International Journal of Food Science and Agriculture, 2025, 9(4), 357-367
https://www.hillpublisher.com/journals/jsfa/
ISSN Online: 2578-3475
ISSN Print: 2578-3467

Physical and Textural Properties of Transglutaminase Treated Protein-enriched Extruded Snacks

Akinbode A. Adedeji[1,*], Felix Akharume[1], Youling L. Xiong[2]

[1]Biosystems and Agricultural Engineering Department, University of Kentucky, Lexington, KY 40506, USA.
[2]Animal and Food Science Department, University of Kentucky, Lexington, KY 40506, USA.

How to cite this paper: Akinbode A. Adedeji, Felix Akharume, Youling L. Xiong. (2025). Physical and Textural Properties of Transglutaminase Treated Protein-enriched Extruded Snacks. *International Journal of Food Science and Agriculture*, 9(4), 357-367.
DOI: 10.26855/ijfsa.2025.12.012

Received: October 31, 2025
Accepted: November 30, 2025
Published: January 14, 2026

Corresponding author: Akinbode A. Adedeji, Biosystems and Agricultural Engineering Department, University of Kentucky, Lexington, KY 40506, USA.

Abstract

In this study, we evaluated the effect of microbial transglutaminase (MTGase), which is often used as a processing aid in formulating food products, on some physical and textural properties of extruded snacks. The base ingredients were cornmeal enriched with protein sources from pea protein and cheddar cheese, which were added at 10% and 5% w/w, respectively. MTGase was added at 0.7% w/w, and the moisture content of the formulation was kept at 16.5 ± 1%, wet basis. The extrusion processing conditions used were a 58 g/min feed rate, a screw speed of 400 rpm, and a die temperature of 140°C. The selected physico-textural properties of the extrudates determined include radial expansion ratio (RER), bulk and apparent densities, water absorption index (WAI), water solubility index (WSI), porosity, fracturability, and hardness. The results showed that extruded snacks made from pure cornmeal in comparison to the extruded snacks made from cornmeal in combination with other added ingredients (MTGase treated proteins and untreated proteins) had the highest radial expansion ratio (2.9 ± 0.2) and porosity (92.7 ± 0.03%) as well as the least apparent density (1.3 ± 0.0 g/cm^3), bulk density (0.1 ± 0.0 g/cm^3), water solubility index (8.8 ± 1.4%), hardness (28.4 ± 8.2 N) and fracturability (29.9 ± 9.1 N). There was a significant ($P<0.05$) difference in the bulk density and porosity, but no apparent difference in density for protein-rich snacks treated MTGase compared to protein-rich snacks without MTGase. No significant ($P<0.05$) difference was observed in the WAI, WSI, RER, hardness, and fracturability due to the addition of MTGase to the protein-rich extruded snacks. While the effect of MTGase treatment on protein was not significant on the physical and textural characteristics of the extruded snacks, the FTIR spectra showed evidence of some protein cross-linking for the MTGase-based sample. Future work will aim to optimize the effect of MTGase addition protein modification in high protein extrudate.

Keywords

Microbial transglutaminase; MTGase; pea protein isolate; cornmeal; extrusion

1. Introduction

Extrusion is a widely used technology for producing ready-to-eat (RTE) snacks. The process occurs at either low (cold extrusion) or high temperatures (hot extrusion). Many extruded snacks are made using hot extrusion from mainly high-starch materials (corn, wheat, millet, and other cereal flours or starches), which confer an expanded, crunchy, and crispy texture to the final product [1]. Owing to the low amino acid profile of starchy foods and the increasing demand for protein-rich foods, many starch-based extruded snacks are now fortified with different protein ingredients, sometimes up to 45% by weight of the final product [1-3]. However, the addition of protein ingredients at higher concentrations (beyond 8% for soy protein isolate, 11 % for wheat gluten) leads to reduced expansion and loss of the desired crunchy and crispy textural quality characteristics of extruded starch-based snacks [2-5]. Generally, this effect on the physical and textural properties of extruded products containing high levels of protein ingredients has been attributed to the fact that protein addition causes a reduction in the extensibility of the starch polymers, discontinuity of the starch cell wall and dispersion of air throughout the extrudate, redistribution of water molecules within the extrudate, and an increase in extrudate viscosity due to protein network formation [2, 4-6]. The reduction in expansion is attributed to protein competing for water with starch, thereby interfering with its gelatinization and swelling [7]. Proteins have also been reported to increase the viscosity of the melt in the extruder, thereby limiting expansion [8]. Chaiyakul et al. [2] reported an inverse correlation between the concentrations of protein (wheat gluten and toasted soy grits) added to a high glutinous rice-based snack and its expansion ratio. In addition, it is directly correlated with its hardness and bulk density [2] and is directly correlated with its hardness and bulk density [2]. Pavani et al. [3] also reported a reduction in the radial expansion ratio and a concurrent increase in the bulk density of the extrudate as the pea protein isolate in the base ingredient (corn flour) increased. Modifying the functional properties of proteins in extruded ingredients could help mitigate the impact of protein addition on expansion. Several methods have been recommended to modify the impact of protein on extrusion, including heat treatment, pH-shifting treatment [9], ultrasonication [10], and enzyme treatment.

Microbial transglutaminase (MTGase) (EC 2.3.2.13, protein-glutamine γ-glutamyl transferase) is an enzyme that is widely used in the food industry to modify proteins to improve their texture and stability, and to also increase their hydrophobicity by inducing cross-linking in the matrix of protein constituents of foods, thereby reducing protein solubility [11, 12]. MTGase catalyzes acyl transfer reactions, deamidation, and crosslinking (polymerization) between protein intra- or inter-chain glutamine (acyl donor) and lysine (acyl acceptor) peptide residues, which leads to higher molecular weight proteins, which could improve the strength of the protein [11, 13]. MTGase is used in all kinds of products that include surimi, meats, noodles, dairy, and pasta to improve their functional properties [14]. They are a family of enzymes found in plants, mammal body fluids, and animal tissues. They modify proteins by incorporating the amine group. Although MTGase has been used in many food products to improve their textural properties, such as chicken meat gel [14] and myofibrillar/soy-protein gel [15], the understanding of its effects on extrudate expansion and textural properties improvement is limited. We hypothesized that using MTGase-pretreated protein in an extruded snack formulation would have comparable physical and textural properties to the control, compared to the pretreated product, by increasing the hydrophobicity of the added protein in a way that does not preclude starch from imbibing added water. Thus, the objective of this study was to investigate the effects of transglutaminase on proteins (pea protein isolate and cheddar cheese) in addition to the physical and textural properties of expanded-extruded product formulation.

2. Methods

2.1 Raw materials

Medium-sized nonorganic cornmeal was purchased from Bob's Red Mill Natural Foods Inc. (Milwaukie, Oregon, USA). A commercial pea protein isolate (Brand: NUTRALYS F85F) with 83% protein concentration on a dry weight basis was obtained from Roquette America Inc. (Geneva, Illinois, USA). Cheddar cheese powder was obtained from Anthony's Good (Glendale, California, USA) and used as a flavor source. The MTGase used in this experiment was Activa TG-TI enzyme (99% maltodextrin and 1% MTGase), donated by Ajinomoto Inc. (Fort Lee, New Jersey, USA).

2.2 Sample preparation

Three different formulation batches were prepared according to the recipes listed in Table 1. Formulation_1 reflects a 0.7% MTGase addition level. This formulation represents optimized conditions based on the literature where MTGase has been added to modify functional properties (hydrophobicity and texturization) of proteins in extruded products [16]. To activate the enzyme, the calculated amount of water necessary to maintain the resultant formulation at 16.65% (w.b.) was added to a mixture of pea protein, cheddar cheese, and MTGase. According to the method of Shand et al. [17], the mixture was stirred thoroughly and incubated at 50 °C for 45 min before blending with a weighted amount of cornmeal in a KitchenAid mixer (Pro 600, MI, USA) for 30 min.

Table 1. Ingredient formulations used in this study

Formulation	Corn meal (g)	PPI (g)	Cheddar Cheese (g)	MTGase (g)	Added Moisture (ml)	Moisture content (%, w.b.)
Formulation_1 (Control)	421.5	0	0	0	26.2	16.65
Formulation_2	421.5	50	25	0	35.0	16.65
Formulation_3	421.5	50	25	3.5	35.5	16.65

Notes. PPI - Pea Protein Isolate; w.b. – wet basis.

2.3 Extrusion procedure

Extrusion was carried out in a twin-screw extruder (Eurolab 16, ThermoFisher Scientific, Karlsruhe, Germany) with a screw diameter of 32 mm, a length-to-diameter ratio (L/D) of 25:1, six-barrel heating zone temperatures of 30/50/80/110/130/140 °C, respectively, with a die temperature of 140 °C and a die hole diameter of 3 mm. The feeding rate of the samples was kept at 32 g/min, and the screw speed was kept at 400 rpm. These extrusion conditions were from previous studies for optimized expansion. All extrusion conditions were selected based on repeated trials to obtain the most expansion, and other desirable properties of extruded products such as hardness, porosity, bulk density, and color [18, 19]. They were kept constant for all experimental treatments.

2.4 Extrudate analysis

2.4.1 Radial expansion ratio (RER), apparent density, bulk density, and porosity

The radial expansion was determined by dividing the cross-sectional area of the extrudate by the cross-sectional area of the die nozzle according to Gopirajah and Muthukumarappan [20]. Because of the unevenness in the circularity of the extrudates, radial measurements were made 10 times per treatment. The bulk density (ρ_b) of the extrudates was determined as the mass-to-volume ratio of five extrudates with volume calculated based on length and diameter measurements taken at 10 random points to determine the volume (Equation 1).

$$\rho_b = \frac{Measure\ weight}{Volume\ of\ container}, kg/m^3 \tag{1}$$

Apparent density and porosity were determined by using a Gas Pycnometer (AccuPyc II 1340, Micromeritics, Norcross, GA). The apparent density (ρ_t) of the extrudates was determined using a gas pycnometer (Model 1340 multivolume, Micromeritics Instrument Corporation, Norcross, GA, USA). Porosity (ε) is defined as the ratio of intergranular void volume to the bulk volume. Porosity was determined using the expression shown in Equation 2 [19].

$$\varepsilon = 1 - \frac{\rho_b}{\rho_t} \tag{2}$$

2.4.2 Water absorption index (WAI) and water solubility index (WSI)

WAI and WSI were determined according to the method of Ding et al. [21] with some modifications. Ten grams of ground extrudate was suspended in 50 ml of water, stirred continuously for 30 min at room temperature, and centrifuged at 3000 x g for 15 min. The supernatant was collected, dried, and weighed. The WSI is the weight of the dry solids in the supernatant expressed as a percentage of the original weight in the sample, while the WAI is the weight of the gel (residual) per unit weight of the original dry sample. The assay was carried out in triplicate. WAI (g/g, dry solid) and WSI (%) were determined from the equations (3 and 4) below:

$$WSI = \frac{Solid\ weight\ in\ supernatant}{dry\ sample\ weight}\ x\ 100\% \tag{3}$$

$$WAI = \frac{Dissolved\ solid\ (Gel)\ weight}{dry\ sample\ weight} \tag{4}$$

2.4.3 Textural properties evaluation

Textural profile analysis (TPA) of the extrudate was performed using a texture analyzer (TA-XTplus, Hamilton, MA, USA) equipped with a 50 kg load-cell. The instrument was interfaced with the Exponent 6.1.4.0 software (Stable Micro Systems, Surrey, UK). Ten pieces of extruded snacks were compressed to 70% of the initial diameter using a 100 mm compression plate that travels at a test speed of 1.0 mm/s. Fracturability was defined as the force at the first crack in the force–displacement curve. Hardness was defined as the maximum compression force (Fmax, N) required to break the sample, following Azzollini et al. [22].

2.5 FTIR Analysis of the samples

FTIR spectroscopy (Thermo Nicolet iS50, Thermo Electron, Madison, WI, USA) was used to characterize structural differences among three extruded formulations: (i) 100% corn meal [Formulation_1], (ii) corn meal supplemented with pea protein isolate and cheddar cheese powder [Formulation 2], and (iii) the same formulation additionally treated with microbial transglutaminase (MTG) [Formulation_3]. Prior to analysis, spectra were baseline-corrected and vector-normalized. Peak assignments followed established food-biopolymer FTIR literature, with particular emphasis on carbohydrate vibrational modes (1200-900 cm^{-1}), protein amide bands (1700-1500 cm^{-1}), and lipid-associated C–H/C=O regions (3000-2800 cm^{-1} and ~1740 cm^{-1}) [23-25]. A small amount of the sample was placed on the diamond crystal of the FTIR accessory (Smart iTR, Thermo Fisher Scientific). The instrument settings included 32 scans per sample, a resolution of 4 cm^{-1}, and absorbance mode. Before measuring the samples, a background spectrum was recorded to compensate for any instrument and environmental influences [26].

2.6 Experimental design and statistical analysis

A completely randomized experimental design was applied for the study, in which three treatments were considered (Formulation_1, Formulation_2, and Formulation_3) and nine response variables were tested (radial expansion ratio, apparent density, bulk density, porosity, WAI, WSI, hardness, and fracturability). This resulted in a total of 3 × 8 data points, each with three replicates. The treatments (extrusion conditions, MTGase, and cheese addition) and replicates were randomized in the experimentation. One-way analysis of variance (ANOVA) was performed on SAS statistical software version 9.4 (SAS Institute, Cary, NC) to test for the to test for significant differences among treatment means. Where it is significant at a 95% confidence interval, mean separation is performed using the Duncan multiple range test [26].

3. Results and Discussion

3.1 Radial expansion ratio, apparent density, bulk density, and porosity

Following extrusion, the radial expansion ratio (RER) decreased significantly ($P<0.05$) in all formulations with added pea protein and/or cheddar cheese with or without MTGase, compared to the control containing 100% cornmeal (Figure 1). The visual observation of the samples shows a distinct difference in expansion that was confirmed by objective measurement. Formulation_1, the control, has a mean RER value of 2.87±0.2, Formulation_2 has a RER value of 1.68±0.2, and Formulation_3 has a RER value of 1.83±0.08. Additionally, products containing MTGase (Formulation_3) showed a higher, but insignificant RER compared to similar products without the enzyme (Formulation_2). While MTGase addition improved the expansion of the extrudate, it is not significant enough compared to the control that contained no added protein. This result contradicts our initial expectation that reduced protein hydrophobicity would allow starch to absorb sufficient water for comparable expansion; the effect of MTGase is visible in the protein-MTGase sample (Formula_3). The outcome is consistent with reports from the literature [6, 27, 28]. Beck et al. [6] reported the least expansion for rice extrudate with the highest content of protein (42%). Shah et al. [27] reported a RER reduction from 3.72 to 2.64 for chickpea fortified corn extrudates. Increased protein content was expected to decrease the glass transition temperature of the extrudate melt, including the bubble size, and might have caused the redistribution of the water molecules [28]. The range (2.02-3.39) of RER reported by Sahu, Patel, and Tripathi [29] for corn extrudate enriched with soy protein is similar to the results presented in this study. Also, the cross-linking of the protein might have caused a firmer protein network instead of softening of the protein-enriched starch extrudates [30]. What we think likely happened was that MTGase catalyzes covalent cross-links between protein chains, reinforcing the melt structure and impeding the sudden vaporization of moisture at the die, therefore causing a reduced expansion. Another critical factor to note for future studies is the impact of the extruder barrel temperature on the MTGase denaturation threshold. The extruder die-end temperature was about 140 °C.

The results of apparent density, bulk density, and porosity of extruded snacks are presented in Figure 2. ANOVA showed that there is a significant effect ($P<0.05$) of the model on the variability of the densities based on the formulations. The apparent density and bulk density increased with the addition of protein and MTGase in the formulation, while the porosity decreased. For example, extrudates made from 100% cornmeal have the least apparent density, bulk density, and the highest porosity of 1.34 g/cm^3, 0.10 g/cm^3, and 92.7 %, respectively, while extruded snacks made from Formulation_3 (added PPI, cheese, and MTGase) has 1.39 g/cm^3 apparent density, 0.24 g/cm^3 bulk density, and a porosity of 85.11%. Extruded snacks made from formulations with added MTGase generally showed a higher apparent density and bulk density compared to similar snacks without MTGase. However, for porosity, an extrudate made with an MTGase treated pea showed a higher porosity compared to a similar product without the enzyme. This behavior was reported by other authors [6, 20]. The high starch content and subsequent superheating under high pressure in the extruder barrel led to high expansion. Hence, the low bulk density of the 100% cornmeal snacks, and conversely, high density in the MTGase treated PPI extrudates, where some of the starch had been replaced by small particle protein isolate.

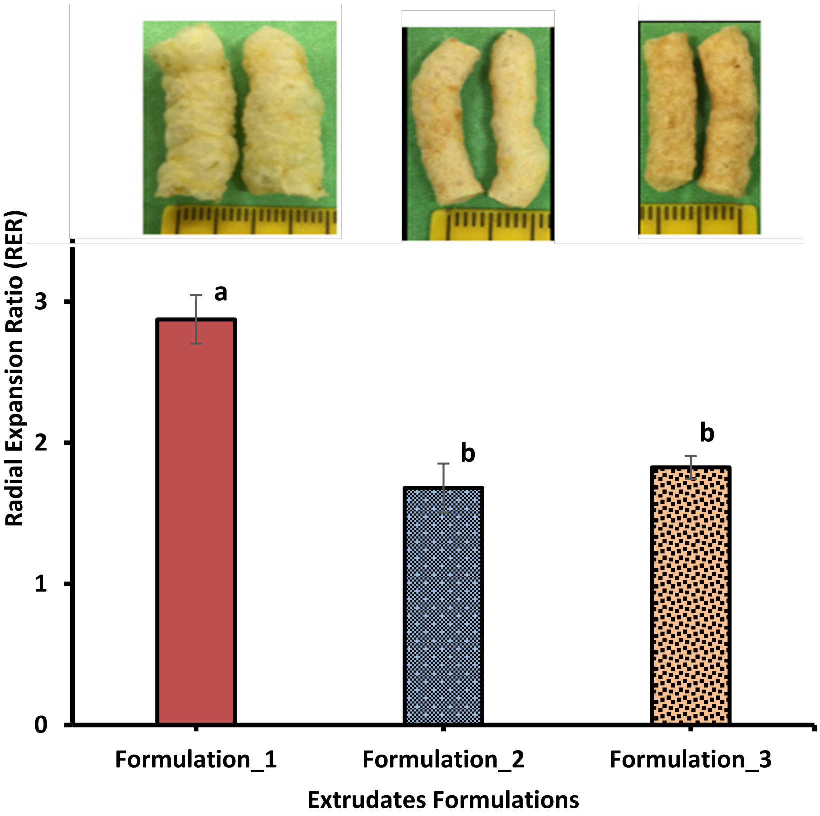

Figure 1. Radial expansion ratio (RER) of extruded snacks made from different formulations (_1: 100% cornmeal [control], _2: cornmeal + cheese + PPI, _3: cornmeal + cheese + PPI + MTGase). Bars with different letters at the top are significantly different at 5% probability of error.

The results of apparent density, bulk density, and porosity of the extruded snacks are presented in Figure 2. Analysis of variance (ANOVA) showed that there is a significant effect ($P<0.05$) of the model on the variability observed in the densities based on the formulations. It can be observed that the apparent density and bulk density increased when protein and enzyme (MTGase) were added to the product formulation, while the porosity decreased across the different formulations. For example, extrudates made from 100% cornmeal had the least apparent density, bulk density, and the highest porosity of 1.34 g/cm^3, 0.10 g/cm^3, and 92.7%, respectively, while extruded snack made from Formulation_3 (added PPI, cheese, and MTGase) had 1.39 g/cm^3 apparent density, 0.24 g/cm^3 bulk density and a porosity of 85.11%. Extruded snacks made from formulations with added MTGase generally showed a higher apparent density and bulk density compared to similar snacks without MTGase. However, for porosity, the extrudate made with MTGase treated pea showed a higher porosity compared to a similar product without the enzyme. This behavior was reported by other authors [6, 20]. Beck et al. [6] reported that high protein addition (42%) significantly increased the bulk density of rice-based extruded products containing added fiber showed a significant increase in bulk density, as reported in this study. It is apparent that MTGase did not significantly modulate the hydrophobicity of a protein that requires multiple times the amount of water by starch to get cooked [31]. Polysaccharides are rich in hydrophilic groups that form hydrogen bonds that help to absorb and retain water. The high starch content and subsequent superheating under high pressure in the extruder barrel led to high expansion in the control, as expected. Hence, the low bulk density of the 100% cornmeal snacks, and conversely high density in the MTGase treated PPI extrudates, where some of the starch had been replaced by small particle protein isolate. A way to further enhance the expansion of protein-rich treatments to reduce the bulkiness is by adding additives like starch or gums (xanthan, sodium alginate, and maltodextrins) that have a large amount of hydroxyl functional groups, with the potential for hydrogen bonding with the potential for more water absorption in the mix [31, 32].

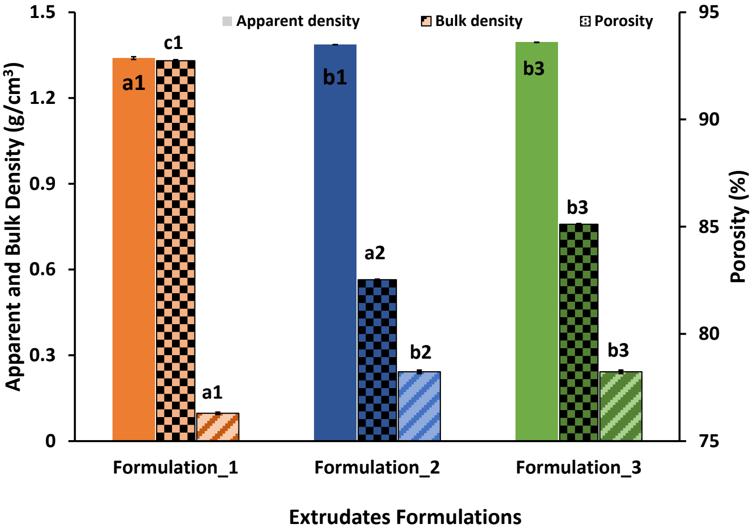

Figure 2. Apparent density, bulk density and porosity of extruded snacks made from different formulations (_1: 100% cornmeal [control], _2: cornmeal + cheese + PPI, _3: cornmeal + cheese + PPI + MTGase). Bar design for the same property with different letters at the top are significantly different at P<0.05. The number next to each letter at the top of the bar indicates the formulation they represent.

3.2 Water absorption index (WAI) and water solubility index (WSI)

The water absorption index (WAI) is a measure of the amount of water absorbed by starch and other hydrophilic constituents in the extrudates. It is a reflection of the index of starch gelatinization [33]. The WAI for all the treatments was not significantly different (P>0.05). This consistent WAI for all three formulations suggests that the structural integrity and starch gelatinization in all three samples remained comparable, possibly due to the dominant presence of cornmeal, a starch-rich ingredient, despite the addition of protein and enzyme to two of the treatments. From Figure 3, we see that the WAI of extruded snacks made from cornmeal (Formulation_3) with added MTGase treated PPI showed the lowest value (3.49±0.1 g/g dry solid), while pure cornmeal (Formulation_1) extrudate showed the highest WAI value (3.88±0.2 g/g dry solid). Generally, snacks containing reduced concentrations of cornmeal as a result of the addition of proteins showed a significantly lower WAI compared to snacks made from pure cornmeal. The more the starch content in the extrudate, the higher the WAI value. This phenomenon has been reported previously by other authors [28, 34, 35]. This outcome is instigated by the same factors that impacted the densities and porosity of the treatments – the level of substances with capacity for water absorption in each sample [31, 32].

The water solubility index (WSI) reflects the extent of macromolecule breakdown during the extrusion process [20]. It is indicative of molecular degradation and solubility of the extrudate's components, such as peptides, sugars, and degraded starch. From Figure 3, the WSI values of extruded snacks containing proteins with or without MTGase treatment are higher than those of snacks made from pure cornmeal (Formulation_1), while MTGase sample (Formulation_3) has a WSI that is significantly (P<0.05) higher than the control. A snack containing MTGase treated cheese and cornmeal only showed a higher WSI than similar products with MTGase treated pea protein. This may be due to the easy breakdown of the flavor and fat compounds in the formulation mix. Pea protein has higher solubility than predominantly starch ingredients, and MTGase, an enzyme that catalyzes protein cross-linking, leads to structural stability, partial hydrolysis, and/ or formation of smaller soluble peptides under extrusion stress that causes an increase in solubility [36, 37]. Both WAI and WSI are indicators of mouthfeel and tooth packing during mastication of extruded snacks [34].

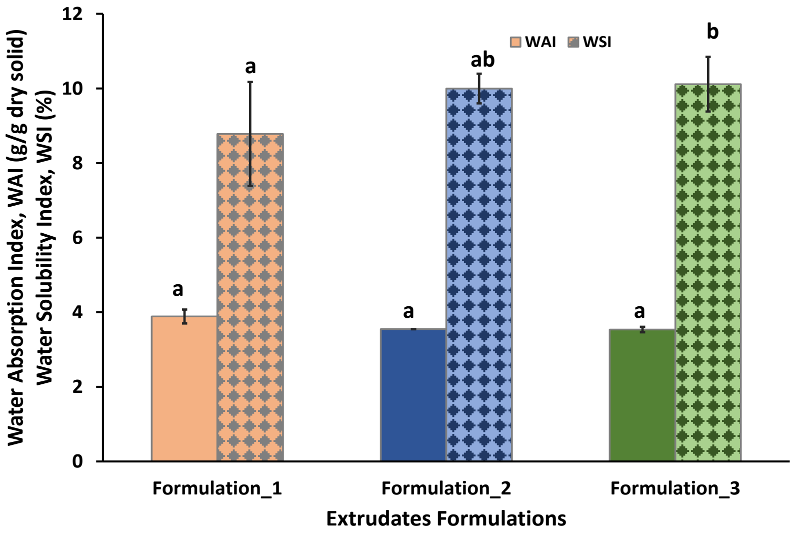

Figure 3. Water absorption index (WAI) and water solubility index (WSI) extruded snacks made from different formulations (_1: 100% cornmeal [control], _2: cornmeal + cheese + PPI, _3: cornmeal + cheese + PPI + MTGase). Bars design for each property with different letters at the top are significantly different at 5% probability of error.

3.3 Textural properties

Both hardness and fracturability are key quality attributes of extruded products that reflect the expansion and cell structure of extrudates [38, 39]. They simulate the force required to make the first crack (fracturability) and grind through (hardness) a food product on the first bite, respectively. Figure 4 presents the hardness and fracturability value of the extrudate from the different formulations. It is apparent that all extrudates containing proteins with or without MTGase treatment showed a significantly higher ($P<0.05$) fracturability and hardness than the extrudate made from pure cornmeal. The significant difference in these important attributes of the extrudates indicates that protein addition increased bulk density and reduced expansion (Formulation_2) as reported earlier, but MTGase addition (Formulation_3) did not significantly reverse this trend as expected. What this implies is that crosslinking stiffens, instead of softening, the protein matrix, increasing resistance to deformation [40]. Extrudates containing both proteins but without MTGase showed a higher value of hardness compared to a similar sample with the enzyme treatment, though not significant ($P>0.05$). Hardness and fracturability correlate well with other attributes of extruded products like RER, porosity, bulk, and apparent densities (Table 2).

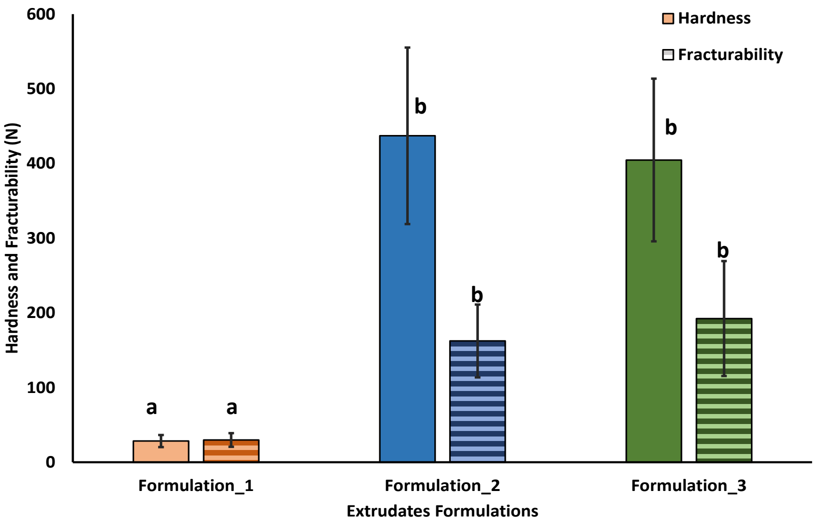

Figure 4. Peak compression force (hardness) and fracturability of extruded snacks made from different formulations (_1: 100% cornmeal [control], _2: cornmeal + cheese + PPI, _3: cornmeal + cheese + PPI + MTGase). Bars for each textural property with different letters at the top are significantly different at 5% probability of error.

Table 2. Correlation analysis between the quality properties of the extrudates

Measured Properties	Hardness	Fracturability	WAI	WSI	Bulk Density	Apparent density	Porosity	RER
Hardness	1	0.97	-1.00	0.99	0.99	0.98	-0.98	-1.00
Fracturability	0.97	1	-0.99	1.00	0.92	1.00	-0.91	-0.96
WAI	-1.00	-0.99	1	-1.00	-0.97	-0.99	0.96	0.99
WSI	0.99	1.00	-1.00	1	0.95	1.00	-0.95	-0.98
Bulk Density	0.99	0.92	-0.97	0.95	1	0.93	-1.00	-0.99
Apparent density	0.98	1.00	-0.99	1.00	0.93	1	-0.93	-0.97
Porosity	-0.98	-0.91	0.96	-0.95	-1.00	-0.93	1	0.99
RER	-1.00	-0.96	0.99	-0.98	-0.99	-0.97	0.99	1

Notes. RER: radial expansion Ratio; WSI: water solubility index; WAI: water absorption index.

All the quality attributes of the extrudates are highly correlated according to the Pearson correlation analysis conducted at a 95% confidence level, shown in Table 2. It is not surprising that RER is highly negatively correlated with hardness. Expanded products usually exhibit minimal resistance to compressive force, so when the extrudate shows minimal expansion, you expect a hard material, and vice versa. A similar negative relationship is seen between porosity, fracturability, and hardness [8]. Highly porous materials are expected to offer less resistance to the deformation force applied to the sample to breach the surface integrity and to compress it [41]. The result also indicates that the hard samples absorb less water and dissolve easily in aqueous solution. There was also a positive correlation between the densities and fracturability, the force to breach the surface integrity of a sample. When a product is dense, it usually takes more force to crack through its surface. These correlations help guide formulation decisions (e.g., starch type, protein addition, enzyme treatment) for desired functional and sensory properties in extruded foods and may influence mastication properties.

3.4 FTIR spectral interpretation for extruded corn–protein systems

The 100% corn extrudate [Forumation_1] exhibited spectral features characteristic of starch-rich matrices and showed the highest relative intensity in the carbohydrate fingerprint region (1150-950 cm^{-1}), corresponding to C–O, C–C, and glycosidic link vibrations typical of gelatinized and retrograded starch. This high intensity in the carbohydrate region is consistent with FTIR characterizations of cereal starches, in which the 1000-950 cm^{-1} region reflects ordered versus amorphous starch domains [25]. The low intensity of the amide I (~1650 cm^{-1}) and amide II (~1540 cm^{-1}) bands in this sample is expected, given the minimal protein content of native corn meal.

In contrast, both protein-enriched extrudates displayed substantially stronger amide I/II bands, indicating a higher abundance of peptide bonds from pea protein and cheese solids. The amide I envelope also showed subtle differences in band shape, including broadened features around 1640-1660 cm^{-1} and 1670-1685 cm^{-1}, suggesting changes in α-helix/β-sheet/turn structures due to thermal extrusion and protein–carbohydrate interactions. Similar extrusion-induced alterations in plant-protein secondary structure have been widely documented using amide I deconvolution [24, 42]. The increase in C–H stretching (2920, 2850 cm^{-1}) and CH_2/CH_3 bending bands (1450-1370 cm^{-1}) in these formulations is consistent with the additional lipid and protein components contributed by cheddar powder [26].

The MTG-treated extrudate [Formulation_3] showed additional, though more subtle, modifications in the amide regions relative to its non-MTG counterpart. Minor shifts and intensity redistributions within the amide I/II bands are consistent with MTG-mediated formation of ε-(γ-glutamyl)-lysine crosslinks, which alter hydrogen bonding and protein conformational organization. These FTIR signatures have been previously reported in dairy and plant protein systems treated with MTG [23]. This change was not sufficient to instigate the was not sufficient to produce significant changes in physical and textural properties anticipated.

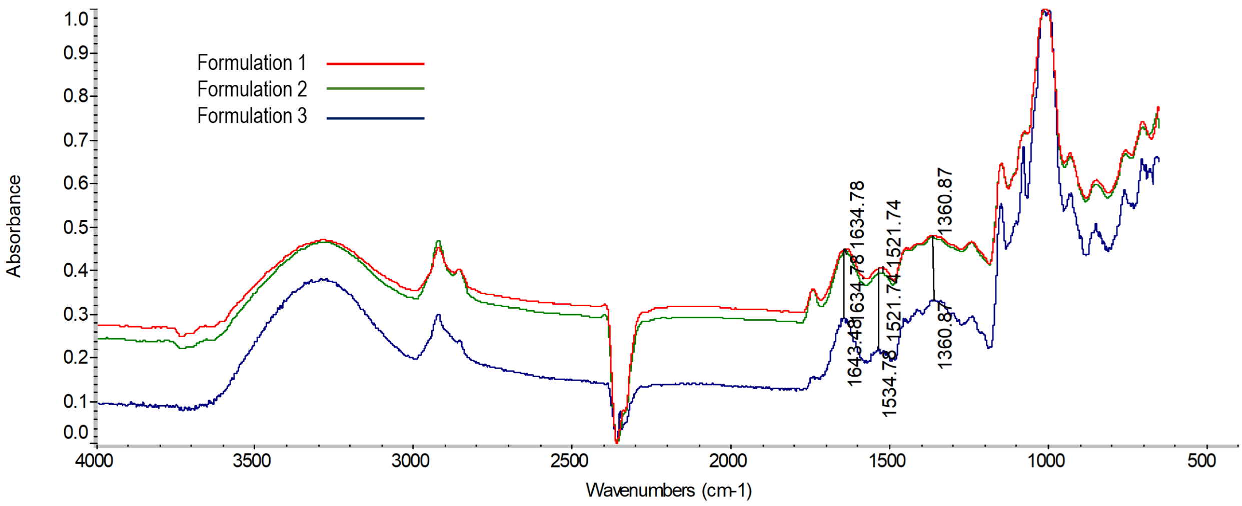

Figure 5. FTIR spectra of extruded samples produced from (i) 100% corn meal [Formulation_1], (ii) corn meal + pea protein isolate + cheddar cheese powder [Formulation_2], and (iii) the same formulation treated with microbial transglutaminase (MTG) [Formulation_3]. Spectra are normalized for fair comparison.

4. Conclusion

The addition of MTGase treated proteins (cheese and PPI) to product formulation prior to extrusion led to products with lower radial expansion ratio, water absorption index, and porosity, as well as higher apparent and bulk density, solubility index, hardness, and fracturability, when compared to extruded snacks made from pure cornmeal (control). All these properties were highly correlated. While the extruded snacks containing proteins treated with or without MTGase have nutritional benefits, the effect of MTGase treatment on the physico-textural properties of the resultant extruded snacks is insignificant (P>0.05). Thus, the formulation with protein treated with MTGase showed a reduction in properties compared to the samples not treated with the enzyme. The established science is that MTGase decreases the water-binding property of protein, without which protein-rich extrudates are expected to show less expansion and other characteristics synonymous with expanded products. MTGase treatment (Formulation_3 compared to Formulation_2) showed slight improvements; however, these differences were not statistically significant. FTIR result showed that Formulation [MTGase treated sample] showed some crosslinking of the proteins, but not in sufficient amount to instigate the desired structure changes. Further investigation is needed to optimize the MTGase application. This may include evaluating enzyme activity at different extrusion stages and incorporating additives such as hydrocolloids or polysaccharides.

Abbreviations

The following abbreviations are used in this manuscript:

MTGase	Microbial transglutaminase
RER	Radial expansion ratio
PPI	Pea protein isolate
WAI	Water absorption index
WSI	Water solubility index

Funding

This work was supported by the Kentucky Agricultural Experiment Station (KAES), the National Institute of Food and Agriculture (NIFA), U.S. Department of Agriculture, Hatch-Multistate project #: 1024529.

Acknowledgements

The authors would like to thank Ajinomoto American Inc. for the gift of Activa TG-TI enzyme.

References

[1] Philipp C, Buckow R, Silcock P, Oey I. Instrumental and sensory properties of pea protein-fortified extruded rice snacks. Food Res Int. 2017;102:658-665.

[2] Chaiyakul S, Jangchud K, Jangchud A, Wuttijumnong P, Winger R. Effect of extrusion conditions on physical and chemical properties of high protein glutinous rice-based snack. LWT-Food Sci Technol. 2009;42(3):781-787.

[3] Pavani M, Singha P, Rajamanickam DT, Singh SK. Impact of extrusion processing on bioactive compound enriched plant-based extrudates: A comprehensive study and optimization using RSM and ANN-GA. Future Foods. 2024;9:100286.

[4] Moraru C, Kokini J. Nucleation and expansion during extrusion and microwave heating of cereal foods. Compr Rev Food Sci Food Saf. 2003;2(4):147-165.

[5] Alefew YD, Tiruneh AT, Yehuala TF. Optimization of extrusion conditions for development of high quality rice-lupin-pumpkin based extruded snack food. Heliyon. 2024;10(24):e40913.

[6] Beck SM, Knoerzer K, Foerster M, et al. Low moisture extrusion of pea protein and pea fibre fortified rice starch blends. J Food Eng. 2018;231:61-71.

[7] Allen KE, Carpenter CE, Walsh MK. Influence of protein level and starch type on an extrusion-expanded whey product. Int J Food Sci Technol. 2007;42(8):953-960.

[8] Yu C, Liu J, Tang X, Shen X, Liu S. Correlations between the physical properties and chemical bonds of extruded corn starch enriched with whey protein concentrate. RSC Adv. 2017;7(20):11979-11986.

[9] Chen W, Wang W, Ma X, et al. Effect of pH-shifting treatment on structural and functional properties of whey protein isolate and its interaction with (−)-epigallocatechin-3-gallate. Food Chem. 2019;274:234-241.

[10] Saran V, Pavithra R, Koli V, et al. Ultrasound modification of techno-functional, structural, and physico-chemical properties of legume proteins: A review. Food Biosci. 2024;60:104456.

[11] Gaspar ALC, de Góes-Favoni SP. Action of microbial transglutaminase (MTGase) in the modification of food proteins: A review. Food Chem. 2015;171:315-322.

[12] Zhan F, Tang X, Sobhy R, Li B, Chen Y. Structural and rheology properties of pea protein isolate-stabilised emulsion gel: Effect of crosslinking with transglutaminase. Int J Food Sci Technol. 2022;57(2):974-982.

[13] Karamoko G, Chèné C, Karoui R. Impact of acidic and basic pH on the interfacial shear rheology and foaming properties of transglutaminase cross-linked casein micelle: Structure-function relationship. Food Chem. 2025;476:143384.

[14] Trespalacios P, Pla R. Simultaneous application of transglutaminase and high pressure to improve functional properties of chicken meat gels. Food Chem. 2007;100(1):264-272.

[15] Ramırez-Suárez JC, Xiong YL. Effect of transglutaminase-induced cross-linking on gelation of myofibrillar/soy protein mixtures. Meat Sci. 2003;65(2):899-907.

[16] Liu X, Qin J-X, Li J-H, Zhou J-W, Chen J. Effect of transglutaminase on the structure and digestive characteristics of high-moisture pea protein extrudates. Shipin Kexue/Food Sci. 2023;44(5):1–8.

[17] Shand PJ, Ya H, Pietrasik Z, Wanasundara PKJP. Transglutaminase treatment of pea proteins: Effect on physicochemical and rheological properties of heat-induced protein gels. Food Chem. 2008;107(2):692-699.

[18] Joseph M, Guo Q, Lindshield B, Adedeji AA, Alavi S. Characterization of extruded sorghum-soy blends to develop pre-cooked and nutritionally dense fortified blended foods. Foods. 2025;14(5):779.

[19] Woomer J, Schendel R, Lovely J, Vijayakumar PP, Adedeji AA. Value-added application of bourbon spent grain and proso millet for high-fiber extruded product. J ASABE. 2023;66(5):1019-1032.

[20] Gopirajah R, Muthukumarappan K. Effect of extrusion process conditions on the physical properties of tef-oat healthy snack extrudates. J Food Process Preserv. 2018;42(3):e13559.

[21] Pardhi SD, Singh B, Nayik GA, Dar BN. Evaluation of functional properties of extruded snacks developed from brown rice grits by using response surface methodology. J Saudi Soc Agric Sci. 2019;18(1):7-16.

[22] Azzollini D, Derossi A, Fogliano V, Lakemond CMM, Severini C. Effects of formulation and process conditions on microstructure, texture and digestibility of extruded insect-riched snacks. Innov Food Sci Emerg Technol. 2018;45:344-353.

[23] Chen Y, Gao X, Su W, et al. Insight into transglutaminase cross-linking induced gel strength and thermal stability improving of gelatin-based emulsion-filled gels. Int J Food Sci Technol. 2023;58(6):2909-2920.

[24] Fetouhi A, Benatallah L, Nawrocka A, et al. Investigation of viscoelastic behaviour of rice-field bean gluten-free dough using the biophysical characterization of proteins and starch: a FT-IR study. J Food Sci Technol. 2019;56(3):1316-1327.

[25] Warren FJ, Gidley MJ, Flanagan BM. Infrared spectroscopy as a tool to characterise starch ordered structure—a joint FTIR–ATR, NMR, XRD and DSC study. Carbohydr Polym. 2016;139:35-42.

[26] Dimitriou L, Koureas M, Pappas CS, et al. Chemometric classification of feta cheese authenticity via ATR-FTIR spectroscopy. Appl Sci. 2025;15(15):8272.

[27] Shah FUH, Sharif MK, Butt MS, Shahid M. Development of protein, dietary fiber, and micronutrient enriched extruded corn snacks. J Texture Stud. 2017;48(3):221-230.

[28] Philipp C, Oey I, Silcock P, Beck SM, Buckow R. Impact of protein content on physical and microstructural properties of extruded rice starch-pea protein snacks. J Food Eng. 2017;212:165-173.

[29] Sahu C, Patel S, Tripathi AK. Effect of extrusion parameters on physical and functional quality of soy protein enriched maize based extruded snack. Appl Food Res. 2022;2(1):100072.

[30] Martin A, Schmidt V, Osen R, et al. Texture, sensory properties and functionality of extruded snacks from pulses and pseudocereal proteins. J Sci Food Agric. 2022;102(12):5011-5021.

[31] Capezza AJ, Robert E, Lundman M, et al. Extrusion of porous protein-based polymers and their liquid absorption characteristics. Polymers (Basel). 2020;12(2):459.

[32] Dekkers BL, Nikiforidis CV, van der Goot AJ. Shear-induced fibrous structure formation from a pectin/SPI blend. Innov Food Sci Emerg Technol. 2016;36:193-200.

[33] Sisay MT, Emire SA, Ramaswamy HS, Workneh TS. Effect of feed components on quality parameters of wheat–tef–sesame–tomato based extruded products. J Food Sci Technol. 2018;55(7):2649-2660.

[34] Patel JR, Patel AA, Singh AK. Production of a protein-rich extruded snack base using tapioca starch, sorghum flour and casein. J Food Sci Technol. 2016;53(1):71-87.

[35] Dlamini N, Solomon W. Effect of ingredients ratio on physicochemical and sensory properties of sorghum, maize and soya protein concentrate blend extrudates. Int J Food Sci Nutr Eng. 2016;6(5):112-120.

[36] Zhang J, Li T, Chen Q, et al. Application of transglutaminase modifications for improving protein fibrous structures from different sources by high-moisture extruding. Food Res Int. 2023;166:112623.

[37] Herz E, Kinne T, Terjung N, Gibis M, Weiss J. Influence of extrudate to SPI-gel-binder ratios and transglutaminase crosslinking on texture of a plant-based salami analogue. Future Foods. 2023;7:100235.

[38] Meng X, Threinen D, Hansen M, Driedger D. Effects of extrusion conditions on system parameters and physical properties of a chickpea flour-based snack. Food Res Int. 2010;43(2):650-658.

[39] Yadav DN, Anand T, Singh AK. Co-extrusion of pearl millet-whey protein concentrate for expanded snacks. Int J Food Sci Technol. 2014;49(3):840-846.

[40] Yu X, Li T, Yue M, et al. Impact of transglutaminase on structural and rheological properties of pea protein-cornmeal-wheat gluten blends for meat analogue production. J Food Eng. 2025;390:112412.

[41] Lisiecka K, Wójtowicz A, Samborska K, et al. Structure and texture characteristics of novel snacks expanded by various methods. Materials. 2023;16(4):1541.

[42] Wang Y, Zheng Z, Zhang C, et al. Comparative structural, digestion and absorption characterization of three common extruded plant proteins. Food Res Int. 2024;177:113852.