Journal of Agriculture and Food Research 18 (2024) 101304

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.sciencedirect.com/journal/journal-of-agriculture-and-food-research

Potentials of Mahachanok mango peel pectin in modulating glycaemic index in simulated *in vitro* carbohydrate digestion of meat product

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ARTICLE INFO

Keywords:
Biomass
Dietary fibre
Meatball
Pectin
Simulate digestion

ABSTRACT

Pectin derived from mango peel biomass offers a noteworthy alternative to starch in food products, potentially assisting in controlling hyperglycaemia by impacting starch digestion. Consequently, this study evaluates the potential of Mahachanok mango peel (MHMP) pectin in glycaemic index (GI) reduction of meat products using simulated *in vitro* carbohydrate digestion. The physicochemical characteristics of MHMP pectin (MHMPP) were assessed using both FTIR and titration techniques, with microarray polymer profiling employed to analyse the glycan profile. In vitro simulations of carbohydrate digestion were carried out to assess its efficacy. Additionally, meatballs fortified with MHMPP were formulated, and the glycaemic index of the resultant products was ascertained. Microarray polymer profiling revealed distinct glycans in different fractions, including galactose, xyloglucan, and glycoprotein. Microwave extraction of pectin yielded 19.04 % MHMPP content with specific characteristics: L* (58.04), a* (12.80), b* (23.50), 6.81 % moisture content, and 78.63 % solubility. The degree of esterification at 55.73 %, an equivalent weight of 789.26 mg/moL, and a methoxyl content of 8.39 %, evidently identified MHMPP as high-methoxyl pectin. In a simulated system of MHMPP, content correlates with reduced digestion, supported by lowered values across the hydrolysis index (HI), rapidly available glucose (RAG), slowly available glucose (SAG), and expected glycaemic index (eGI). Higher MHMPP levels consistently exhibit a decreased impact on these digestive factors. In a simulated meat product system, increased MHMPP content corresponded to slower digestion rates, indicating its potential to retard digestion, as supported by HI, RAG, SAG, and eGI. The supplementation of 25 % pectin to meatballs is the most successful treatment, as it results in eGI, RAG, and SAG values of 8.71 (mg/g_{sample}), 6.65 (mg/g_{sample}), and 1.85 (mg/g_{sample}), respectively. This study highlights the advantage of MHMP-derived dietary fibre in product development from industrial byproducts, aligning with sustainable development goals by reducing reliance on non-renewable materials.

1. Introduction

Mango (*Mangifera indica* Linn.) is a fruit produced on a global scale in substantial quantities, catering to both fresh and processed consumption, with an annual yield reaching up to 25 million metric tons with Thailand standing as one of the top producers [1]. Within the ranges of the commercial Thai cultivars, Mahachanok mango is recognised as a preferred cultivar for processing and export, with arrogant distinctive ripened characteristics such as a deep yellow colour, a fragrant aroma, and a delightful taste [2–4]. This cultivar of mango has undergone

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https://doi.org/10.1016/j.jafr.2024.101304
Received 24 April 2024; Received in revised form 6 June 2024; Accepted 18 July 2024
Available online 23 July 2024
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processing to yield a range of products, including frozen mango dices, dehydrated mango snacks, and beverages, thereby adding the value of excess produce. However, during the processing, a high volume of mango biomass (ca. 200,000 tons) was obtained, with its peels accounting for up to 24 % of this total volume [5]. Mango peel is a valuable resource in the food industry, capable of being processed into a selection of supplementary products for both food and beverages, including powders, extracts, and concentrates [6]. Furthermore, the extracts derived from mango peel hold promise in the creation of functional foods, nutraceuticals, and dietary supplements, owing to their potential health benefits [7]. Mahachanok mango peel (MHMP) composes a significant proportion of soluble dietary fibre, particularly pectin, with levels reaching up to 20 % [8]. Pectin, categorised as a soluble fibre, undertakes effective fermentation by bacteria in the large intestine, extending the duration of food in the stomach. This results in diminished glucose absorption through the intestinal mucosa and a reduction in fat absorption, offering benefits for the regulation of glucose and fat levels [9]. Pectin and its derived products have been explored for their potential as prebiotics, with pectin functioning as a dietary fibre that imparts beneficial physiological effects on the gastrointestinal tract [10,11]. Presently, pectin is employed as a fat substitute in various meat products, contributing to enhanced juiciness and smoothness of the food texture, similar to the role of fats in these formulations. It preserves characteristics close to the original products in processed food items that fortify dietary fibre with pectin as well [12]. Additionally, according to the European Food Safety Authority (EFSA) report, a cause-and-effect relationship has been confirmed between the consumption of pectin and a reduction in post-prandial glycaemic response [13]. The glycaemic glucose fraction of food, exclusive of lactose, represents the cumulative glucose content within its glycaemic carbohydrate fraction [14]. This glucose content, inherent in carbohydrate-rich foods, is further delineated as either rapidly available (RAG) or slowly available (SAG), denoting their respective potential rates of release and absorption [15]. The glycaemic index (GI) serves to categorise carbohydrate foods based on their impact on postprandial plasma glucose response [16]. During digestion, carbohydrates undergo rapid conversion to sugar, with the GI value ranging from 0 to 100, reflecting the extent to which a specific food influences the increase in blood sugar levels within 2–3 after consumption [17]. The consumption of low-GI foods is associated with enhanced regulation of blood sugar levels and a reduced risk of type 2 diabetes. Meatballs commonly favour processed meat items, especially in Asia and select European nations [18]. Their primary components consist predominantly of protein-rich meat, supplemented with carbohydrates, especially starch. The advantage of incorporating starch in processed meat products lies in its cost-effectiveness and favourable properties for shaping, while preserving the original flavour, aroma, and sensory characteristics of the food [19]. However, carbohydrates primarily consist of glucose, they can lead to elevated blood sugar and insulin levels, potentially increasing the risk of developing diabetes with excessive consumption [20].

While the potential of pectin in the development of low-glycaemic index foods, along with its structure, applications, and sources, has been previously elucidated [21,22], there is currently no available data, to the best of our knowledge, on the *in vitro* carbohydrate digestion and glycaemic index (GI) of pectin extracted from MHMP. With this rationale, the aim of this study is to extract and analyse the properties of pectin from MHMP, develop meatball products supplemented with MHMPP as a replacement for tapioca starch, and investigate the mechanism of reducing glycaemic index values through simulating *in vitro* carbohydrate digestion of the meat product. This knowledge gap highlights the potential significance of obtaining such information for the future development of products utilising dietary fibre derived from the biomass generated through industrial processing. In addition, the anticipated results of this study align with the overarching goal of global sustainable development by offering an alternative approach to minimise reliance on non-renewable materials. This, in turn, can contribute to cost reductions in management practices and mitigate the carbon footprint associated with mango processing.

2. Materials and methods

2.1. Preparation of Mahachanok mango peel powder

MHMP, sourced from Union Fresh Company Limited (2003) in Chiang Mai, Thailand, were harvested at a commercially ripe stage, characterised by specific gravities ranging from 1.01 to 1.02, following the methodology outlined by Wongkaew et al. [23]. The peels were cut into small pieces, washed, blanched in hot water at 95 °C for 10 min, and then rapidly cooled to room temperature. Following this, the peels were dried at 60 ± 1 °C until achieving a moisture content of 4–6% and finely powdered using a high-speed food processor. (Electrolux E5HB1-59 GG, Stockholm, Sweden) [12].

2.2. Microarray polymer profiling (MAPP) for glycan analysis

The glycan analysis methods using microarray polymer profiling (MAPP) were followed according to the method of Bakshani, Sangta [24]. MHMP powder was lyophilised, and its alcohol-insoluble residue (AIR) was prepared by adding 1.5 mL of 70 % ethanol to the sample powder, followed by centrifugation. The supernatant was discarded, 1.5 mL of methanol and chloroform (1:1 (v/v) were added, and then the supernatant was centrifuged and discarded. Finally, 1.5 mL of 100 % acetone was added, centrifuged, and the pellet was allowed to dry. The pallet was rinsed with DIH_2O (deionised water), centrifuged, and the supernatant was removed. The glycan was extracted by adding 30 μL/mg of 50 mM CDTA to the AIR material, followed by vigorous shaking. After centrifugation, the supernatant was retained. Thirty μL/mg of 4 M NaOH mixed with 0.1 % $NaBH_4$ was added to the remaining pellet, the supernatant was centrifuged and retained the pellet. Then, 30 μL/mg of cellulase was added to the remaining pellet, centrifuged, and retained. The samples were printed onto nitrocellulose membranes using a microarray printing robot (MicrogridII, Genomic Solutions, UK). The printed microarrays were incubated with monoclonal antibodies (listed in Supplementary Table S1) or other probes for 2 h. The arrays were washed to remove unbound probes. Antibody binding was detected by incubating with the NBT/BCIP colour development solution until purple precipitate spots developed. The glycoarrays were analysed using the scanner at 2400 dpi, converting to TIFF and then negatives. Using microarray analysis software, a grid is overlaid to measure colour intensity at antigen binding sites, removing background. The grid data, exported as.txt, is integrated into Excel. There, mean spot signal intensities per sample are computed by averaging spot values across dilutions and replicates. Normalisation sets the highest intensity as 100, allowing a thorough comparison of spot intensity variations across samples.

2.3. Extraction of Mahachanok mango peel pectin (MHMPP) using microwave-assisted

A modified extraction method based on the technique described by Gama, Silva [25]. Twenty grams of MHMP powder were extracted in a 1:20 ratio using 6 % citric acid. The extraction was operated using the microwave technique (LG brand MS2127CW microwave oven, Thailand) with a power rating of 360 W for 30 min [26]. The pectin was precipitated using 95 % ethanol, then filtered and dried at 40 °C until the final moisture content remained below 4–6% [12]. Subsequently, the physicochemical characteristics of the pectin were analysed, and the yield can be determined using the following equation (1):

$$\text{Yield } (\%) = \frac{W_0}{W} \times 100 \tag{1}$$

Where; W_0 (g) = the weight of dried pectin.

W (g) = the weight of dried mango peel powder

2.4. Physicochemical characterisation of MHMPP

2.4.1. FTIR analysis and degree of esterification (FTIRDE)

FTIRDE was employed to analyse the chemical structure of the extracted pectin using Infrared Spectroscopy (FTIR), PerkinElmer, USA. The percentage of DE was then calculated using Wang, Du [27] using equation (2):

$$\text{FTIRDE}\ (\%) = \frac{A_{1745}}{A_{1745} + A_{1630}} \times 100 \quad (2)$$

Where; A_{1630} and A_{1745} represent the absorption intensities at 1630 and 1745 cm^{-1}, respectively, corresponding to non-methyl-esterified and methyl-esterified carboxyl groups.

2.4.2. Physicochemical characteristics

2.4.2.1. Physical characteristics. The colour analysis of MHMPP was conducted in accordance with CIE Lab system using HunterLab Colour Flex EZ Spectrophotometer, Reston, Virginia, where L* represents lightness on a scale from 0 to 100, indicating variations from black to white; a* signifies the presence of red (+) or green (−), and b* indicates the presence of yellow (+) or blue (−) [12]. Water activity was measured using a water activity metre or hygrometre (LANDTEK band WA-60A, Thailand). The sample moisture content was determined using a hot air oven set at 105 °C [28]. The moisture content of pectin can be calculated using equation (3):

$$\text{Moisture content}\ (\%) = \frac{W_0}{W} \times 100 \quad (3)$$

Where; W_0 (g) = the weight of MHMPP before drying.

W (g) = the weight of MHMPP after drying

The solubility of pectin was evaluated by immersing a 1 g sample in 40 mL of DIH_2O, allowing it to rest at room temperature for 60 min, and then centrifuging it at 6000 rpm for 15 min. Subsequently, the supernatant was removed, and the solid residue was left to drain for 30 min at room temperature. The residue was then dried until it reaching a constant weight [29,30]. The water holding capacity (WHC) and solubility of pectin can be evaluated using equations (4) and (5):

$$\text{WHC} = \frac{m_w - m_d}{m_d}\ (\text{g } H_2O / \text{g dried pectin}) \quad (4)$$

$$\text{Solubility}\ (\%) = m_i - m_d \quad (5)$$

Where; m_w = wet weight of pectin samples.

m_i = initial weight of dried pectin samples before WHC processing
m_d = dried weight of pectin samples after WHC processing

2.4.2.2. Equivalent weight. The equivalent weight (Eq.W.) was determined using the method of Virk and Sogi [31]. This determination was achieved through a titration with sodium hydroxide (NaOH) to reach a pH of 7.5, with the assistance of phenol red as an indicator. The procedure involves weighing 0.5 g of the pectin sample into a 250 mL conical flask, which is then moistened with 5 mL of ethanol. Subsequently, 1.0 g of NaCl is added to the mixture, followed by the addition of 100 mL of DIH_2O. Three drops of phenol red indicator and an additional six drops of phenol red were added. The solution was titrated against 0.1 N NaOH until the colour of the solution changed to pink, indicating the endpoint of the titration. It is important to ensure that the pink colour change persists for at least 30 s. The Eq.W. can be calculated using equation (6):

$$\text{Eq.W. (mg / moL)} = \frac{\text{Weight of sample}}{\text{mL of Alkali} \times \text{Normality of alkali}} \times 100 \quad (6)$$

2.4.2.3. Methoxyl content. Methoxyl content (Mox) was determined using the method of Virk and Sogi [31]. The pectin (5.0 g) was weighed, and 25 mL of 0.25 N NaOH was added to the pectic substance. The pectin mixture was stirred and allowed to stand for 30 min at room temperature. Then, 25 mL of 0.25 N HCl was added to the pectin mixture, and titration was performed against 0.1 N NaOH to reach the same endpoint as previously described [31]. The Mox can be assessed using equation (7):

$$\text{Mox}\ (\%) = \frac{\text{mL of Alkali} \times \text{Normality of alkali} \times 31}{\text{Weight of sample} \times 1000} \times 100 \quad (7)$$

2.4.2.4. Total anhydrouronic acid content. The total content of anhydrouronic acid (AUA) is a critical parameter for assessing purity, degree of esterification, and physical properties. To calculate the AUA content, the values of the equivalent weight and the methoxyl content were employed, followed by the method of Virk and Sogi [31]. The AUA of pectin can be calculated using equation (8):

$$\text{AUA}\ (\%) = \frac{176. \times 0.1A \times 100}{\text{Weight of sample} \times 1000} + \frac{176 \times 0.1B \times 100}{\text{Weight of sample} \times 1000} \quad (8)$$

Where; molecular unit of AUA (1 unit) = 176 g.

A = mL (titre) of NaOH from equivalent weight determination
B = mL (titre) of NaOH from methoxyl content determination

2.4.2.5. Degree of esterification. The degree of esterification (DE) of MHMPP was evaluated based on both of Mox and AUA [31]. This calculation was carried out using equation (9):

$$\text{DE}\ (\%) = \frac{176 \times \%\text{Mox}}{31 \times \%\text{AUA}} \times 100 \quad (9)$$

2.5. Biochemical characterisation of MHMPP

2.5.1. In vitro simulated gastrointestinal digestion and expected glycaemic index (eGI) of MMHPP

An *in vitro* simulated gastrointestinal digestion method was implemented based on the procedures of Tu et al. [32] and Arslan-Tontul et al. [33]. MHMPP powder at different contents (0.25, 0.50, 0.75, and 1.00 g) was mixed with 5 mL of phosphate buffer and heated at 95 °C for 5 min, followed by cooling to form gel. Afterward, this gel was combined with 5 mL of simulated oral juice containing α-amylase (75 U/mL) and digested for 2 min at 37 °C. The pH of the resulting digest was adjusted to 1.5, initiating gastric digestion by adding 10 mL of gastric juice (containing pepsin at 4000 U/mL, pH 1.5). After 1 h of incubation under continuous shaking at 37 °C (120 rpm), the pH was immediately raised to 7.0 with 4 mol/L NaOH. The intestinal juice (20 mL, containing α-amylase at 200 U/mL) was then mixed with the gastric digest and incubated at 37 °C for an additional 2 h. Samples were collected at 30-min intervals (0, 30, 60, 90, 120, 150, and 180 min), and the glucose content was measured using the DNS method. The calculation of released reducing sugar and the total area under the curve was based on the utilisation of the rate constant 'k' in the context of the kinetics of MHMPP gel digestion, as described by equation (10):

$$\text{Ln}\ (dC_{(t)}/dt) = \text{Ln}\ (C - C_0) - kt \quad (10)$$

Where; $C_{(t)}$, C, and k standing for the greatest amount of starch digested over an extended specific time, the percentage of MHMPP digested at that time, and the digestion rate.

Therefore, the kinetic equation was employed to elucidate the progression of MHMPP using equation (11).

$$C = C\infty \times \left[1 - e^{(-Kt)}\right] \quad (11)$$

Where; C = concentration of each time, C∞ = concentration at equilibrium, k = kinetic constant, t = time.

A nonlinear model was used to compute of the area under the curve (AUC) following equation (12):

$$AUC = C\infty \times (t_f - t_0) - \left(\frac{C\infty}{k}\right) \times \left[1 - e^{-k(t_f - t_0)}\right] \quad (12)$$

Where; C∞ = concentration at equilibrium, t_f = the end time of the experiment, t_0 = the start time of the experiment, k = kinetic constant.

The hydrolysis index (HI) was determined by comparing the AUC of MHMPP with that of a standard control material, which was white bread [34]. Subsequently, the estimated glycaemic index (eGI) was calculate using equation (13).

$$GI = (0.862 \times calcHI \times 100) + 8.198 \quad (13)$$

Where; calcHI = The MHMPP, which is calculated as the sample $AUC_{sample}/AUC_{control}$.

2.5.2. Rapidly available glucose (RAG) and slowly available glucose (SAG) of MHMPP

The assessment of the quantity of glucose that is quickly absorbed from ingested food in the initial 20 min, known as rapidly available glucose (RAG), and the quantity of glucose that undergoes slower digestion in the following 100 min (at 120 min), referred to as slowly available glucose (SAG). The procedure was employed based on the methodology outlined by Englyst et al. [35]. The MHMPP powder at different levels (0.25, 0.50, 0.75, and 1.00 g) was weighed and mixed with 5 mL of DIH_2O. Afterward, a 2.5 mL solution of enzyme mix (comprising amylase, amyloglucosidase, and maltase) and 10 mL of phosphate buffer solution were added to the tube and thoroughly mixed. The tubes were incubated at 37 °C and shaken at 120 rpm. After 20 s, 0.2 mL of the sample was removed and mixed with 4 mL of absolute ethanol to stop the reaction. The solution was then analysed the reducing sugars (glucose) using the DNS method. The residual mixture in the tube was continuously incubated and shaken for a total duration of 120 min. After that, ethanol was added to stop the reaction, and the solution was sampled for the assessment of reducing sugars (G120). The values of RAG and SAG were calculated using equations (14) and (15), respectively.

$$RAG = G20 \quad (14)$$

$$SAG = G120 - G20 \quad (15)$$

Where; G20 = amount of glucose at 20 min.

G120 = amount of glucose at 120 min

2.6. Supplementation of MHMPP in meat product

The meatball recipe was obtained from the Chiang Mai Livestock Product Research and Development Centre mainly consisted of pork, starch, salt, monosodium glutamate, and water at (%w/w) 70.0, 10.0, 4.0, 1.0, and 15.0, respectively. Pork and starch were ground and combined with all ingredients in a chiller at 5 ± 1 °C during processing. The pectin powder, replacing starch at levels of 25 %, 50 %, 75 %, and 100 % (w/w), was initially dissolved in 2 g of DIH_2O and subsequently mixed with the prepared ingredients for 10 min using a cutter mixer (QS600, Baicheng, China). The pork mixture was refrigerated for 30 min, shaped into balls, and cooked in water at 90–95 °C for 5 min. After cooling at room temperature, the meatballs were packed in vacuum-sealed nylon bags and stored at 4 ± 1 °C until testing.

2.6.1. In vitro simulated gastrointestinal digestion of glycaemic index meatballs

One gram of the MHMPP meatballs were analysed using an *in vitro* digestion method according to the method in 2.5.1.

2.6.2. Rapidly available glucose (RAG) and slowly available glucose (SAG) of glycaemic index meatballs

The 0.25 g of MHMPP meatballs were assessed for the values of RAG and SAG following the method in 2.5.2.

2.6.3. Physical and chemical quality assessments of MHMPP meatballs

Each treatment of glycaemic index meatballs was analysed using texture profile analysis (TPA) with a CT3 texture analyser. This analysis encompassed the determination of various texture parameters, including hardness (g), adhesiveness (mJ), resilience, fracturability (g), cohesiveness, springiness (mm), gumminess (g), and chewiness (mJ), which were calculated using the software provided with the instrument. Additionally, water activity was measured and analysed. Lastly, colour analysis was conducted [36]. To assess the overall colour differences between the samples (L*, a*, b*) and control (L_0*, a_0*, b_0*), the total colour difference (ΔE_{ab}) was depicted using equation (16):

$$\Delta E_{ab} = \sqrt{(L^* - L_0^*)^{2+} (a^* - a_0^*)^{2+} (b^* - b_0^*)^2} \quad (16)$$

2.6.4. Proximate composition analysis

MHMPP meatballs were tested for proximate analyses according to methods outlined by the Association of Official Analytical Chemists [28]. The moisture content of the sample was assessed through the hot air oven method at 105 °C. The estimation of crude fat content involved extracting the sample in a Soxhlet apparatus using dichloromethane as the solvent. To quantify crude fibre, the defatted sample underwent digestion with 0.128 M H_2SO_4 and 0.223 M NaOH. The ash content was determined by burning it in an oven at 600 °C for 4 h. Subsequently, the total carbohydrate content can be calculated using equation (17):

Carbohydrate (%) = 100 – [moisture content (%) + crude protein (%) + ash (%) + crude fat (%) + crude fibre (%)] (17)

2.7. Statistical analyses

The analyses of physical and chemical data were carried out at least in biological and technical triplicates. Data were analysed using one-way analysis of variance and Duncan's test, with significance determined at $P < 0.05$. Statistical analysis was performed using the SPSS program (version 22.0, Armonk, NY, USA). Additionally, Principal Component Analysis (PCA) was conducted to explore the relationship between monoclonal antibodies and extracts, employing XLSTAT version 2021.4.1 (Suite NY, New York, NY, USA).

3. Results and discussion

3.1. Microarray polymer profiling (MAPP) for glycan analysis

MAPP is a reliable method for assessing glycans and glycoconjugates in biological samples, influenced by reagent selection for polysaccharide separation [24]. Plant cell wall analysis requires specialised, multi-step extraction processes for releasing diverse glycan classes effectively. All solvents chosen for the sequential extraction of cell-wall glycans (DIH_2O, CDTA, NaOH, and cellulase) were selected based on their known abilities [37]. DIH_2O and CDTA were used to solubilise pectin due to its characteristic of being a dietary fibre soluble in water and acid [38,39]. Once the pectin was extracted from the sample, NaOH was

utilised to extract hemicelluloses [40], followed by cellulase, which was used for the final extraction of residual cellulose [41]. This outcome implies that the structure of MHMP comprises both proteins and a wide variety of types of sugars. The results can be visually represented in the heat-map diagram depicted in Fig. 1A. Both CDTA and DIH_2O fractions exhibit similar glycans recognised by specific antibodies (JIM7, JIM5, LM18, LM5), while the sodium hydroxide and cellulase fractions present a distinct pattern with antibodies (LM15, LM1, LM25, JIM20). To investigate the interaction between monoclonal antibodies and extracts, a thorough analysis was performed utilising principal component analysis (PCA), as depicted in the loading plot of Fig. 1B. The first two dimensions of the PCA described a total of 94.30 % of the variance across the PCA score plot (PC1 = 50.36 % and PC2 = 43.94 % of the variance). In-line with the heat map result, the CDTA and DIH_2O extracts projected together, while the extracts obtained from sodium hydroxide and cellulase clustered in a separate group. The biplot in Fig. 1C illustrates the recognition patterns of these extracts by antibodies. The glycosides derived from CDTA and DIH_2O exhibit recognition patterns by the antibodies JIM5, JIM7, LM5, and LM18. In earlier studies on pectin structure, JIM5 and JIM7 were employed to identify partially low methyl-esterified homogalacturonan (HG) epitopes [24]. Additionally, LM5 and LM18 were specifically utilised to detect β-(1,4)-galactan [1–4, 42]-β-D-galactan [43], targeting essential components in the pectin structure. In contrast, glycosides derived from sodium hydroxide and cellulase display a separate pattern recognised by the antibodies LM1, LM15, LM25, and JIM20. These antibodies identified epitopes associated with extensin (hydroxyproline-rich glycoproteins (HRGPs), xyloglucan, galactosylated xyloglucan, polysaccharides, and β-glucan epitopes [42,44–46].

3.2. MHMPP characterisation

3.2.1. Yield and physical characteristics

The MHMPP characteristics using microwave-assisted techniques are presented in Table 1. The average pectin content was 19.04 %. The yield depends on various parameters, including mango variety, extraction methods, and maturity stages [47,48]. This variation could be attributed to the distinct roles of pectin methyl esterase and polygalacturonase in fruit development [49]. MHMPP colour was assessed using the CIELAB system, yielding values of L* = 58.04, a* = 12.80, and b* = 23.50. The colour of MHMPP exhibited a yellowish hue. Extraction conditions significantly affect pectin colour [50], which derives primarily from natural colorants in raw materials, with some compounds attaching to and precipitating with the pectin [51]. Additionally, alterations in sample-to-solution ratio and precipitation time affect pectin colour [48]. MHMPP colour should be carefully considered for food ingredient applications, as it influences final product colour [12].

Table 1
Physicochemical characteristics of MHMPP.

Parameters	Value
Yield (%)	19.04 ± 0.59
Colour value (L*)	58.04 ± 0.51
Colour value (a*)	12.80 ± 0.39
Colour value (b*)	23.50 ± 0.28
Moisture content (%)	6.81 ± 0.12
Water activity	0.56 ± 0.01
Solubility (%)	78.63 ± 0.02

Values are presented as mean values with their corresponding standard deviations (n = 3).

The moisture content of the MHMPP was 6.81 %. These experimental findings align with previous research conducted by Naveena et al. [48], which reported that the moisture content of mango peel pectin typically falls within the range of 6 %–8 %. Meanwhile, the water activity of MHMPP was measured at 0.56, a value lower than the conventional point of 0.6 for rehydrated food products [52]. Water activity, an index of free water content in food, contributes to the proliferation of harmful microorganisms, spoilage, fly infestation, and diminished product quality in environments with elevated water activity levels [53].

Another important physical property of pectin is its solubility, which refers to its ability to dissolve in various solvents, including water. MHMPP solubility was 78.63 %, consistent with Nguyen et al. [49], who found mango peel pectin solubility ranging from 77.4 % to 86.0 % across three varieties. Pectin fractions, water-soluble (WSP), chelator-soluble (CSP), and diluted alkali-soluble (DASP), form gels depending on the solvent used [54,55]. Solubility is influenced by a wide variety of enzymes during the fruit ripening stage, including polygalacturonase, pectin methyl esterase, and β-galactosidase. These enzymes collectively

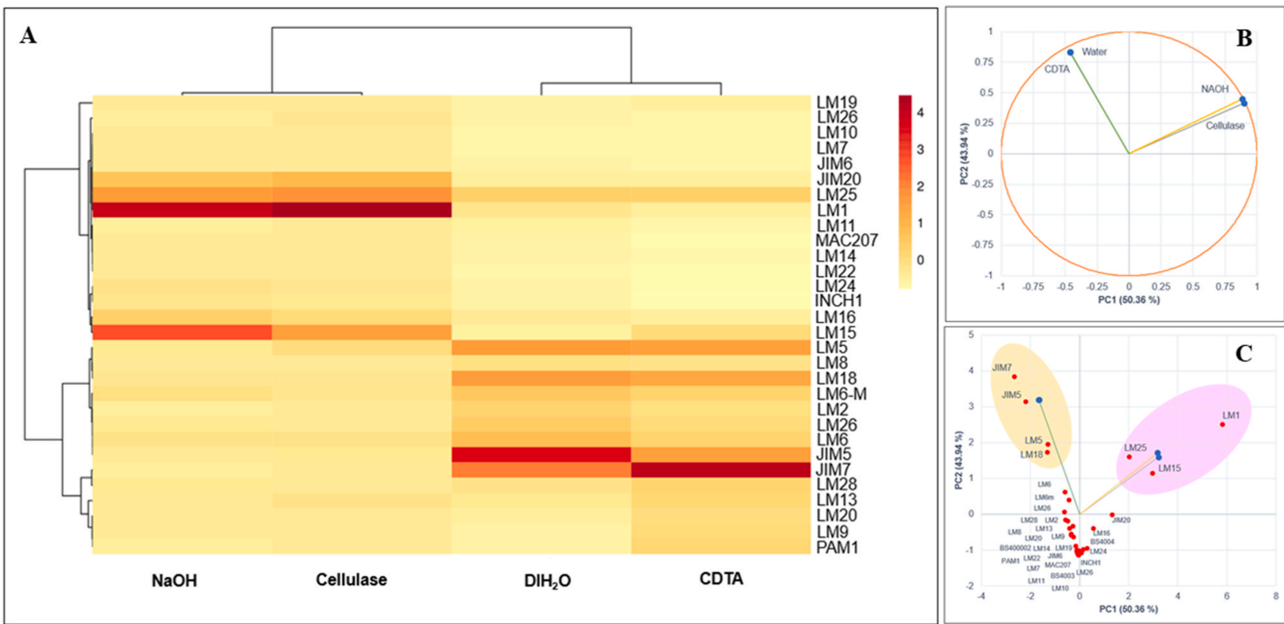

Fig. 1. Heat-map diagram (A) illustrating glycoactive components recovered by CDTA, NaOH, DIH_2O, and cellulase reagents and the 30 antibodies, along with the loading plot (B) and biplot (C) of monoclonal antibodies in a comprehensive microarray polymer profiling.

contribute to the breakdown of long and complex chains within the pectin structure, resulting in shorter fragments and thereby enhancing the solubility of pectin in water [56,57]. In addition, pectin exhibits enhanced solubility in acidic surroundings and plays a role in cell adhesion. When the pH decreases and the environment becomes more acidic, pectin solubilizes more effectively [58].

3.2.2. FTIRDE

FTIRDE provided a comprehensive understanding of the structural properties of the crude pectin extracts. FTIRDE was used to identify pectin extracts and estimate their degree of esterification (DE). The FTIRDE spectrum between 950 and 1200 cm^{-1} is recognised as the 'fingerprint' region for carbohydrates, which is crucial for detecting carbohydrate chemical groups in polysaccharides [59]. The DE in pectin was calculated by comparing the peak of 1630 cm^{-1} areas, or free carboxyl groups (COO^-) and the peak of 1745 cm^{-1} areas, or esterified groups (C=O) [55]. The result in Fig. 2 showed that the DE values of MHMPP were found to be 55.73. If absorbance in the FTIR spectrum is higher at 1750 cm^{-1}than at 1650 cm^{-1}, it indicates high methoxyl pectin (HMP) [60]. HMP, characterised by increased esterified carboxyl group intensities and areas with higher DE values, forms junction zones via hydrogen bridges and hydrophobic forces among methoxyl groups [61]. This pectin type gels under acidic conditions (pH 2.0–3.5). Regarding the distinctive structure of MHMPP, peaks at 1022, 1106, and to a lesser extent 1149 cm^{-1} indicate elevated homogalacturonan (HG) content, attributed to C–C, C–O, CCH, and OCH vibrations [62,63]. The band at 1149 cm^{-1} is notable for C–O–C vibrations linked to glycosidic bonds and the glycosidic ring [64]. Consequently, it is also indicative of pectin backbone structures. The bands at 1045 and 1076 cm^{-1} indicate the presence of neutral sugars like arabinose, xylose, and galactose [65], primarily in the side chains of rhamnogalacturonan I (RGI) domains within pectin molecules [66]. A carboxylate band at 1460 cm^{-1} suggests the presence of lowly esterified pectin [62,64,65]. The spectral range of 1100–1200 cm^{-1} originates from ether (R-O-R) and cyclic C–C bonds in pectin's ring structure. Peaks at 1500 cm^{-1} indicate OH bending, 1540–1560 cm^{-1} correspond to protein amide in pectin, and 1590–1600 cm^{-1} regions suggest aromatic ring stretching. A slight peak around 2900–3000 cm^{-1} arises from C–H stretching in sugars (CH, CH_2, and CH_3 groups), with a broader band from 2300 to 3600 cm^{-1} representing O–H stretching. Similar band patterns were detected in pectin extracted from mango peel and passion fruit [8,67].

3.3. Chemical characteristics of MHMPP

Based on the chemical attributes of MHMPP, assessments were conducted for Eq.W., Mox, AUA, and DE, as detailed in Table 2. A higher Eq.W. level corresponds to an increased capacity for gel formation [68,69]. The Eq.W. of MHMPP was 789.26 mg/moL. It aligned with the range of values for lime peel pectin extracted using acid-assisted microwave methods (790.28 and 1395.03 mg/moL) [70]. Nevertheless, pectin extracted from fruit byproducts exhibits varying functionality due to the presence of free –OH groups influencing water-holding capacity. Gelling behaviour is determined by factors such as molecular weight [71]. Consequently, the quality of the extracted pectin varies under different extraction conditions [72]. Mox levels classify pectin as low Mox (≤7 %) and high Mox (>7 %). MHMPP, with a Mox level of 8.39 %, falls into the high Mox category, typically ranging from 7.06 % to 7.09 % in commercial pectin [73]. High Mox pectin has the ability to form gels under conditions of high sugar concentrations (>65 % sugar), while pectin with low Mox content (≤7 %) can generate gels with reduced sugar content [74]. AUA is an index of pectin purity, and a recommended value of no less than 65 % is suggested for pectin intended for use as food additives or pharmaceutical purposes [75]. MHMPP showed an AUA value of 66.90 %, slightly below commercial pectin standards, indicating higher concentrations of proteins, starch, and sugars [76]. The protein content in fruit peel influences pectin purity, potentially remaining after alcohol precipitation. Sugar levels in mango peel also contribute significantly to observed purity levels [77]. DE reflects the ratio of esterified galacturonic acid groups to total galacturonic acid. Pectin with DE > 50 % is high methoxyl pectin (HMP), while DE < 50 %

Table 2
Chemical characteristics of MHMPP.

Parameters	Value
Eq.W (mg/moL)	789.26 ± 3.46
Mox (%)	8.39 ± 0.07
AUA (%)	61.90 ± 0.33
DE (%)	57.86 ± 0.23

Values are presented as mean values with their corresponding standard deviations (n = 3); Eq.W = equivalent weight; Mox = methoxyl content; AUA = total anhydrouronic acid content; DE = degree of esterification.

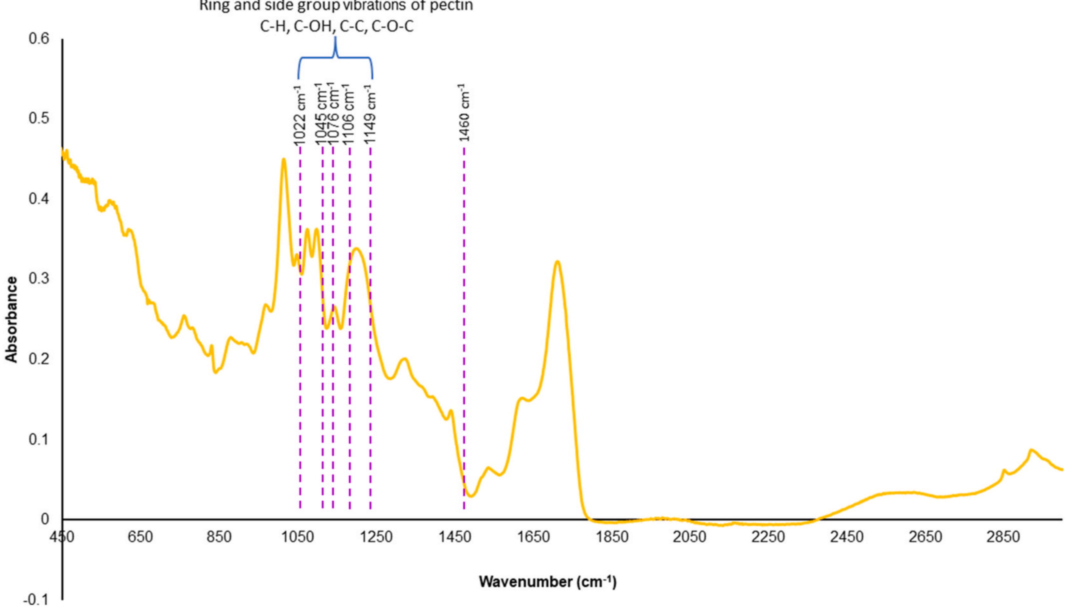

Fig. 2. The FTIRDE spectra of MHMPP from 450 to 3000 cm^{-1} (x-axis) in terms of absorbance units (y-axis).

categorizes as low methoxyl pectin (LMP) [75,78]. MHMPP was categorised as high Mox pectin, given its DE value of 57.86 %. This indicates that MHMPP has the capability to form gels in an acidic condition or at a low pH value [79]. When compared with the DE from the FTIRDE result, the DE from both expressions has a similar result that confirms the MHMPP was HMP. Nonetheless, the type of pectin obtained may vary even when extracted from the same raw material, as it is influenced by various extraction factors, including the choice of solvent, temperatures, and extraction times [80].

3.4. In vitro simulated gastrointestinal digestion of MHMPP

3.4.1. Simulated digestion rate

In simulated gastrointestinal digestion of MHMPP (Table 3), the digestion rate varied significantly depending on MHMPP concentration levels. The logarithm of the slope plot, relating reducing sugar content to digestion time, provides a crucial mathematical model for determining the digestion rate constant (k, min^{-1}) [81]. The digestion rate constant, k, was notably higher for lower concentrations of MHMPP when compared to samples with higher MHMPP content. Higher MHMPP content correlates with a lower digestion rate, indicating its potential to hinder the digestive system and slow down digestion. This effect may involve inhibition of amylase activity [82], alteration of gel structure affecting gelatinization degree [83], acting as a physical barrier [84], and interacting with polysaccharide hydrolysates. Pectin indirectly influences hyperglycaemia control by interacting with starch digestion, a process strongly linked to metabolic disorders like obesity and diabetes. Bai et al. [85] revealed that pectin from citrus with a high GalA content and a high proportion of homogalacturonan acts as an inhibitor of starch digestion, leading to reduced glucose absorption. Pectin has also been shown to decrease starch digestion rates without causing any changes in the amylose/amylopectin ratio [21]. The deceleration of the digestion rate is attributed to the interaction between amyloglucosidase and pectin, involving electrostatic complexation and/or hydrogen bonding, which is highly likely to modify the enzyme's conformation and/or delay its interaction with starch [85]. Therefore, an increased content of polysaccharides in the form of soluble dietary fibre is linked to decreased digestion, likely playing a pivotal role in the slowdown of digestion levels as well as blood sugar levels.

Additionally, the correlation is further supported by the hydrolysis index (HI) values, rapidly available glucose (RAG), slowly available glucose (SAG), and expected glycaemic index (eGI). The elevated levels of MHMPP had a decreased impact on all factors. The glycaemic index (GI) or expected glycaemic index (eGI) categorizes carbohydrate-rich foods according to their impact on postprandial plasma glucose response [16,86]. Both RAG and SAG measurements of carbohydrate-containing foods can serve as supplements to the GI approach, offering additional insights. These measurements may provide valuable information for understanding the varying impacts of different carbohydrate-rich foods on blood glucose and insulin levels [14]. However, the RAG value should be combined with the GI value, as the RAG value signifies the quantity of glucose expected to be rapidly absorbed in the human small intestine [87]. Moreover, Bai et al. [88] also reported that the digestion of pumpkin flour, characterised by a high content of starch and pectin, is influenced by the intricate network of pumpkin polysaccharides. In this context, pectin acts as a barrier between amylase and starch, distinguishing it from the digestion of pure starch, where the digestibility is primarily determined by the starch structure.

Table 3
Simulated digestion rate of MHMPP.

MHMPP concentration %	k (min^{-1})	HI (%)	RAG (mg/g_{sample})	SAG (mg/g_{sample})	eGI (mg/g_{sample})
25	2.26 ± 0.09^a	102.10 ± 0.18^a	11.87 ± 0.02^a	26.44 ± 0.55^a	96.20 ± 0.15^a
50	1.33 ± 0.01^b	45.80 ± 0.27^b	6.87 ± 0.05^b	12.39 ± 0.03^b	47.68 ± 0.23^b
75	1.23 ± 0.01^b	29.28 ± 0.17^c	4.56 ± 0.03^c	7.60 ± 0.17^c	33.44 ± 0.14^c
100	0.81 ± 0.12^c	19.64 ± 0.06^d	3.49 ± 0.11^d	3.90 ± 0.07^d	25.13 ± 0.05^d

Digestion rate constant (k, min^{-1}), HI = hydrolysis index, eGI = estimated glycemic index, RAG = rapidly available glucose, SAG = slowly available glucose.
[a-d] Mean ± SD with same superscripts in the same row indicates no significant difference ($P < 0.05$) and values with different superscript in the same row indicate significant differences.

3.5. MHMPP supplemented meat product

3.5.1. In vitro simulated gastrointestinal digestion of MHMPP meatball products

Studying simulated gastrointestinal digestion of MHMPP meatballs (Table 4) revealed that digestion rate is significantly influenced by MHMPP levels, consistent with the results in Section 3.3.1. The digestion rate constant, k, showed a significant increase in the products with lower levels of MHMPP supplementation in contrast to those with higher levels of MHMPP. This phenomenon can be explained by the physicochemical properties of pectin, a water-soluble polysaccharide chain known for its viscosity resulting from physical interactions [89,90]. When pectin is used as an ingredient in food products, it leads to reduced rates of starch breakdown and glucose absorption due to the impact of water-soluble polysaccharides on the upper digestive system's viscosity [91]. Additionally, viscosity served as a physical hindrance, limiting the movement of the fluid in the intestinal space. This limitation led to a decrease in enzyme diffusion towards starch substrates and/or hindered efficient mixing. Therefore, the interaction between digestive enzymes and starch was diminished, causing a slowdown in the process of amylolysis [92]. Sanchez et al. [93] findings suggested that increased viscosity from orange and apple pectin may reduce intestinal glucose absorption.

In terms of HI, RAG, SAG, and eGI, the increased levels of MHMPP had a diminishing impact on all these factors. The values of HI, RAG, and SAG ranged between 0.21 and 0.74, 0.52 to 6.87, and 1.53 to 2.89, respectively. According to Ma et al. [94], an increased pectin ratio resulted in decreased HI and SAG values for starch. This occurred because the pectin's arrangement around starch granules hindered their contact with digestive fluids, reducing the rate of starch breakdown and limiting enzyme access to the starch [94]. Moreover, Englyst et al. [95] reported that RAG had a positive correlation with GI, whereas SAG exhibited a negative correlation with GI. This indicates that RAG and

Table 4
Simulated digestion rate of MHMPP meatballs with varied mango peel pectin additions.

MHMPP concentration %	k (min^{-1})	HI (%)	RAG (mg/g_{sample})	SAG (mg/g_{sample})	eGI (mg/g_{sample})
0	0.0283 ± 0.00^a	0.74 ± 0.02^a	6.87 ± 0.05^a	2.89 ± 0.05^a	8.84 ± 0.02^a
25	0.0225 ± 0.00^b	0.59 ± 0.01^b	6.65 ± 0.03^b	1.85 ± 0.03^b	8.71 ± 0.00^b
50	0.0136 ± 0.00^c	0.21 ± 0.00^c	6.60 ± 0.01^b	1.80 ± 0.02^b	8.38 ± 0.00^c
75	0.0131 ± 0.00^c	0.21 ± 0.02^c	5.32 ± 0.02^c	1.53 ± 0.04^a	8.37 ± 0.01^c
100	n/m	n/m	n/m	n/m	n/m

n/m = not measured.
Digestion rate constant (k, min^{-1}), HI = hydrolysis index, eGI = estimated glycaemic index, RAG = rapidly available glucose, SAG = slowly available glucose, n/d = not detectable.
[a-c] Mean ± SD with same superscripts in the same row indicates no significant difference ($P < 0.05$) and values with different superscript in the same row indicate significant differences.

SAG values are significant variables in indicating the GI in food. GI reflects the quality and quantity of carbohydrates in foods, which defines the blood glucose response to a test food, measured as the incremental area under the curve (iAUC) within 2 h after ingestion [96]. Foods can be categorised as low (<55), medium [55–69], or high (>70) GI foods based on their GI values [97]. In the experiment, as the proportion of pectin in MHMPP meatballs increased, their eGI values tended to decrease. Across all treatments, eGI ranged from 8.84 to 8.37, classifying these products as low GI foods. Jaisut et al. [98] highlighted that low values of kinetic constant (k) and equilibrium concentration ($C\infty$) indicate low digestibility of glutinous rice starch, suggesting low eGI for such foods.

3.5.2. *Physical and chemical quality assessments of MHMPP meatballs*

3.5.2.1. Textural profile analysis. The textural profile analysis (TPA) of MHMPP products are shown in Table 5. The gelation properties of pectin interfere with meatball structure formation at higher MHMPP supplements, rendering the evaluation of texture properties impractical for these treatments based on product shape. From the hardness, adhesiveness, resilience, fracturability, cohesiveness, springiness, gumminess, and chewiness, all values exhibit a statistically significant decrease with increasing MHMPP levels ($P < 0.05$). The findings of this work align with those of Henning et al. [99] and Pereira et al. [100] who noted that incorporating HMP in partially emulsified meat products resulted in lower hardness, springiness, and cohesiveness values. Generally, pectin exhibits strong interaction with meat proteins, primarily through hydrogen bond formation and free binding energy, particularly with myofibrillar proteins. The proteins accumulate around oil droplets, participate in forming a three-dimensional network gel during meat processing, and enhance emulsion stability, water-holding capacity, and texture in meat products [101,102]. However, an elevated pectin content may hinder the bonding of myofibrillar proteins, resulting in the formation of gels that are not properly bound to the protein. Pectin, being primarily hydrophilic, typically exhibits surface activity due to the high hydrophobicity of residual protein moieties linked to arabinogalactan and, to some extent, the hydrophobic nature of its methyl, acetyl, and ferulic acid esters [103]. This phenomenon contributes to the lack of cohesion in meat products. Therefore, the meatballs supplemented with a small quantity of MHMPP demonstrate texture properties similar to those of conventional meatballs (CTRL).

3.5.2.2. Colour and water activity. The physicochemical and nutritional attributes of MHMPP meatballs are shown in Table 5. Among the significant physical properties of the food product, colour was specifically examined. As the MHMPP content increased, there was a notable improvement in all values of L*, a*, and b*, signifying a significant enhancement in these parameters. This colour variation in the meat product might be linked to the pigments in mango peel, which are essential bioactive compounds found in mango peel [104]. In accordance with Zapata and Pava [105] findings, the addition of quinoa flour to frankfurter sausages significantly intensifies the product colour. Moreover, the colour value can be used to calculate the total colour difference (ΔE_{ab}). The result showed that the ΔE of MHMPP supplementation at 25 % closely resembles that of the CTRL, and increased therein in the formulations with 50 % and 75 % inclusions. This experiment clearly demonstrates that an increased pigment quantity notably impacts the colour variations in the meatball samples [106]. In terms of the water activity, all treatments of MHMPP meatballs fall within a range of 0.80–0.83 %. Values range from 0.00 to 1.00, with higher values indicating more water and potentially shorter shelf life [107].

3.5.3. *Proximal profile*

For the nutritional properties of MHMPP meatballs, the levels of protein, fat, carbohydrates, fibre, and ash are shown in Table 6. The moisture content significantly increased ($P < 0.05$) with higher levels of MHMPP replacement, attributed to meat emulsion interaction in the meatball. Water affinity for charged compounds, like proteins, enhances water holding capacity, especially with myofibril proteins in pork [108]. Increased meat emulsion added more charged proteins, further enhancing water holding capacity and moisture content. Additionally, protein, fat, and fibre contents notably increased ($P < 0.05$) with higher MHMPP levels in the meatball formulation. According to a study by Namir et al. [109] a rise in the quantity of okra pectin in low-fat beef burger products subsequently causes an increase in the levels of ash and protein. The increasing values in fat and protein are due to the high protein and fat content of the meat emulsion [110]. Younis et al. [111] reported that the supplementation of pectin in meat sausages and patties significantly increased their dietary fibre content ($P < 0.05$). This is attributed to pectin being classified as a soluble dietary fibre [112]. Conversely, the carbohydrate content decreased with the higher addition of MHMPP. It is because sugar makes up the majority of

Table 5
Assessing texture characteristics in MHMPP meatballs with varied mango peel pectin additions.

Treatments					
TPA parameters	0 % (CTRL)	25 %	50 %	75 %	100 %
MHMPP meatball feature					
Hardness (g)	2871 ± 159.05^{a}	1066 ± 65.09^{b}	312.33 ± 5.47^{c}	n/d	n/d
Adhesiveness (mJ)	0.40 ± 0.17^{a}	0.23 ± 0.15^{b}	0.13 ± 0.12^{c}	n/d	n/d
Resilience	0.39 ± 0.12^{a}	0.25 ± 0.02^{b}	0.17 ± 0.00^{c}	n/d	n/d
Fracturability (g)	2817 ± 145.03^{a}	1067 ± 63.65^{b}	311.33 ± 5.23^{c}	n/d	n/d
Cohesiveness	0.59 ± 0.13^{a}	0.45 ± 0.02^{b}	0.41 ± 0.02^{ab}	n/d	n/d
Springiness (mm)	8.41 ± 0.97^{a}	7.42 ± 0.11^{b}	5.67 ± 0.21^{c}	n/d	n/d
Gumminess (g)	478.33 ± 17.62^{a}	128.00 ± 3.46^{b}	7.42 ± 0.11^{c}	n/d	n/d
Chewiness (mJ)	155.60 ± 104.57^{a}	34.80 ± 121^{b}	7.13 ± 0.46^{c}	n/d	n/d
Colour value					
- L*	34.03 ± 0.66^{d}	36.91 ± 0.33^{c}	39.18 ± 0.15^{b}	50.87 ± 0.21^{a}	n/m
- a*	0.53 ± 0.01^{d}	1.40 ± 0.06^{c}	2.20 ± 0.01^{b}	2.99 ± 0.01^{a}	n/m
- b*	7.18 ± 0.11^{d}	9.51 ± 0.23^{c}	11.75 ± 0.05^{b}	15.06 ± 0.01^{a}	n/m
ΔE	–	3.80 ± 0.23^{c}	7.08 ± 0.08^{b}	18.76 ± 0.11^{a}	n/m
Water activity	0.80^{c}	0.80^{c}	0.81^{b}	0.83^{a}	n/m

n/d = not detectable.
[a-c] Mean ± SD with same superscripts in the same row indicates no significant difference ($P < 0.05$) and values with different superscript in the same row indicate significant differences. TPA: texture profile analysis.

Table 6
Assessing physicochemical and nutritional quality in meatballs with various MHMPP supplementation.

	Treatments				
Parameters	0 % (CTRL)	25 %	50 %	75 %	100 %
Moisture content (%)	73.18 ± 0.16[a]	74.71 ± 0.20[a]	75.89 ± 0.14[b]	76.26 ± 0.25[b]	n/m
Protein (%)	36.85 ± 0.10[d]	37.87 ± 0.10[c]	39.42 ± 0.37[b]	40.73 ± 0.35[a]	n/m
Fat (%)	1.05 ± 0.03[c]	1.31 ± 0.15[bc]	1.55 ± 0.03[b]	2.56 ± 0.09[a]	n/m
Carbohydrate (%)	35.72 ± 0.27[a]	34.82 ± 0.34[ab]	34.42 ± 0.28[b]	31.20 ± 0.30[c]	n/m
Fibre (%)	0.02 ± 0.01[d]	0.33 ± 0.0.03[c]	0.71 ± 0.0.04[b]	1.38 ± 0.15[a]	n/m
Ash (%)	n/s	n/s	n/s	n/s	n/m

n/m = not measured.
n/s = no significant.
[a-d] Mean ± SD with same superscripts in the same row indicates no significant difference ($P < 0.05$) and values with different superscript in the same row indicate significant differences.

carbohydrates in nutritional foods [113]. As a result, the carbohydrate content dropped with the incremental level of MHMPP. Nevertheless, the ash content levels did not exhibit significant differences across all MHMPP meatball treatments. Typically, ash content is an indicator of mineral content in agricultural and food products [114].

From the analysis of MHMPP meatballs, we found that increasing the supplementation of MHMPP dietary fibres resulted in higher nutritional values for the meatballs. This also enhanced their ability to reduce sugar digestion, leading to positive health effects. However, a high quantity of dietary fibre supplementation negatively impacted the texture of the meatballs, making them less desirable. Therefore, when selecting meatball formulations, consideration should be given to nutritional value, sugar digestion capability, and the texture of the meatballs. In this case, a 25 % supplementation of dietary fibre is deemed the most suitable.

4. Conclusion

The study underscores the potential of MHMPP in mitigating GI in meat products via simulated *in vitro* carbohydrate digestion. MHMPP, with its distinct properties confirmed through analysis, exhibits a correlation between increased content and reduced digestion rates, notably affecting factors such as HI, RAG, SAG, and eGI. However, challenges arose in meatball structure at higher MHMPP levels, affecting texture assessment yet enhancing colour values. Despite these challenges, the increase in protein, fat, and fibre with MHMPP addition, coupled with consistent ash content, suggests its potential in enriching meat products and promoting sustainable development through the utilisation of industrial byproducts. This study illuminates the promising role of MHMP-derived dietary fibre in advancing product innovation while aligning with goals aimed at diminishing reliance on non-renewable resources, thereby contributing to a sustainable future.

Funding

This research project was partially supported by Chiang Mai University. Additionally, the research project received funding from Targeted Research 2023 (R66IN00339).

Ethical approval

The authors confirm that this paper has not been published elsewhere, is not simultaneously under consideration for publication elsewhere, and the results presented in this work are genuine and unaltered.

Consent for participate

All the individual participants involved in the study have received informed consent.

Consent for publication

The participant has provided consent for the submission of the study to the journal.

CRediT authorship contribution statement

Chonlada Srikamwang: Writing – original draft, Formal analysis, Data curation. **William G.T. Willats:** Formal analysis, Data curation. **Cassie R. Bakshani:** Formal analysis, Data curation. **Sarana Rose Sommano:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Malaiporn Wongkaew:** Validation, Supervision, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jafr.2024.101304.

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