Aerial parts of maca (*Lepidium meyenii* Walp.) as functional vegetables with gastrointestinal prokinetic efficacy *in vivo*

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**Abstract:** *Lepidium meyenii* Walp. (maca) has been utilized in the Andean region because of its edibleness and medicinal value. The aerial parts of maca (APM) were analyzed for protein, total sugar, vitamins, amino acids, and minerals and its characteristic active ingredients at five different growth stages. Results showed the high protein, total sugar, vitamin C, niacin, potassium, and calcium contents of APM. All 17 amino acids and the characteristic active ingredients, namely, macamide, glucosinolates, adenosine, and total saponins, were detected. We examined the effects of maca plant powders on gastric emptying and intestinal propulsion and the levels of serum motilin and gastrin in atropine-treated mice. Benzyl isothiocyanate (BITC) was investigated to identify the potential active material foundation of APM. Results revealed that both maca plant powders and BITC can promote gastrointestinal prokinetic efficacy. Thus, APM feature potential as new functional vegetable sources.

**Keywords:** *Lepidium meyenii*; Maca; Benzyl isothiocyanate; Gastrointestinal motility; Functional vegetables

1. **Introduction**

*Lepidium meyenii* Walp., commonly referred to as “maca,“ is an annual herbaceous plant of
the Brassicaceae family, which is native to the central Andes with medicinal and economic values. This plant is a major economic crop in Peru, and its cultivation is carried out under conditions of low temperature and high altitude (3500–4000 m). Maca root has been widely consumed as a common vegetable in the Andean highlands for thousands of years. This plant part is considered as a healthy food that is rich in sugar, protein, starch, minerals and secondary metabolites with significant bioactivities, such as anti-fatigue, anti-cancer, and memory-inflammatory, anti-osteoporosis, improving memory and neuroprotective effects. The aerial parts of maca (APM) also possess high biomass and their taste is distinctive. However, little attention has been devoted to APM, especially on their phytochemical characterization, biological activity and medical value. Therefore, to improve the edibleness value of APM, systematic analysis should center on the nutritional ingredients of APM and further explore their potential as functional vegetables.

Gastrointestinal motility disorder is one of the most common pathologies of the gastrointestinal tract, including delayed gastric emptying (GE), gastrointestinal transit, impaired gastric accommodation, visceral hypersensitivity, and inflammation have indicated that vegetable intake, especially intake of dietary cruciferous vegetables, may treat gastrointestinal motility disorder. Cruciferous vegetables are rich in glucosinolates. Glucosinolates exhibit no biological activities but can be hydrolyzed to a range of bioactive compounds such as isothiocyanates, thiocyanates, nitriles and indoles by the plant-based enzyme myrosinase, or less efficiently by the colonic microflora. The main breakdown products include isothiocyanates under the neutral pH condition or gut microbial communities under conditions similar to those in animals.
The aerial parts of maca (APM) also belong to the glucosinolate-containing family of cruciferous vegetables. To the best of our knowledge, no study has reported the effectiveness of APM in gastrointestinal motility disorder. Therefore, in the present work, we detected the basic nutritional compositions and main characteristic active ingredients of APM and examined for the first time the effects of APM powders on gastrointestinal motility and gastrointestinal hormone levels in atropine-induced gastrointestinal motility disorder in mice. Gastrointestinal prokinetic efficacy of benzyl isothiocyanate (BITC) was also investigated to identify the potential active material foundation of APM, providing a theoretical reference for the development of APM as functional vegetable sources.

2. Material and methods

2.1. Materials

2,6-Dichloroindophenol (DCIP), atropine, domperidone, and blue dextran 2000 were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Niacin, amino acid mixture, BITC, macamide, benzylglucosinolate, adenosine and dioscin standard compound were purchased from Sigma–Aldrich (St. Louis, MO, USA).

All other reagents used in the experiments were of analytical or high-performance liquid chromatography (HPLC) grade.

2.2. Plant material and cultivation

Maca seeds (Fig. 1a) were purchased from Wuhan Huashite Industrial Biotechnology Development Co., Ltd. (Wuhan, China). Before planting, the seeds were rinsed with water and stirred in the fine soil, and then distributed evenly into the experimental field. After one week, the sprouts germinated, and the bud production rate reached 99%. The plants were
identified as *L. meyenii* Walp. by Prof. Jun Xiang at Huanggang Normal University (Huanggang, China).

Considering the change in biomass at different growth stages, five experimental materials (Figs. 1 b–1f) were collected; these materials included the seedlings at the seeding stage (marked as sample No.1), leaves in two time periods at the reproductive stage (marked as sample Nos. 2 and 3), stems at the bolting stage (marked as sample No. 4), and inflorescences at the flowering stage (marked as sample No. 5).

### 2.3. Animals

Mice were treated according to the Ethical Guidelines for Animal Experimentation, Huazhong University of Science and Technology and the Guide for the Care and Use of Laboratory Animals of the National Institute of Health. The animal experiments were approved by the Animal Ethics Committee, College of Life Science and Technology, Huazhong University of Science and Technology (Code: #2020015).

Half male and half female Kunming mice (20 ± 2 g) were purchased from the Laboratory Animal Research Center of Hubei province (Wuhan, China; Certificate No. 42000600024677), housed for a week in a room controlled at a temperature of 25 ± 1 °C and 35%–75% relative humidity under a 12 h light/12 h dark cycle. The mice were ventilated with filtered fresh air and allowed free access to tap water and pelletized diets. To acclimatize the mice, a widely used standard diet was given during the first week, and the diets prepared for this experiment were used thereafter.

### 2.4. Analytical methods

Fresh plant materials were analyzed for dry weight (DW). After freeze drying, the plants were
finely ground, homogenized with a laboratory mill for small amounts, and stored for further use.

### 2.4.1. Analysis of moisture, protein, total sugar, and vitamin contents

To determine the moisture contents, the plant materials were weighed before and after freeze drying. The residual moisture content was determined by drying at 105 °C overnight (17 h)\(^\text{41}\). Protein contents of the freeze-dried materials were calculated through the Kjeldahl procedure using the N factor 6.25 based on the Association of Official Analytical Chemists method\(^\text{42}\).

Total sugar content was determined by anthrone–sulfuric acid colorimetry\(^\text{43}\). Vitamin C (VC) content was determined by DCIP titration method using a dye solution\(^\text{44}\). Niacin content analyses were performed by HPLC using an analytical reversed phase C18 (250 × 4.6 mm, 5 µm) column with the mobile phase consisting of a mixture of buffer (hexane sulphonlic acid sodium, potassium dihydrogen phosphate, and triethylamine, pH 3.0) and methanol at a ratio of 96: 4 (v/v) and flow rate of 1.0 mL/min with UV detection at 210 nm\(^\text{45}\).

### 2.4.2. Analysis of element content

The fresh weight (FW) of minerals and heavy metals were investigated. The minerals (mg/100 g FW), including sodium, potassium, calcium, magnesium, and iron (Na, K, Ca, Mg, and Fe), and heavy metals (mg/kg FW), including lead, cadmium, mercury, arsenic, and chromium (Pb, Cd, Hg, As, and Cr, respectively), were detected by inductively coupled plasma source mass spectrometry (MS) (Optima 7000DV, Perkin Elmer) after acid digestion of the ash under pressure of the samples in a microwave digestion system\(^\text{46}\).

### 2.4.3. Analysis of amino acid content

Quantitative determination of amino acids (g/100 g DW) was conducted by HPLC method
based on phenyl isothiocyanate (PITC) derivatization. The freeze-dried plant powders (20 mg) were treated with 10 mL of 6 mol/L solution containing 5% phenolic hydrochloric acid under nitrogen atmosphere for 24 h at 110 °C for acid hydrolysis.

For derivatization, 200 µL of the sample or amino acid standard mixture was combined with 100 µL of 0.1 mol/L PITC reagent (PITC dissolved in acetonitrile) and 100 µL of 0.1 mol/L triethylamine–acetonitrile solution. The samples were mixed and stood for 1 h at room temperature. Then, 600 µL of n-hexane reagent was added. After immediate mixing, the samples were allowed to stand for 10 min. Then, the samples were filtered through a 0.45 µm membrane filter. Subsequently, 20 µL of sample was injected into the HPLC system. Chromatographic analysis of the amino acid derivatives was conducted with an amino acid analysis special chromatographic column (4.6 mm × 250 mm, 5 µm). The mobile phase comprised sodium acetate–acetonitrile aqueous solution (pH 6.5):acetonitrile–water (80:20, v/v) maintained at 40 °C, and the wavelength was kept at 254 nm. Flow rate was 1.0 mL/min with gradient elute.

2.4.4. Analysis of macamide content

The extraction and detection procedure of macamide involved modification of the method reported by Pan et al. The dried plant material (1.0 g) was ultrasonicated in 20 mL of petroleum ether for 40 min at room temperature. The extracts were evaporated to obtain a residue under reduced pressure; the residue was dissolved in 10 mL of methanol. The solutions were filtered through a 0.22 µm membrane filter and were stored at 4 °C for HPLC analysis. HPLC experiments were performed on an HP 1200 Series (Agilent, USA) equipped with a G1314BVWD UV–vis detector and an AT-130 column oven and controlled by Agilent.
ChemStation. The chromatographic column was a ZORBAX Eclipse Plus C18 column (4.6 mm × 250 mm, 5 µm). The mobile phase consisted of acetonitrile: 0.05% formic acid in water (75: 25, v/v) with isocratic elution at a flow rate of 0.6 mL/min. Column temperature was maintained at 40 °C. The chromatograms were acquired at 205 nm and the injection volume was 20 µL.

2.4.5. Analysis of glucosinolate content

Glucosinolates were determined by HPLC following a procedure previously optimized and described by Li et al.49 and Verkerk et al.50 with some modifications. The dried plant material (0.5 g) was extracted in 10 mL of boiling methanol (70%) in a water bath at 75 °C for 20 min, and then centrifuged at 5000 rpm for 10 min. The supernatant was collected, and the residue was re-extracted twice in the same manner. Both supernatants were mixed and metered to a volume of 25 mL. A total of 2.0 mL of the above solution, 5.0 mL of NaAc–HAc buffer solution (pH 4.0), and 1.0 mL of freshly prepared sulfatase (1.0 mg/mL) were mixed in a reagent bottle. Enzymolysis was carried out overnight at 37 °C. Finally, the enzymatic hydrolysate was metered to a volume of 10 mL and filtered through a 0.22 µm membrane filter for HPLC analysis. HPLC experiments were performed on an HP 1200 Series (Agilent, USA) equipped with a G1314BVWD UV–vis detector and an AT-130 column oven and controlled by Agilent ChemStation. The chromatographic column was an Eclipse XDB C18 column (4.6 mm × 250 mm, 5 µm). The mobile phase consisted of water and methanol (90:10 v/v, containing 0.05% trifluoroacetic acid) run in isocratic mode at a flow rate of 1.0 mL/min. Column temperature was maintained at 30 °C. The chromatograms were acquired at 235 nm, and the injection volume was 20 µL.
2.4.6. Analysis of adenosine content

Adenosine was determined following the method as previously described but with modifications\textsuperscript{51–52}. A total of 1.0 g of dried plant material was placed in a 50 mL volumetric flask, and 30 mL of 10% methanol solution was added. The mixture was shaken for 30 min at room temperature using an ultrasonic processor and centrifuged at 5000 rpm for 10 min. The residue was re-extracted twice in the same manner. Both supernatants were mixed and concentrated under vacuum to obtain the residue, which was dissolved in 10 mL of methanol. The solutions were filtered through a 0.22 µm membrane filter and stored at 4 °C for HPLC analysis. HPLC experiments were performed on a Waters Series (Waters, USA) equipped with a 2489 UV–vis detector and a 1525-F pump. A SunFire\textsuperscript{TM} C18 column (4.6 mm × 250 mm, 5 µm) was used. Column temperature was maintained at 30 °C. The mobile phase consisted of 0.4% phosphoric acid:methanol (90: 10, v/v) with isocratic elution at a flow rate of 1.0 mL/min. The chromatograms were acquired at 260 nm, and the injection volume was 20 µL.

2.4.7. Analysis of total saponin content

A total of 1.0 g of dried plant material was ultrasonically extracted with 20 mL of 80% ethanol solution at room temperature for 40 min according to the procedure reported by Hui et al\textsuperscript{53}. Then, the mixture was centrifuged at 5000 rpm for 15 min. The supernatant was collected, and the residue was re-extracted twice in the same manner. Both supernatants were mixed and concentrated under vacuum to obtain the residue, which was dissolved in 20 mL of ultrapure water and multiple extracted by water-saturated butanol in the same volume until the butanol layer became colorless. The butanol layers were mixed and concentrated under vacuum to obtain the residue, which was dissolved in anhydrous ethanol and metered to a
volume of 25 mL. A total of 1.0 mL of the above solution was dried using nitrogen, added with 0.2 mL of 5% vanillin–acetic acid solution and 0.8 mL of perchloric acid, and heated in a water bath at 55 °C for 15 min. After cooling for 2 min, 5 mL of acetic acid was added, and the mixture was allowed to stand for 15 min for spectrophotometry assay (547 nm). Total saponins were identified as steroidal saponins through Liebermann–Burchard reaction.

2.5. Gastrointestinal prokinetic efficacy of APM

Gastric emptying (GE) and intestinal propulsion (IP) were measured according to a previously described method \(^5^4\). Considering the taste of vegetables and the content of glucosinolates, sample No. 2 was used in the animal experiment. Before the experiments, the APM powders were diluted with 0.5% sodium carboxymethyl cellulose aqueous solution containing 1% Tween-80. Solutions of 144.0, 72.0, and 36.0 mg/mL served as high-, middle- and low-dose (2.16, 1.08 and 0.54 g/kg) groups, respectively. BITC (0.18 mg/kg) was dissolved in 0.5% sodium carboxymethyl cellulose solution containing 1% Tween-80, kept in a sealed container, pre-warmed to approximately 40 ℃ every day before administration and served as a group to verify the potential active material foundation. Dose selection for the BITC group was based on the content in the middle-dose APM group by headspace gas chromatography–MS (HS-GC-MS) (Agilent 7890A/5975C, USA) \(^5^5\).

After acclimatization for a week, a total of 70 mice were randomly divided into seven groups \((n = 10)\) with similar body weights. The model and control groups were treated with 0.5% sodium carboxymethyl cellulose solution containing 1% Tween-80 (vehicle), and the positive control group was given the domperidone suspension (12 mg/kg). The plant material solutions were set in water bath to 40 ℃ heating and allowed to stand for 10 min by gavage
once a day for seven days.

The animals were not fed for 24 h prior to the experiment but were allowed free access to tap water. After normal gavage on the ninth day, the mice in the other groups were injected with atropine (1.0 g/kg) except the control group, which was injected with vehicle in the same volume by intraperitoneal injection. Twenty minutes later, all mice were given 0.3 mL of 2% blue dextran 2000 solution. Then, each mouse was sacrificed by cervical rupture after 30 min. A total of 0.5 mL of blood was collected from the mice eye socket vein and centrifuged at 4 °C by 3000 rpm for 30 min. Then, the supernatant was centrifuged again to obtain the serum, which was preserved in a refrigerator at −20 °C. According to enzyme-linked immunosorbent assay kit instructions, serum motilin and gastrin (GAS) content were detected.

Subsequently, abdominal incision was carried out and the cardiac and pyloric regions were ligatured to separate the stomach and intestines from the cardia to the distal ileum. The stomach was cut into several pieces in phosphate-buffered saline (PBS, pH 7.0) (4 mL) to collect the gastric contents. The gastric contents were then centrifuged at 4000 rpm for 20 min, and the supernatant was measured at 620 nm to determine the residual pigment content in the stomach. Then, 0.3 mL 2% blue dextran 2000 solution was added to 4 mL of PBS (pH 7.0) and measured at 620 nm as the base value.

GE was calculated as follows:

\[
\text{Residual rate of pigment in stomach } \% = \frac{\text{value of residual pigment in stomach}}{\text{base value}} \times 100
\]

Simultaneously, all intestine segments were naturally straightened on the laboratory bench, and the following measurements were recorded: (i) total length of pyloric sphincter to the cecum (\(L_0\)) and (ii) length of pyloric sphincter to the forefront of blue dextran 2000 (\(L_1\)).
2.6. Statistical analysis

All the obtained data were expressed as means ± standard error (SE). The statistical significance of the results was evaluated by one-way ANOVA using SPSS statistical software (Version 13.0, SPSS. Inc., Chicago, USA). The data were also analyzed by Student’s t-tests. Least significant differences smaller than 0.05 were considered significant.

3. Results and Discussion

3.1. Overview of nutritional quality of APM in the different growth stages

Fig. 2 shows the results of nutrient analyses of AMP at five growth stages. Notably, all maca samples analyzed in this study were produced in the same cultivated experimental field using standard handling procedures. As shown in Fig. 2, moisture content ranged from 79.79% to 88.50%. Fig. 2 shows that the stems in the bolting stage featured the highest protein abundance (38.48 g/100 g DW), whereas sample No. 2 in the growth stage yielded the lowest content (23.02 g/100 g DW). The protein content in the flowering stage was slightly lower than that in the bolting stage but higher than that in the seedling and reproductive stages. Total sugar content is intimately linked with plant growth and development. The change in total sugar content of APM is due to differences in photosynthesis, respiration, transportation, and distribution of assimilates. Total sugar content ranged between 1.01 g/100 g DW to 2.21 g/100 g DW, with an average of 1.65 g/100 g DW. The vitamin content is a preferential factor for evaluation of the quality of a number of vegetables. Although vitamins are required only in small amounts per day, they play a vital role in human health. Nutritional deficiency in VC has long been known to cause scurvy, a disease characterized by bleeding gums, impaired
wound healing, anemia, fatigue, and depression which without proper care, can eventually lead to fatality \textsuperscript{58}. In the present study, VC ranged from 162.9 mg/100 g DW to 236.3 mg/100 g DW, with an average of 200.6 mg/100 g DW. Compared with other common vegetables, the APM are rich in VC, with higher contents than those found in cabbage, lettuce, and crown daisy \textsuperscript{59}. B vitamins are water-soluble vitamins that play important roles in cell metabolism \textsuperscript{60}. Several B vitamins act mainly as coenzymes in the metabolism of foodstuffs to produce energy. As shown in Fig. 2, the niacin content ranged from 35.21 mg/100 g DW to 134.5 mg/100 g DW, with an average of 85.59 mg/100 g DW, which is considerably higher than those of caraway, cabbage, spinach, and lettuce \textsuperscript{59}.

The results showed the inhomogeneous level of nutrient distribution in different growth stages due to changes in biomass. However, the APM maintained a high level of nutrient distribution as a whole. Compared with many common vegetables, the APM are enriched with nutrients, can be prospective vegetables, and can be cooked by frying, stewing, short-time heating in boiling water and pickling.

3.2. Minerals and heavy metals content

Figs. 3 and 4 display the minerals and heavy metal contents, respectively. The FW of mineral and heavy metal contents of representatives from the five different growth stages were investigated. As shown in Fig. 3, Na, K, Ca, Mg, and Fe contents were evaluated. Notably, K content ranged from 358.778 mg/100 g to 752.748 mg/100 g, whereas Na content ranged from 1.99 mg/100 g to 17.21 mg/100 g. High K and low Na contents prevent hypertension, coronary heart disease, and other cardiovascular diseases. Ca content ranged from 133.979 mg/100 g to 323.135 mg/100 g, and Mg content ranged from 26.695 mg/100 g to 54.285
mg/100 g, which are higher than those in other common vegetables. Ca and Mg are vital components of the bones and teeth. Thus, high Ca and Mg contents are useful sources of the human body. At the same time, Fe content ranged from 1.789 mg/100 g to 8.426 mg/100 g. Fe is essential to the human body and is involved in hematopoiesis and performs extensive physiological functions and biological effects. Overall, Na, K, Ca, Mg, and Fe contents showed APM as excellent sources of bioelements and as functional vegetables.

Pb, Cd, Hg, As, and Cr are common heavy metal pollutants in vegetables. As shown in Fig. 4, Pb, Cd, As, and Cr were detected in all samples, whereas Hg was not detected in all samples. Pb content ranged from 0.245 mg/kg to 1.26 mg/kg, which exceeded the detection limits of Chinese National Standards (CNS) (0.1 mg/kg, CNS GB 2762-2005). Cd content ranged from 0.02 mg/kg to 0.048 mg/kg, and As content ranged from 0.024 mg/kg to 0.0945 mg/kg, which both accorded with national standards. Sample Nos. 2 and 3 contained 0.67 and 0.842 mg/kg Cr, respectively, which exceeded the detection limits (0.5 mg/kg, CNS GB 2762-2005). On the other hand, our research group has analyzed the heavy metals of maca leaves which grow in high-cold and high-altitude areas, such as Yunnan and Xizang, China. Analysis results indicated that Pb, Cd, Hg, As, and Cr all accorded with CNS, and this result may be attributed to the good air quality and soil efforts.

3.3. Analysis of amino acid content

Table 1 presents the amino acid composition of five samples. All 17 amino acids were detected in the APM, and the contents were higher than those in common vegetables. The results showed that the essential amino acid contents accounted for 41%-47% of total amino acids, among which the percentage of sample No. 3 was the highest, and whereas that of No.
1 was the lowest. The distribution of the 17 amino acids varied slightly in different samples, but the data still showed that the APM are good sources of essential amino acids and high-quality protein.

<table>
<thead>
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<th>Amino acid</th>
<th>No. 1</th>
<th>No. 2</th>
<th>No. 3</th>
<th>No. 4</th>
<th>No. 5</th>
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<td>2.829</td>
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</table>

* Essential amino acids for humans.

3.4. Analysis of characteristic active ingredients

Functional foods have attracted worldwide attention, particularly in the fields of nutrition, phytochemistry, pharmacology, and other disciplines. Metabolites isolated from functional
foods are possibly responsible for their efficacy \(^{48}\). To evaluate the functional value of the APM, a detailed analyses of macamide, glucosinolates, total saponins, and adenosine were performed.

As shown in Fig. 5, the macamide content ranged from 45.10 mg/100 g DW to 70.33 mg/100 g DW, with sample No. 4 showing the highest and the inflorescences in the flowering stage showing the lowest content. Glucosinolates content ranged from 0.625 g/100 g DW to 1.813 g/100 g DW, with sample No. 2 showing the highest content, and samples in the seedling stage showing the lowest content. Adenosine content ranged from 2.157 mg/100 g DW to 4.068 mg/100 g DW. Although the contents of these active ingredients in the aerial parts were lower than those in maca roots, their functional value was as significant as those in vegetables. Glucosinolates lead to pungency. Thus, their low level can improve the taste and increase the amount of actual intake. Notably, total saponin content ranged from 3.318 g/100 g DW to 5.183 g/100 g DW, with sample No. 5 in inflorescences showing the highest at three- to fivefold that of maca roots.

3.5. Gastrointestinal prokinetic efficacy of APM

3.5.1. GE and IP

GE is the process by which food is delivered to the small intestine at a certain rate and in a form that optimize intestinal absorption of nutrients. GE rate is regulated by tonic contraction of the proximal stomach (fundus), contraction of the distal stomach (antrum), and inhibitory forces of pyloric and duodenal contraction. GE and IP are the most direct manifestations of gastrointestinal motility, and their rates are key indicators of gastrointestinal motility. The gastrointestinal motility disorder caused by various factors can reduce the rates of GE and IP.
Compared with the normal control group, the residual rate of pigment in mice stomachs of the model group significantly increased ($P < 0.01$, Fig. 6), indicating that the model was successful. When a small dose of APM was added, the residual rate of pigment reduced to 42.68%, whereas the rates of middle and high doses reached 42.12% and 44.17%, respectively. The statistics we gathered indicated that small-dose APM notably promoted GE. Remarkably, the residual rate of the pigment of the BITC group was also statistically significant compared with the model group ($P < 0.05$). In addition, as shown in Fig. S1, BITC was a breakdown product from the middle-dose group of APM by HS-GC-MS.

The rate of IP of the model group decreased significantly ($P < 0.01$, Fig. 6) compared with that of the normal control group. The APM and BITC groups yielded higher rates than the model group ($P < 0.05$). Among all groups, the low-dose APM group exhibited a conspicuous effect, and a significant difference was observed compared with the model group ($P < 0.05$). This effect was not dose-dependent among the APM groups.

The results showed that the low-dose APM group played a certain role in promoting GE and IP. As vegetables, APM can help human GE and IP and will exert positive effects on human gastrointestinal health. We also speculated that BITC may work in APM.

### 3.5.2. Levels of MTL and GAS

MTL, an endogenous prokinetic hormone, is secreted by gastrointestinal endocrine cells. MTL is released during fasting at cyclical bursts and in association with phase III of the migrating motor complex (MMC). Since its isolation in 1971, evidence suggested that MTL performs a pivotal role in clearing the stomach and intestine from any undigested food remnants. GAS is excreted by G cells in the antrum of the stomach, duodenum, and pancreas.
GAS release is stimulated by stomach distension, vagal stimulation, ingested proteins, and amino acids and is produced in highest concentrations in the ileum and colon; in addition to its other functions, GAS may exert a trophic influence on the gastrointestinal tract

Lower levels of MTL and GAS were observed in the serum of the model group than the normal control group \((P < 0.01, \text{ Fig. 6})\). The levels of APM and BITC groups increased significantly compared with those of the model group \((P < 0.05)\). These results suggest that the APM and BITC may stimulate the endocrine cells of gastric sinus and duodenal to promote MTL and GAS secretion, smooth muscle contraction, and creep of gastrointestinal tract. No significant differences were observed among the APM and BITC groups, consistent with the results of GE and IP. Recent studies have reported that isothiocyanates exert certain effects on gastrointestinal motility. For example, the allyl isothiocyanate modulates the gastrointestinal motility of mouse \(^{66}\) and protectes ethanol-induced gastric lesions by pretreatment with indomethacin in rats \(^{67}\). Allyl isothiocyanate also stimulates colonic motility and defecation in conscious dogs \(^{68}\). Mustard oil may be effective in both clonidine- and loperamide-induced delays in colonic transit constipation \(^{69}\). BITC is the main enzymatic hydrolysis product of benzylglucosinolate \(^{70-71}\), which is the most abundant glucosinolate in maca, accounting for 80%-90% of the total glucosinolates \(^{72}\). Therefore, except for the anti-cancer activity, the APM may enhance gastrointestinal motility. In conclusion, BITC may play a role in APM. However, further studies are needed to clarify the mechanism of BITC in gastrointestinal prokinetic efficacy.

Considering these results, low-dose intake of APM may be necessary for gastrointestinal prokinetic efficacy in humans or mice.
4. Conclusion

Information on the potential use of APM as a vegetable is limited. Therefore, this study aimed to demonstrate the phytochemical characterization and nutritional data for APM to popularize their consumption and utilization. Results showed that nutrients and minerals are particularly abundant and diverse in APM. Amino acid compositions were coordinated, with the essential amino acids accounting for 41%–47% of the total amino acids. The characteristic active ingredients, including macamide, glucosinolates, adenosine, and saponin were all identified, and total saponin content in APM was higher than that in maca roots. Therefore, APM not only feature the nutritional value of common vegetables but also functional values, such as anti-fatigue and anti-cancer properties.

Gastrointestinal prokinetic studies of APM showed that APM significantly promotes gastrointestinal activity. Thus, APM can be used as gastrointestinal prokinetic vegetables. BITC may be an active ingredient in this vegetable. However, the possible action mechanisms of APM and BITC in gastrointestinal motility in vivo requires in-depth investigation in the future. We plan to investigate the origins of the above-standard Pb and Cr contents in APM under the present experimental conditions and the metabolism of APM chemical compositions in the gastrointestinal tract to control the quality of APM and evaluate the effect of dietary APM on gastrointestinal health.

Overall, the study demonstrated that APM are not only good sources of health food that is rich in nutrition but are also good sources of functional vegetables that can promote gastrointestinal peristalsis.

Conflicts of interest
The authors declare that they have no conflicts of interest.

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Aerial parts of maca (*Lepidium meyenii* Walp.) as functional vegetables with gastrointestinal prokinetic efficacy *in vivo*

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Fig. 1 Maca seeds and representative samples obtained from five different growth stages. (a) Maca seeds; (b) sample No. 1 (seedlings at the seeding stage); (c) sample No. 2 (leaves at the reproductive stage); (d) sample No. 3 (leaves at the reproductive stage); (e) sample No. 4 (stems at the bolting stage); sample No. 5 (inflorescences at the flowering stage).
Fig. 2 Basic composition of APM at different growth stages. (A) Moisture content, (B) total protein content, (C) total sugar content, and (D) vitamin content. Data (B–D) are related to DW. Means ± SE are given.
Fig. 3 Minerals of five samples at different growth stages. Data are related to FW. Means ± SE are given.

Fig. 4 Heavy metal contents of five samples at different growth stages. Data are related to FW. Means ± SE are given.
Fig. 5 Active ingredients of APM at different growth stages. (A) Macamide content, (B) glucosinolate content, (C) adenosine content, and (D) total saponins content. Data (A–D) are related to DW. Means ± SE are given.

Fig. 6 Effects of APM on gastrointestinal prokinetic efficacy in atropine-treated mice. (a) Residual rate of pigment in mice stomach (%); (b) rate of IP in mice (%); (c) serum MTL level (pg/mL); (d) serum...
GAS level (pg/mL). A: Model group; B: positive control group; C: low-dose group of APM; D: middle-dose group of APM; E: high-dose group of APM; F: BITC group. Data are expressed as mean ± SE (n = 10) and compared with the control group, *P < 0.05, **P < 0.01; compared with the model group, †P < 0.05, ‡P < 0.01.
The aerial parts of maca powders and benzyl isothiocyanate promote the gastrointestinal prokinetic efficacy in atropine-treated mice.