively correlated with springiness (r: 0.90). Grahl *et al.* (2018) added spirulina as a protein source to their mixtures and found that its content only affected springiness, so they suggest that it is likely that soy protein provides the products the ability to return to their original size after compression.[44] This is in line with the results that analogues with a high soy protein content have a good correlation with springiness. Webb *et al.* (2023) in their instrumental texture profile analyses demonstrate how the springiness, cohesion and chewiness of textured extrudates based on pea proteins are lower than those of SPI.[48]

These results reaffirm the properties of SPI to obtain meat analogues with good textural properties. Cohesiveness, which is defined as the strength of the internal bonds that make up the material, was found to be positively correlated with protein content (r: 0.93), since the higher the protein content, the easier it will be for adjacent protein molecules to form bonds.[41] A positive correlation was also found between protein content and resilience (r: 0.96). All of this leads to a firmer and more consistent analogue, thus avoiding a facilitated disintegration of the product. Other minor compounds, such as minerals and vitamins, influence the textural attribute. In this research, ash content was positively correlated with the hardness of the material (r: 0.95). These results correspond to what was proposed by Zhang *et al.* (2023) that a high ash content leads to stronger interactions between proteins through the presence of more ionic bonds, leading to a harder meat analogue.[49]

From all the analyses carried out, specifically scanning electron microscopy on the analogues obtained by high moisture extrusion, it is concluded that the sample that had 4% by weight of pectin (DMD 3) showed a more evident fibrous structure. Quantification of intermolecular disulfide bridges (t–g–t) (which are related to the formation of the fibrous structure), by Gaussian adjustment of the Raman bands, showed that sample DMD 3 had a greater amount of intermolecular disulfide bridges and, therefore, a greater fibrous structure. It was also the case for the sample that had a moisture content closer to 55% in addition to better chewiness. It is important to highlight that the samples that had 2 and 6% by weight of pectin also presented a fibrous structure and their textural properties were also within the range of those provided by the literature.

Finally, we acknowledge that a limitation of this study is the absence of sensory evaluation, which is a crucial component for understanding consumer acceptance of meat analogue products. Given the primary focus on the structural and functional characterization of the samples, along with logistical constraints during the research period, sensory testing was not conducted. However, we recognize its importance and plan to include comprehensive sensory analysis in future studies to complement the physicochemical and functional findings presented here.

4 Conclusions

Fibrous samples were produced *via* high-moisture extrusion using mixtures of soy protein isolate and pectin. The addition of avocado oil improved tenderness and texture, while the oil-free formulation yielded a dry, rough product. Proximate analysis of well-structured analogues showed ∼55% moisture, 27% protein, 10% carbohydrates, and 4% lipids. Scanning electron microscopy revealed that samples without pectin lacked fibrous structure, whereas formulations with 2–6% pectin exhibited elongated fibers due to biopolymer incompatibility. The 4% pectin sample showed the most pronounced fibrous structure and was also the hardest and chewiest, according to texture profile analysis. Raman spectroscopy confirmed the predominance of intramolecular disulfide bonds, typical of soy proteins, with the highest proportion of intermolecular bonds—associated with fiber formation—observed in the 4% pectin sample. Moisture content negatively affected hardness, chewiness, cohesiveness, springiness, and resilience, while protein content had little effect on hardness but positively influenced the other texture parameters.

Author contributions

Darianna Ur-Mora: investigation, methodology, writing the original draft. Oscar Hernández-Melendez: methodology, validation, project administration, supervision. Eduardo Bárzana: conceptualization, supervision, resources, writing-review & editing.

Conflicts of interest

There are no conflicts to declare.

Data availability

The datasets supporting this manuscript are available in this paper. The required information is available upon reasonable request from the authors. All data extracted from the literature, where applicable, have been duly acknowledged and cited.

Acknowledgements

CONAHCyT is acknowledged for grant CVU 1270184 to DUM. The authors would like to thank the following persons for their advice and technical support: M. C. María Berenit Mendoza of IB-UNAM (Critical Point Drying), Drs Selene R. Islas Sánchez and José M. Sániger of ICAT-UNAM (Raman Spectroscopy), Iván Puente (SEM), Julieta Sandoval and Verónica García of FQ-UNAM (various laboratory tests).

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