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Product development of protein drink containing maslinic acid for the elderly

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ABSTRACT

Background: The world population is aging rapidly, leading to various health problems such as malnutrition and insufficient protein intake. These issues contribute to reduced muscle mass and a decline in physical function among older adults. The high prevalence of muscle atrophy and oxidative stress-related muscle dysfunction among elderly people highlights the urgent need for effective strategies to promote healthy aging and improve quality of life.

Objective: This research aims to develop a protein drink containing maslinic acid (MA) for the elderly and to investigate its muscle growth-promoting effects using cell line models. The nutritional content was evaluated, and product prototypes were subsequently developed.

Methods: Cricket protein was selected as the main ingredient for the product formulation, combined with inulin, cocoa powder, vitamins, minerals, and commercially available MA, in accordance with Thai Recommended Daily Intake (Thai RDI) guidelines. Standard methods for nutritional value and microbial analysis were applied to evaluate the product. The prototype was designed for consumer appeal and subsequently subjected to sensory evaluation. Cytotoxicity, cytoprotective effects, and reactive oxygen species (ROS) generation of the developed product were evaluated in C2C12 mouse myoblast cells using MTT and ROS assays at various concentrations.

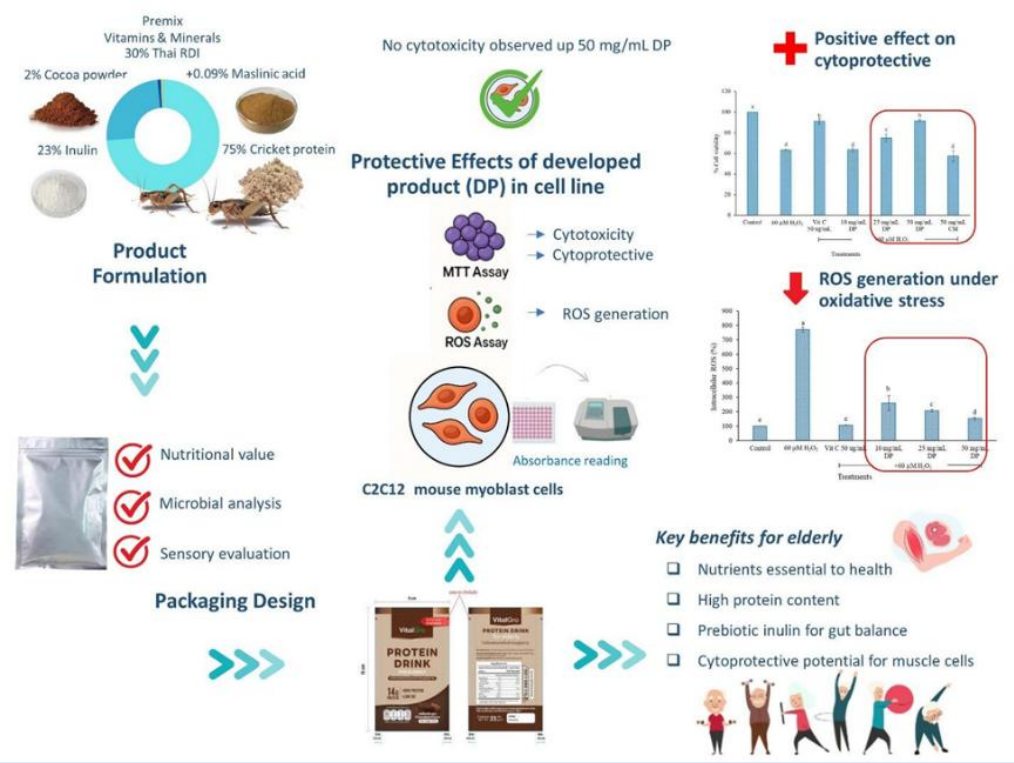
Results: The MA-containing protein drink for the elderly was formulated with cricket protein and inulin powder as the main ingredients, with chocolate flavoring added to improve palatability. It contained 58.79% protein, 21.2% carbohydrates from inulin (a dietary fiber and prebiotic), 0.87% total sugar, and 12.7% fat, providing 120 kcal per 23 g serving. Fortified with vitamins and minerals at 30% of Thai RDI per serving, it represents a nutrient-dense option for

older adults. Microbiological tests confirmed compliance with Thai FDA safety standards. Sensory evaluation indicated high acceptance compared to the commercial product. The product packaging was designed with attractive colors and clear labeling, providing both appeal and comprehensive product information. Moreover, the developed product (DP) showed no cytotoxicity up to 50 mg/mL and significantly reduced ROS levels in C2C12 cells under oxidative stress. At concentrations of 25 and 50 mg/mL, DP enhanced cell viability and demonstrated strong cytoprotective effects superior to a commercial protein product without MA.

Conclusion: The MA-containing protein drink for the elderly, formulated with cricket protein, inulin, and MA, provides high protein content and essential nutrients with good safety and sensory acceptance. It delivers nutrients relevant to muscle function and digestion with packaging designed for the elderly. In cell studies, the product showed no toxicity up to 50 mg/mL and effectively reduced oxidative stress, enhancing cell viability. Its cytoprotective effects surpassed those of a commercial protein product without MA.

Novelty of the Study: This study introduces a novel protein drink for the elderly, combining cricket protein, MA, and inulin to support muscle function and reduce oxidative stress. It is among the first products aligned with Thai dietary guidelines to integrate these components, demonstrating cytoprotective effects in muscle cell models and offering commercial potential as a functional beverage for aging populations.

Keywords: muscle, maslinic acid, elderly, cell line, protein drink, C2C12



Graphical Abstract: Product development of protein drink containing maslinic acid for the elderly

INTRODUCTION

The worldwide population is aging at an unprecedented rate, representing one of the major challenges of this century. The World Health Organization predicts that the population aged 60 years and above will increase to 1.4 billion by 2050 [1]. Among the numerous health issues that affect aging individuals, poor nutrition together with insufficient protein intake stands as a primary factor that impacts their physical health and functional ability and overall well-being. Sarcopenia represents a progressive muscle loss that affects 6–22% of elderly people across the world. The condition develops primarily because of insufficient protein intake [2]. Current guidelines recommend a daily protein intake of 1.0-1.2 grams per kilogram of body weight for healthy older adults, which exceeds the requirements of younger individuals. The body needs higher protein intake because aging leads to decreased protein synthesis together with elevated metabolic requirements [3]. Recent studies demonstrate that resistance training plays a vital role in protecting muscle health and enhancing physical capabilities in seniors with sarcopenia. When combined with adequate protein intake, resistance exercise offers an effective strategy to combat muscle deterioration and weakness, thereby enabling older adults to maintain independence and a better quality of life [4].

Bioactive compounds, key components of functional foods that provide benefits beyond basic nutrition, may also help prevent muscle mass loss through their anti-inflammatory properties, as inflammatory cytokines contribute to muscle fiber atrophy [5–7]. MA (2-α, 3-β-dihydroxyolean-12-en-28-oic acid), a plant-derived pentacyclic triterpenoid, exhibits antioxidant and anti-inflammatory activity by reducing oxidative stress and inhibiting pro-inflammatory cytokine production [8–10]. Oral administration of MA from olive fruit reduces the expression of inflammation-related genes in joint tissues [11]. The mTOR signaling pathway is activated by MA, thereby promoting muscle growth [12]. Moreover, MA reduces the expression of *Atrogin-1* and *MuRF 1* genes to inhibit muscle atrophy, while IGF-1 expression is enhanced to maintain muscle mass by balancing protein synthesis and degradation [13]. Therefore, the application of MA in dietary supplement development and its potential medical benefits have become increasingly important area of research. This study examines the combined effects of MA as a bioactive compound when paired with commercial cricket protein.

The protein found in crickets serves as an excellent alternative protein source because it contains all essential amino acids at levels comparable to those in animal proteins. The digestibility of cricket protein is similar to that of plant foods but remains slightly below that of animal protein [14], while also offering environmental sustainability benefits over traditional mammalian sources. The nutritional profile of cricket protein includes beneficial antioxidants and essential minerals [15], with higher levels of iron, zinc, and potassium than standard plant and animal sources, as well as superior calcium compared to both alternatives.

A recent study showed that cricket protein supplementation combined with concurrent training significantly improved muscle mass, strength, inflammatory modulation, anabolic signaling, cardiorespiratory fitness, and quality of life in older women. These results highlight the synergistic benefits of insect-derived protein and structured exercise, supporting the use of cricket protein as a sustainable nutritional strategy to counteract sarcopenia and maintain physical independence in aging populations [16]. The unique properties of cricket protein make it an excellent choice to investigate synergistic effects with MA, potentially leading to promising functional food and nutraceutical developments with enhanced therapeutic benefits.

Therefore, this study aims to develop a formulation for MA-containing protein drink for the elderly. In

addition, the research seeks to evaluate the product's ability to promote muscle growth in cell line models. Furthermore, the study intends to assess nutritional value and develop a prototype with a nutrition label.

METHODS

This research was conducted in accordance with ethical standards and was approved by the Human Research Ethics Committee of Sirindhorn College of Public Health Yala (reference number: SCPHYLIRB-2567/238).

Nutritional Formulation: The nutritional formula consisted mainly of cricket-derived protein powder (75%), inulin (23%), and cocoa powder (2%), serving as a natural chocolate flavoring agent. Additionally, commercial grade MA powder, vitamins, and minerals were incorporated into the formulation. All nutritional ingredients were purchased from certified suppliers. A premixed blend of vitamins and minerals was added to provide 30% of Thai RDI, including vitamins A, D, E, K, B1, B2, niacin, B6, B12, and C, as well as iron, calcium, phosphorus, magnesium, iodine, and potassium [17]. The finished product contained 0.09% MA by weight and was in powder form. The product was stored in sterile aluminum foil pouches under refrigerated conditions until nutritional analysis was conducted.

Nutritional Quality Assessment: The nutritional composition of the final product was analyzed by SGS (Thailand) Co., Ltd., located in Songkhla, Thailand [17]. Fat and saturated fat were determined using AOAC (2019) 996.06 with GC/FID, and cholesterol was measured according to SOP LBCH-00259 based on AOAC (2019) 994.10. Protein content was measured according to SOP LBAG-14002, referencing AOAC 990.03, 992.15, and 992.23. Carbohydrates and energy were analyzed using the Method of Analysis for Nutrition Labeling (1993), while sugars were determined according to SOP LBLC-17001 based on AOAC (2019) 982.14 with HPLC-RI. Sodium and potassium were measured according to SOP LBCH-13532 based on AOAC (2019) 999.10 and 2011.14.

Product packaging concept: The MA-containing protein drink is packaged in single-serving sachets (23 g each) intended for mixing with 240 mL of water. Each box contains 15 sachets. Both sachets and boxes display complete product information, including nutrition facts, ingredients, and manufacturer information. The packaging incorporates vibrant colors to enhance visual appeal and perceived market value.

Analysis of microbial profiles in product: The final product underwent microbiological testing [17]. A 1 g sample was serially diluted (1:10) and cultured using the pour plate method. Plate count agar was used for bacteria, Potato Dextrose Agar with 0.01% chloramphenicol for yeasts, and Sabouraud Dextrose Agar for molds [18]. Bacterial plates were incubated at 37°C for 48 hours, while yeast and mold plates were incubated at 27°C for 3 days. Quality assessment followed FDA BAM and ISO standards, including tests for yeasts and molds (FDA BAM, 2021), *Salmonella spp.* (ISO 6579-1:2017/Amd.1:2020), *Staphylococcus aureus* (FDA BAM, 2016), *Listeria monocytogenes* (ISO 11290-1:2017), *Bacillus cereus* (FDA BAM, 2020), and *Clostridium perfringens* (FDA BAM, 2001) [17].

Sensory assessment of product quality: The sensory evaluation of the protein drink containing MA was conducted with 50 elderly panelists (aged 60–80 years) from Yala City Municipality, Thailand. The sample size aligns with standard practice for 9-point hedonic scale testing [19–20], ensuring statistical reliability. Participants were purposively selected based on health status (no serious diseases or protein allergies) and voluntary consent, confirmed via interviews. A 9-point hedonic scale (1 = extremely disliked to 9 = extremely liked) was used, and the evaluation form was validated

by three food product development experts. The Index of Item-Objective Congruence (IOC) ranged from 0.67 to 1.00 (mean = 0.91), exceeding the acceptable threshold (≥0.50). Sensory attributes assessed included appearance, thickness, aroma, taste, mouthfeel, aftertaste, and overall preference. The developed product was compared with two commercial products (cricket protein powder and whey protein powder) to determine consumer preference and market potential.

Cell culture: In this study, the C2C12 mouse myoblast cell line (ATCC, Manassas, VA, USA) was selected as a primary research tool in muscle biology, having been widely applied in various studies to investigate muscle growth and differentiation [21–22]. These cells initially exist as myoblasts, then proliferate and differentiate into multinucleated myotubes that closely resemble skeletal muscle fibers under specific conditions. This makes C2C12 an ideal in vitro model for exploring muscle development and the biochemical processes involved in muscle function. The cells were maintained under standard culture conditions using ATCC-formulated Dulbecco's Modified Eagle's Medium (DMEM, Catalog No. 30-2002), supplemented with 10% fetal bovine serum (FBS) [12]. The culture medium was refreshed every 2–3 days to maintain optimal growth conditions.

Cytotoxicity and cell viability determination: The cytotoxicity of the MA-containing protein drink was evaluated using the MTT assay, based on a previously reported method [23] with slight modifications. C2C12 cells were seeded into 96-well culture plates at a density of 5×10^4 cells/well. Plates were incubated at 37 °C in a humidified atmosphere of 5% CO_2 for 24 hours to allow cell attachment. After the initial incubation, the culture medium was replaced with 100 µL of the MA-containing protein drink at various concentrations (0.1–100 mg/mL), and the cells were further incubated for an additional 24 hours. Subsequently, 20 µL of MTT solution (5 mg/mL in PBS; Sigma-Aldrich, St. Louis, MO, USA) was added to each well, and the plates were incubated for 2 hours. The resulting purple formazan crystals were solubilized by adding 100 µL of dimethyl sulfoxide (DMSO). Absorbance was measured using a microplate reader at a wavelength of 570 nm and 650 nm.

Assessment of Cytoprotective Effect and ROS Generation: The method for determining ROS production in hydrogen peroxide (H_2O_2)-stressed C2C12 cells was modified from previous studies [24]. C2C12 cells were seeded into black-walled, clear-bottom 96-well plates at a density of 5×10^4 cells per well and incubated for 24 hours at 37 °C to allow cell attachment. After incubation, the cells were treated with various concentrations of the MA-containing protein drink (10, 25, and 50 mg/mL) along with 100 µL of 1.57 mM H_2O_2 as a co-treatment. The plates were then incubated at 37 °C for 30 minutes. Subsequently, 10 µL of 100 µM 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA; Sigma-Aldrich, St. Louis, MO, USA) was added to each well, and the cells were incubated for an additional 30 minutes under the same conditions. After incubation, intracellular ROS production was quantified by measuring the fluorescence intensity using a multimode microplate reader (Varioskan™ LUX, Thermo Fisher Scientific) at an excitation wavelength of 485 nm and an emission wavelength of 530 nm.

Statistical analysis: Experimental data were obtained from three independent replications and are presented as mean ± standard deviation. Statistical significance was determined by ANOVA at the $p < 0.05$ level. Duncan's multiple range test was applied for post-hoc comparisons between treatment groups to identify specific mean differences. All statistical computations and analyses were conducted using SPSS software (version 18).

RESULTS AND DISCUSSION

Product formulation: The development of the MA-containing protein drink for the elderly demonstrated that a formulation consisting of 75% cricket protein and 25% inulin, with chocolate flavoring added to mask the cricket protein odor, resulted in a product with a texture of fine powder, dark color, distinct chocolate aroma, and good solubility in water. The final concentration of MA was set at 0.09%, based on prior evidence supporting its safety, stability in protein-based beverages, and potential health-promoting effects in the elderly. At this level, each 23 g serving of the product provides approximately 20.7 mg of MA. With the recommended intake of 2–3 servings per day, depending on body weight and protein requirements, this corresponds to an estimated daily intake of 40–60 mg, which aligns with previous findings suggesting beneficial effects of olive-derived MA in community-dwelling elderly (60 mg/day) [25]. Importantly, the addition of commercial-grade MA at this concentration did not alter the color, aroma, or taste of the product. The formulation was further fortified with a premixed blend of vitamins and minerals, providing 30% of Thai RDI per serving. These included vitamins A, D, E, K, B1, B2, niacin, B6, B12, and C, along with essential minerals such as iron, calcium, phosphorus, magnesium, iodine, and potassium [17]. The finished product was packaged in sterile aluminum foil pouches to protect against light and moisture, thereby preserving nutrient stability. It was stored under refrigerated conditions prior to analysis. Overall, the experimental results indicate that this formulation possesses optimal nutritional value, sensory characteristics (color, aroma, and taste), as well as physical properties, demonstrating strong potential for commercial development.

Nutrition Facts: A certified food laboratory performed nutritional analysis on the formulated MA-containing protein drink to ensure accuracy and compliance with relevant standards. The analysis revealed that the developed product contains a high protein content of 58.79%, indicating its potential as a high-protein supplement to support muscle maintenance and recovery in aging populations, particularly those at risk of sarcopenia. Moreover, substantial scientific evidence supports the use of protein supplementation combined with resistance exercise to increase and maintain muscle mass, strength, and physical function in both older adults and athletes [16, 26–27]. In this context, cricket protein, the main component of the developed product, shows strong potential as an alternative protein source for the elderly, offering complete nutritional value comparable to that of conventional animal- or plant-based proteins [28]. The protein quality evaluation revealed that cricket protein contains abundant essential amino acids of high quality. It is easy to process, has good solubility, and performs well in food applications. Furthermore, it is a sustainable and eco-friendly protein source, making it a promising alternative for the future [29]. The evaluation of carbohydrate composition showed that the product contains 21.2% carbohydrates from inulin, which serves as dietary fiber and a prebiotic, while the total sugar content is only 0.87%. These results indicate that the product supports digestive health through its prebiotic properties, which promote the growth of beneficial gut microbiota–a factor especially important for older adults. The recommended daily dietary fiber intake for men and women in Europe and the United States is 30–35 g for men and 25–32 g for women [17]. Thai RDI recommends 25g of dietary fiber per day for individuals aged six years and older. The dietary fiber content of the product developed in this study was approximately 5 g per serving. It is recommended to consume 2–3 servings per day to achieve beneficial dietary fiber intake. Maintaining good gastrointestinal health is crucial for older adults because it enables proper nutrient absorption and immune function, leading to better quality of life and a reduced risk of age-related diseases. This is particularly important since dietary fiber, essential for gut health, is

typically obtained from various food sources [30]. Furthermore, the product contains 12.7% fat, which helps deliver essential fatty acids and enhances the absorption of fat-soluble vitamins A, D, E and K [31]. Overall, the energy contribution of the product is 434 kilocalories per 100 g. The developed product was designed to be a nutrient-dense option suitable for elderly individuals who have higher protein needs but potentially lower total food intake. The nutritional labeling was developed based on the product's nutrient content and Thai RDI for the population aged 6 years and older, which is based on 2,000 kilocalories per day [17]. A 23 g serving of this product contains 120 kilocalories, 14 g of protein, 0.33 g of total fat, 3 g of saturated fat, 56 mg of cholesterol, and 5 g of carbohydrates. The proportions per serving compared with Thai RDI are 5% for total fat, 5% for saturated fat, 12% for cholesterol, and 2% for carbohydrates. The mineral content per serving provides 4% and 6% of Thai RDI for sodium and potassium, respectively. This formulation aims to provide both nutritional support and functional health benefits through the inclusion of bioactive compounds like MA. Previous studies have shown that MA has the potential to prevent muscle loss after nerve injury in mice by reducing inflammation and suppressing TGF-β signaling, a pathway linked to muscle atrophy [32]. These findings are consistent with earlier research in older adults, where MA combined with resistance training helped preserve muscle mass [25]. This further supports MA's anti-inflammatory role in preventing muscle atrophy and highlights its potential for future therapeutic applications.

Microbiological analysis: The finished product underwent microbiological quality testing at a certified laboratory that met the FDA registration standards for Thailand [17–18]. The laboratory results demonstrated that yeast and mold, as well as *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, and *Clostridium perfringens* were either very low or undetectable throughout the entire study period, as shown in Table 1. The product fulfilled the Thai FDA's microbial quality standards for powdered products regarding these pathogens and spoilage organisms. The results indicate that strict quality control procedures throughout production, storage and handling are essential to ensure both microbial safety and product stability. The recommended approach includes ongoing monitoring and process optimization to achieve both high microbial quality and full regulatory compliance.

Table 1. Microbiological quality of the MA-containing protein drink for the elderly.

Items	Specification	Resulted	Units
Yeast & Mold count	Less than 10	< 10	CFU/g
Salmonella sp.	Not detected	Not detected	Per 25g
Listeria monocytogenes	Not detected	Not detected	Per 25g
Staphylococus aureus	Less than 100	< 3	CFU/g
Bacillus cereus	Less than 100	< 10	CFU/g
Clostridium perfringens	Less than 100	< 10	CFU/g

Product prototype: The maintenance of muscle mass in older adults depends primarily on their dietary protein intake [33]. Medical research shows that protein requirements vary significantly between age groups, especially in older adults, whose needs depend on their health status and physical activity level [34–35]. Expert guidelines for muscle health and general wellness in older adults recommend individualized daily protein intake. To address the needs of elderly consumers, our team created a simple protein drink containing MA, with

each 23 g serving of the product powder providing 14 g of premium-quality protein, which is dissolvable in 240 mL of water. Although MA is poorly soluble in water, the small amount added to the product means that the floating appearance is not pronounced. Nevertheless, some of the compound may remain suspended within the product. Therefore, it is recommended to shake the product before consumption to ensure even dispersion, without affecting consumption. Improving the dispersion properties of MA remains an area for further development. The chocolate flavor of the product provides a pleasant taste that encourages regular consumption. The product comes in single-serve aluminum foil sachets which include clear nutritional information for easy reading. The label presents the essential macronutrients, vitamins and minerals that match Thai RDI standards. In addition, the packaging displays all essential information about brand identity, ingredients, manufacturer details, and unique health benefits on both front and back. The packaging design emphasizes the high protein content and low sugar levels while showcasing how MA and probiotics work together to support muscle function and digestive health. The sachet design provides simple access and mobility features which make this protein drink suitable for elderly people who want to preserve their strength and wellness.

Sensory acceptability: Fifty panelists evaluated the sensory acceptability of the developed MA-containing protein drink for the elderly (DP) using a 9-point hedonic scale. All sensory attributes received favorable ratings: appearance (7.86 ± 1.67), color (7.48 ± 1.16), thickness (7.78 ± 1.57), aroma (8.08 ± 1.54), taste (7.92 ± 1.86), mouthfeel (7.84 ± 2.13), aftertaste (7.98 ± 1.60), and overall preference (8.48±0.97), as shown in Table 2. The prototype outperformed both the control and commercial products (CM) in all attributes ($p < 0.05$), indicating that the formulation enhanced both nutritional value and sensory acceptability. This may be attributed to the pleasant chocolate color and mild sweetness derived from inulin, one of the key ingredients in the product, which likely contributed to its favorable reception. Although the inherent color and taste of cricket protein may be perceived as unappealing, the use of chocolate flavoring and coloring effectively masked these undesirable characteristics, thereby enhancing overall consumer acceptability. Sensory attributes serve as essential factors in determining consumer acceptance of functional foods, particularly for elderly populations, because these products need to deliver health benefits while tasting pleasant [36]. The successful formulation strategy of this study achieved a balance between nutritional function and consumer preference, as reflected by the high sensory scores across various attributes. The product received outstanding scores for its visual aspects, including appearance and overall preference. Although the importance of visual appeal diminishes for elderly consumers due to age-related visual impairments affecting contrast sensitivity and color discrimination [37–38], the high visual attribute scores indicate both the objective quality of the product's appearance and the success of designing it for visual recognition by people with declining vision. The combination of visual contrast enhancement with familiar colors, such as chocolate brown, helps elderly consumers maintain interest in the product despite sensory challenges. Positive taste and aroma scores indicate that the flavor-masking approach successfully concealed any unpleasant tastes from cricket protein and MA bioactive ingredients. Notably, a product's health benefits cannot compensate for negative sensory traits, that might prevent older adults from continuing to use it [38]. Ensuring the product's palatability is therefore crucial for long-term acceptance, which is vital for achieving functional outcomes.

Table 2. Sensory scores of the developed MA-containing protein drink for elderly (DP).

Evaluation criteria	Control	CM	DP
Appearance	6.48±2.01^{b}	6.36±1.74^{b}	7.86±1.67^{a}
Color	6.28±0.95^{b}	5.16±1.11^{c}	7.48±1.16^{a}
Thickness	6.58±1.68^{b}	5.94±1.67^{b}	7.78±1.57^{a}
Aroma	6.50±1.76^{b}	5.84±1.96^{b}	8.08±1.54^{a}
Taste	6.12±2.20^{b}	5.66±2.08^{b}	7.92±1.86^{a}
Mouthfeel	6.04±2.21^{b}	5.68±2.08^{b}	7.84±1.58^{a}
Aftertaste	6.10±2.30^{b}	5.72±2.28^{b}	7.98±1.60^{a}
Overall preference	6.84±1.68^{b}	6.48±2.07^{b}	8.48±0.97^{a}

*Different lowercase letters above the numbers in each row indicate significant difference between samples ($p < 0.05$)

Effect of the DP on cytotoxicity effects: The assessment of cell viability is a fundamental step in evaluating the impact of MA on cell survival, which is closely related to its potential cytotoxic or cytoprotective effects. The MTT assay represents a standard technique which measures cellular metabolic activity to determine the number of living cells [10]. The test reveals MA cytotoxic effects at various concentrations while identifying appropriate concentrations for subsequent experiments. Experimental results from this study confirm that the developed product (DP) positively affects the viability of C2C12 cells, as shown in Figure 1. The results showed that the concentration of DP from 0.1 to 50 mg/mL did not significantly affect cell viability ($p > 0.05$) compared to the untreated control group. However, at concentrations above 100 mg/mL, cell viability decreased to 73.12%. This may be attributed to cytotoxic effects at higher concentrations, possibly resulting from the accumulation of bioactive compounds exceeding the cellular tolerance threshold. The MTT assay evaluates mitochondrial dehydrogenase activity in living cells, which reduces MTT to purple formazan crystals, thereby indicating greater cell viability. At elevated concentrations, bioactive compounds exert cytotoxic effects, damaging mitochondrial function and reducing enzymatic activity, which leads to less reduction, consequently decreasing absorbance values [39]. This inverse relationship between compound concentration and cell viability is utilized in cytotoxicity assessments to determine the optimal concentration for use. Therefore, concentrations below 50 mg/mL of the DP were selected for subsequent experiments.

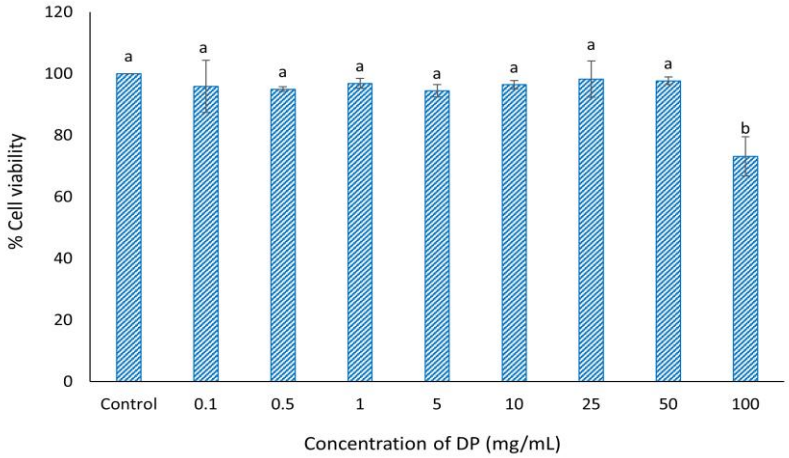


Figure 1. Cytotoxic effects of DP after 24 h of treatment. C2C12 cells were incubated with or without DP (0.1–100 mg/mL). All data were expressed as mean with standard deviation of three experimental replications ($n = 3$). Means that do not share a common letter are significantly different ($p < 0.05$), as determined by one-way analysis of variance (ANOVA) followed by Duncan's post-hoc test.

ROS generation and cytoprotective effect: MA occurs naturally in olives and other plant sources [40, 41], and various studies have investigated its multiple health-promoting properties, including anti-obesogenic, anti-tumor, hypoglycemic, neuroprotective, anti-inflammatory, antioxidant effects, and various other biological activities [41–42]. Among these benefits, several studies specifically indicate that MA helps improve muscle strength as well as muscle mass. Given that the essential function of skeletal muscle is to support both athletic performance and quality of life, preservation of muscle mass is vital for athletes and aging individuals [43]. Research has demonstrated that MA supplementation can enhance muscle protein synthesis and reduce muscle protein breakdown, contributing to overall muscle health and function. Experimental evidence supporting these mechanisms comes from studies showing that the combination of MA supplementation with resistance training led to stronger grip strength and greater arm muscle mass in elderly participants compared to those in the placebo group [31]. These findings suggest that MA may serve as a beneficial agent for protecting against age-related muscle loss and improving physical performance in older adults. Additional support for this conclusion is provided by research demonstrating that elderly women who received MA, while undergoing whole-body vibration training, showed superior quadriceps strength [44]. Animal studies further support these outcomes by demonstrating mechanisms involving enhanced protein synthesis [31].

Despite these promising effects, recent studies evaluating MA supplementation in athletes revealed that, although no direct improvements in physical performance were observed, there was a notable reduction in perceived fatigue and muscle soreness. This discrepancy may be attributed to the relatively low levels of inflammation, oxidative stress, and muscle damage [45]. Given that oxidative stress plays a key role in mediating cellular function, inflammation, and muscle recovery, further investigation into the effect of MA, a bioactive compound included in the DP, on ROS production was warranted to verify its cytoprotective potential. Therefore, this step aimed to confirm the effect of DP on intracellular ROS generation. Intracellular ROS levels were quantified using a fluorescence microplate reader, and the results are presented in Figure 2.

The study found that in the H_2O_2-treated group, ROS levels significantly increased to 771.86% compared to the untreated control group, which was set at 100% ($p < 0.05$). In the positive control group, where 60 µM H_2O_2 was co-treated with vitamin C (60 µg/mL) to mitigate ROS production, ROS levels were reduced to the point that there was no significant difference from the untreated control group ($p > 0.05$). Furthermore, to evaluate the antioxidant potential of DP in reducing ROS production, the results showed that treatment with DP at concentrations of 10, 25, and 50 mg/mL (corresponding to 9, 22.5, and 45 µg/mL of MA present in the formulation) significantly decreased ROS levels compared to the H_2O_2-treated group, (negative control). However, the ROS-reducing effect of DP was significantly less potent than that observed in the vitamin C group. Specifically, ROS production was reduced to 261.05%, 207.77%, and 153.46%, respectively, compared to the control group, which served as a reference antioxidant due to its well-established efficacy in neutralizing free radicals and mitigating oxidative stress.

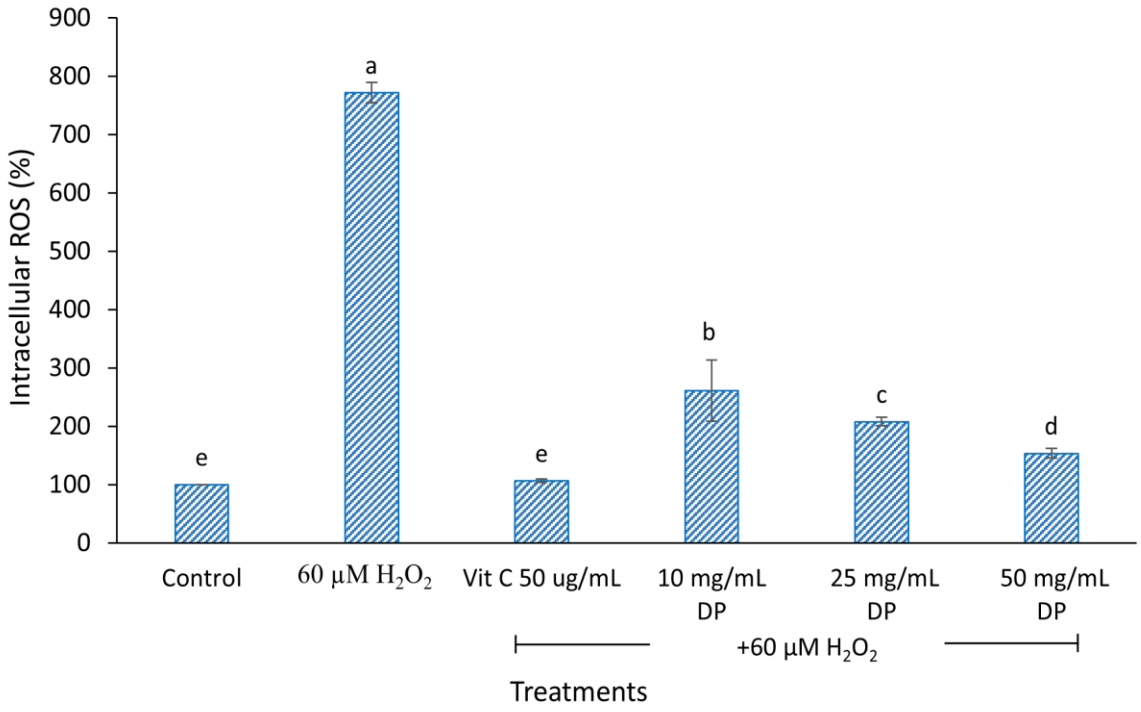


Figure 2. ROS generation after 24 hours of treatment with DP. C2C12 cells were incubated with or without DP at concentrations of 10, 25, and 100 mg/mL. Ascorbic acid (50 µg/mL) was used as a positive control (Vit C), and H_2O_2 (60 µM) was used to induce oxidative stress. Data are presented as mean ± standard deviation from three independent experiments (n = 3). Different letters above bars indicate statistically significant differences ($p < 0.05$), as determined by one-way analysis of variance (ANOVA) followed by Duncan's post-hoc test.

ROS are major contributors to oxidative stress, a condition that plays a significant role in the development of aging and various diseases. During oxidative stress, excessive ROS can damage vital macromolecules such as proteins and nucleic acids, by inducing cross-linking and polymerization. This molecular damage can disrupt protein function, inhibit RNA synthesis, and alter normal cellular structures. Consequently, ROS may interfere with critical biological processes, including the cell cycle, chromosome segregation, hormone synthesis, cell proliferation, and apoptosis [42].

Moreover, excessive oxidative stress from ROS can impair skeletal muscle by disrupting muscle formation, regeneration, and altering cell fate. High ROS levels reduce antioxidant defenses, activate nuclear factor kappa B (NF-κB) signaling, and suppress key regulators such as myogenic differentiation 1 (MyoD1), leading to increased muscle cell death and decreased contractile function [46]. During muscle repair, ROS damage satellite cells, reducing their ability to proliferate and regenerate tissue. Furthermore, oxidative stress can contribute to muscle atrophy or fat infiltration in older adults. ROS promotes adipogenic differentiation of muscle-associated cells through NF-κB activation and the upregulation of proteins such as S100 calcium-binding protein B (S100B), shifting cell fate from muscle to fat [47]. It has been reported that MA exhibits an antioxidant effect and can mitigate oxidative damage caused by oxidative stress [48]. MA primarily prevents the generation of oxidative stress induced by excessive H_2O_2, thereby reducing ROS production [48], inhibiting low-density lipoprotein (LDL) peroxidation and suppressing nitric oxide (NO) production induced by lipopolysaccharide (LPS) [42]. However, it is worth noting that although MA possesses antioxidant properties, high doses can lead to an increase in reactive oxygen species

in cells, further inducing cell damage and apoptosis [49]. This observation is consistent with the findings of the present study, which demonstrated that concentrations above 100 mg/mL of DP containing 0.09% MA resulted in more than an 80% reduction in cell viability.

However, to verify MA's capacity to safeguard muscle cells from ROS-induced damage, its cytoprotective effects were evaluated. The cytoprotective effects of the tested compounds were assessed based on the percentage of cell viability maintained under oxidative stress**,** reflecting their ability to prevent or reduce ROS-induced cell damage. The experimental results showed that C2C12 cells incubated with 60 μM H_2O_2 to induce oxidative stress exhibited a significant decrease in cell viability compared to the control group without H_2O_2 treatment ($p < 0.05$). In contrast, the addition of vitamin C at a concentration of 50 μg/mL together with 60 μM H_2O_2 significantly enhanced cell viability to 91.19%, indicating a strong cytoprotective effect. When assessing the cytoprotective potential of DP at concentrations of 10, 25, and 50 mg/mL on muscle cells, DP at 10 mg/mL did not significantly improve cell viability compared to the H_2O_2-treated group ($p > 0.05$). DP at concentrations of 25 and 50 mg/mL, on the other hand, showed significant cytoprotective effects, with cell viability percentages of 75.50% and 91.46%, respectively, compared to the H_2O_2 group ($p < 0.05$), as shown in Figure 3. Notably, the effect of 50 mg/mL DP was not significantly different from that of the positive control (vitamin C) ($p > 0.05$), suggesting comparable cytoprotective efficacy. To further confirm DP's cytoprotective properties, it was compared with a commercial protein product (CM) lacking added MA, using the same evaluation method. The results demonstrated that DP exhibited significantly greater cytoprotective effects at the same concentration of 50 mg/mL ($p < 0.05$), as shown in Figure 3. These results indicate that DP has superior cytoprotective potential compared to CM.

These findings align with recent studies emphasizing the antioxidant and cytoprotective roles of MA in muscle and other cell types under oxidative stress. Previous research has demonstrated that MA can restore mitochondrial function impaired by damage, reduce excessive autophagy, and alleviate oxidative stress in cells. Notably, MA activates the adenosine monophosphate-activated protein kinase/sirtuin 1 (AMPK/SIRT1) signaling pathway, a crucial regulator of these processes, particularly under conditions suppressed by carbonyl cyanide m-chlorophenyl hydrazone (CCCP), a mitochondrial damaging agent. MA exerts high efficacy in these conditions [41]. Additionally, Uemichi et al. [50] reported that MA, a pentacyclic triterpene, promotes phosphorylation of ribosomal protein S6 (rpS6) via the mechanistic target of rapamycin (mTOR) signaling pathway in skeletal muscle following acute resistance exercise. This suggests that MA stimulates exercise-induced protein synthesis and translation. Coupled with its potent anti-inflammatory and antioxidant effects, MA shows promise as a functional supplement to enhance muscle hypertrophy for both athletic performance and general health. Taken together, these studies support the present findings indicating that DP enriched with MA significantly improves muscle cell viability under oxidative stress. Nonetheless, further studies using animal models or clinical trials are warranted to fully validate these cytoprotective effects and clarify the underlying mechanisms.

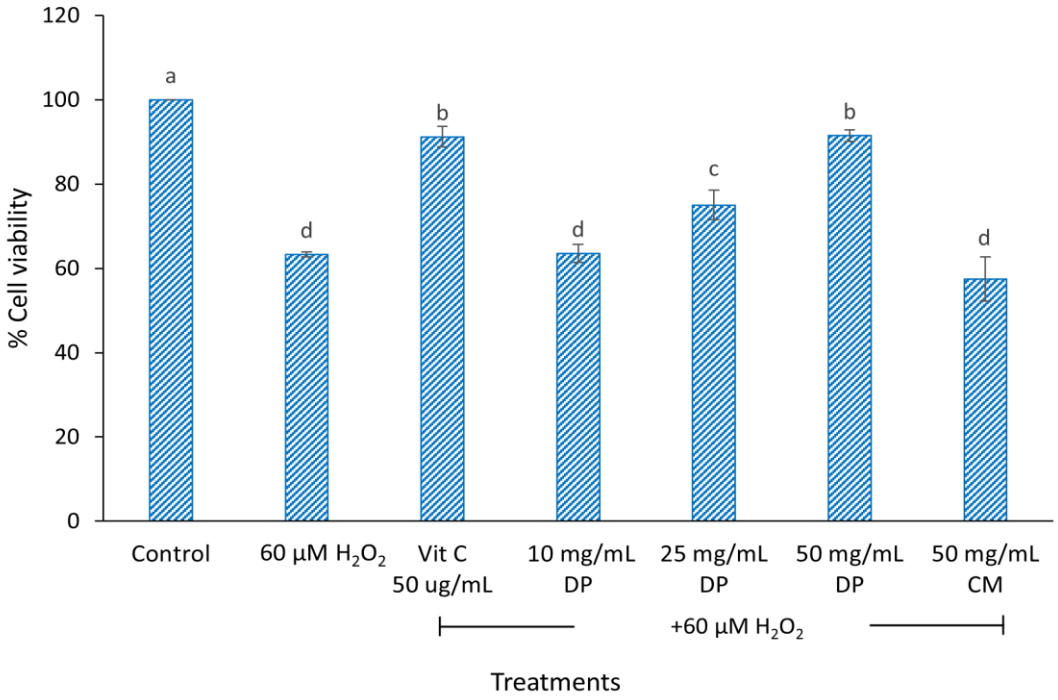


Figure 3. Cytoprotective effects of DP after 24 h of treatment. C2C12 cells were incubated with or without DP at concentrations of 10, 25, and 50 mg/mL. A commercial product (CM, 50 mg/mL) was used as a comparative control, ascorbic acid (Vit C, 50 µg/mL) was used as a positive control, and H_2O_2 (100 µM) was used to induce oxidative stress. Data are presented as mean ± standard deviation from three independent experiments (n = 3). Different letters above bars indicate statistically significant differences ($p < 0.05$), as determined by one-way analysis of variance (ANOVA) followed by Duncan's post-hoc test.

Interestingly, recent studies report that MA can inhibit the growth of HT29 human colon cancer cells by causing cell cycle arrest at the G0/G1 phase, the resting stage before cells enter the division process, thereby suppressing cancer cell proliferation. Additionally, MA can induce apoptosis through the mitochondrial pathway regulated by the JNK-Bid proteins, in conjunction with activation of the p53 protein, which plays a crucial role in controlling cell growth and eliminating abnormal cells [51]. In Caco-2 colon cancer cells lacking p53, MA rapidly activates caspase-8 and caspase-3, followed by subsequent caspase-9 activation, while Bax protein levels remain unchanged. These findings suggest that MA exerts anti-cancer effects through both intrinsic mitochondrial and extrinsic apoptotic pathways. Based on these findings, the present study developed a product that combines MA with inulin powder, a well-known prebiotic dietary fiber. Inulin selectively stimulates the growth of beneficial gut microbiota, such as *Bifidobacterium* and *Lactobacillus* spp., resulting in the production of short-chain fatty acids (SCFAs), including butyrate, acetate, and propionate [52–54]. These SCFAs exert anti-inflammatory effects, enhance gut barrier integrity, and have been linked to suppression of colorectal cancer progression [17]. Although these effects were not directly tested in the present study, they are plausible based on established mechanisms and prior evidence. Thus, the formulation developed herein offers a synergistic dual mechanism: MA directly inhibits colon cancer cell proliferation and induces apoptosis, while inulin supports gut microbiota modulation and SCFA production, collectively promoting colon health and immune regulation. This represents an intriguing area for further research and holds promise for future studies to explore its full potential and therapeutic applications.

Scientific Innovation And Practical Implications: This study provides novel insights into elderly nutrition through the development of a protein drink combining MA and cricket protein. The formulation takes advantage of the antioxidant and cell-protective properties of MA, along with the high-quality protein from crickets, representing a unique advancement in functional foods for older adults, consistent with definitions and guidance from the Functional Food Center (FFC). By delivering bioactive compounds and essential nutrients in an accessible form, the drink demonstrates its potential application in nutritional programs and dietary strategies to support long-term health.

CONCLUSIONS

A protein drink containing MA for the elderly with chocolate flavor effectively masks any undesirable odors from the cricket protein. The product features a fine powder texture and can be dissolved in water, with high nutritional value containing 58.79% protein. It includes inulin as dietary fiber and prebiotic, which may contribute to gut microbiota regulation. The developed product exhibits antioxidant properties, helping protect muscle cells from oxidative damage by reducing ROS production. Microbiological safety tests and sensory evaluations showed favorable results, indicating the product's suitability for commercial development as a nutrient-dense option for the elderly.

Abbreviations: MA, maslinic acid; ML, milliliter; Thai RDI, Thai Recommended Daily Intakes; Kcal, kilocalorie; CFU, colony-forming unit; ROS, reactive oxygen species; SCFAs, short-chain fatty acids; AMPK/SIRT1, adenosine monophosphate-activated protein kinase/ sirtuin 1; CCCP, carbonyl cyanide m-chlorophenyl hydrazone; rpS6, ribosomal protein S6; mTOR, the mechanistic target of rapamycin.

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