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Research Article

Development of A Plant-Based Meat Product Supplemented With Red Amaranth Antioxidants For The Elderly

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ABSTRACT

This study was aimed at developing a protein-rich food formulation for the elderly using ingredients derived from soybean, sacha inchi, wheat flour, quinoa, and perilla seed. First, the protein content of all ingredients was analyzed. The results showed that the highest protein level (48.54%) was seen in sacha inchi. Then, sensory test by elderly adults was evaluated. The formulation which had the highest sensory acceptance comprised 33% soybean, 40% sacha inchi, 20% wheat flour, 5% quinoa, and 2% perilla seed. The effect of stabilizers (xanthan gum and sodium alginate) at levels of 0.1 and 0.2%, respectively, was studied. It was found that 0.1% sodium alginate produced the highest sensory score. Measurements of the texture and water absorption of the formulation showed that the values for hardness, cohesiveness, springiness, adhesiveness, chewiness and gumminess were 1003, 0.25, 0.45, 0.17, 110, and 222, respectively, while water absorption was 51.10%. An aqueous extract of *Amaranthus dubius* was subjected to analysis of levels of polyphenols and anthocyanins, as well as antioxidant capacity and cytotoxicity. The aqueous extract had polyphenol, anthocyanin, DPPH radical-scavenging and FRAP levels of 41.13 µg GAE/mL, 458 mg/L, 62.7%, and 14.8 µg Trolox/mL, respectively. At a concentration of 2000 µg/mL, the crude extract exerted 22% average anti-proliferative effect on P388, KB, Hela and HepG2 cells. Different extract levels were added to the product, and the acceptability of the concentrated extract was re-evaluated. The results showed that the concentrated extract at 0.5% level of incorporation had the highest acceptance rating as a meat analogue. The energy per 100 g of the plant meat sample was 247.95 kcal, while its contents of protein, fat, carbohydrate, ash, and dietary fibre were 24.71, 6.27, 23.17, 2.52 and 6.76%, respectively. Thus, the plant meat product supplemented with *A. dubius* extract could be an alternative and safe diet for the elderly.

Key words: *Amaranthus dubius*, antioxidant, elderly, plant-based meat, red amaranth

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INTRODUCTION

The World Health Organization (WHO) has reported that the number of elderly people is continuously increasing and that by 2030, the elderly population will increase to 1.4 billion, and will be doubled to 2.1 billion in 2050. During aging, physical changes occur, resulting in chronic disease, hearing impairment, and visual problems (American Psychological Association, 2017). Moreover, aging is associated with reductions in chewing and swallowing abilities (Hammond *et al*., 2004). Elderly individuals have decreased functional teeth and decreased number of teeth, resulting in slower swallowing and dysphagia which make it difficult for them to chew and swallow (Kshetrimayum, 2013). One basic treatment strategy for dysphagia is a textural modification of foods (Icht *et al*, 2018). Therefore, one important challenge for food scientists and food creators is the design of food textures suitable for the elderly. Moreover, nutrition plays an important role in the health and functionality of elderly people. Several studies have identified proteins as key nutrients for elderly adults. The benefits of increased protein intake include improved muscle function and the prevention of chronic diseases which enhance the quality of life in healthy elderly people (Wolfe, 2012). In addition to the consumption of macronutrients, foods supplemented with vitamins, minerals, and bioactive compounds are of huge health benefits for elderly people (Calligaris *et al*., 2022).

Meat-like alternatives may be classified as plant-based (e.g., soy, pea, gluten, etc.); cell-based (*in vitro* or cultured meat), and fermentation-based (mycoproteins) (Sha, 2020). Newer developments in plant-based protein products include other plant protein sources, for example, microalgae protein extracts (Soto-Sierra, 2018). Plant-based meat alternatives have been developed in a variety of products in response to the growing demand by consumers and the sustainability of future food supply, and the market has grown exponentially in recent years. The market for meat substitutes was valued at USD 10.10 billion in 2018, and its value is expected to reach USD 30.9 billion by the year 2026 (Watson, 2019). Meat consumption may be reduced by consumers for many reasons such as climate change, health reasons, and new novel food trials (Collier, 2021). However, plants are not as high in nutritive value as meat, concerning essential amino acids (Gorissen *et* al., 2018). Therefore, it is necessary to use a variety of grain-based raw materials to boost levels of protein, amino acids, and other nutrients in these raw materials. A study of meat analogs has been carried out on sustainable products, not only concerning nutritive values, but also in terms of appearance, smell, taste, and texture, to satisfy consumer needs (Kyriakopoulou, 2019).

The development of protein-rich plant-based foods has largely been limited to re-structured products such as meats. Due to their structural characteristics, myofibrillar proteins in meat play a dominant role in tenderness and juiciness. In plant-based alternative products, carbohydrate polymers or hydrocolloids are generally needed to improve texture consistency, bind water, improve rheology, and reduce syneresis (Sha & Xiong, 2020). Extrusion is the most commonly used commercial technique for the transformation of plant-based materials into fibrous products. The process is divided into two classes of structuring with extrusion: low-moisture structuring and high-moisture structuring. Low-moisture (<50%) structuring produces dry and slightly expanded products which gain moisture thereafter, while in the high-moisture category, the fibrous products are produced with moisture contents above 50%. During extrusion, proteins are denatured, unfolded, and cross-linked due to shearing, as well as heat and pressure in the barrel (Zhang *et al*., 2019).

Antioxidants protect both foods and pharmaceutical products against oxidative stress. *Amaranthus dubius,* also known as red Amaranth, is rich in polyphenols which are strong antioxidants due to their high concentrations of betacyanin and phenolic compounds (Khandaker & Oba, 2008). The plant has been used in traditional medicine. *In vivo* studies on *Amaranthus dubius* have revealed that it has anti-inflammatory, anti-helminthic, and antioxidant properties (Iftikhar & Khan, 2019). The incorporation of bioactive compounds into food formulations is necessary due to their antioxidant properties and beneficial effects. The objective of this research was to formulate a new functional plant-based meat product using protein ingredients (soybean, sacha inchi, wheat flour, quinoa, and perilla seed); different hydrocolloids (sodium alginate, xanthan gum), and antioxidants, for enhancing immunity and preventing disease in the elderly population while serving as an alternative food for them.

MATERIALS AND METHODS

Materials and preparation

Soybean, sacha inchi, and perilla seed were purchased from Chiang Rai Province (Thailand). Quinoa seed was bought from Healthy Choice Asia Company (Thailand). Wheat flour was purchased from United Flour Mill Public Co. Ltd., (Thailand). Sodium alginate and xanthan gum were bought from TCS Pacific Company (Thailand). *Amaranthus dubius* was obtained from Prince Chakraband Pensiri Center for Plant (Chiang Rai Province). The seasoning powder was the product of Four Foods Co. Ltd. (Thailand). All materials were washed and oven-dried at 50 ± 1 °C for 24 h or until dry. Thereafter, whole materials were ground into flour using a hammer mill. Sacha inchi was pressed to separate the oil before use, and the residue was dried and ground.

Preparation of plant-based meat

Four formulations are shown in Table 1. All formulations had the same levels of wheat flour, quinoa flour, and perilla seed, i.e., 20, 5 and 2 %, respectively. However, the formulations differed in the amounts of soybean and sacha inchi flour. Salt and sugar at the level of 0.5% were added to all formulations. The components were weighed and mixed, and water was added to adjust the moisture content to about 50%, followed by blending and kneading for about 5 min until it formed a dough. Each formulation was transferred into a single screw extruder machine. The conditions under which extrusion was done were as follows: the temperatures of barrels 1, 2, 3, and died were 60, 120, 160, and 160 °C, respectively, and the screw speed was 130 rpm.

Preparation of crude extract

The stem and leaves of *A. dubius* were washed and blanched at 90 °C for 30 s, and drained and dried in a tray dryer at 50 °C until dry. Thereafter, the dried samples were finely ground using an ultracentrifuge, followed by extraction with distilled water at the ratio of 1:10 (20 g of dried plant sample and 200 mL of water). Each mixture was extracted with microwave at 350 W for 1 min, after which it was filtered. The filtrate was freeze-dried. Finally, the dried extract was solubilized at a concentration of 2000 μg/mL and kept frozen at -35 °C before analysis.

Proximate analysis

The protein contents of the raw materials i.e., soybean, sacha inchi, quinoa, wheat flour, and perilla seeds were determined according to the method of AOAC (2000). Nitrogen content was determined using the Kjeldahl digestion method, and protein content was obtained by multiplying the percent nitrogen with a factor of 6.25. The plant-based meats were analyzed concerning protein, fat, ash, and dietary fiber using the procedure of AOAC (2019).

Table 1. Protein contents of plant samples and the formulations of plant-based meat

Materials	Protein content (%)	Formula 1 (%)	Formula 2 (%)	Formula 3 (%)	Formula 4 (%)
Soybean	30.37±0.45	23	33	43	53
Sacha inchi	48.54±0.28	50	40	30	20
Wheat flour	13.61±0.01	20	20	20	20
Quinoa	13.35±0.05	5	5	5	5
Perilla seed	23.73±0.02	2	2	2	2

Phytochemical composition and antioxidant capacities

Polyphenols

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method as modified by Singleton *et* al. (1999). The extract was diluted with distilled water. Then, 0.5 ml of extract was put in a test tube and adjusted to a volume of 10 mL with distilled water. Folin–Ciocalteu reagent (0.5 mL) was added and incubated at room temperature for 5 min, after which 2 mL of 10 % sodium carbonate was added, with mixing. After incubating in the dark for 10 min, the absorbance of the solution was measured at 760 nm. The TPC level was expressed as milligrams of gallic acid equivalent (GAE)/mL of extract.

Anthocyanins

Total monomeric anthocyanin content was determined using the pH-differential method of Giusti & Wrolstad (2001). In this procedure, 50 g of dried plant sample was mixed with 150 mL of distilled water, shaken for 1 h, and filtered. The residue was exhaustively re-extracted until the extracted color faded. The extracts were pooled, filtered, evaporated at 40 °C, and diluted with water, before analysis. Two sample solutions were prepared. Both samples were dissolved in 0.025 M potassium chloride buffer, pH 1.0, and 0.4 M sodium acetate buffer, pH 4.5. The absorbance values of the solutions were read at 449.2 and 700 nm, with distilled water as blank. The total monomeric anthocyanin was calculated as shown in equations 1 and 2.

Equation 1:

$$\text{Absorbance} = (A_{449.2} - A_{700})_{\text{at pH 1.0}} - (A_{449.2} - A_{700})_{\text{at pH 4.5}}$$

Equation 2:

$$\text{Anthocyanin content} = \left(\frac{A \times MW \times DF \times 1000}{\varepsilon \times 1} \right)$$

Where *MW* is the molecular weight of cyanidin-3-glucoside (449.2), *DF* is the dilution factor, and ε is the molar extinction coefficient (26,900).

DPPH radical scavenging assay

The DPPH radical scavenging assay was done as described by Brand-Williams *et al*., (1995). In this assay, 3 mL of 0.2 mM DPPH was added to 1 mL of extract in a tube and kept in the dark place at room temperature for 30 min. The control tube contained a mixture of 1 mL of ethanol and 3 mL of DPPH. The absorbance of each tube was measured at 517 nm, and the percentage inhibition of DPPH radical was calculated as shown in Equation 3.

Equation 3:

$$\text{DPPH radical scavenging (\%)} = \frac{Ac - As}{Ac} \times 100$$

Where Ac is the absorbance of the control, and As is the absorbance of the treated sample.

Ferric Reducing Antioxidant Power (FRAP)

The procedure described by Benzie and Strain (1996) was used for the determination of FRAP, with minor modifications. The extract (0.1 mL) was mixed with 3 mL of FRAP solution (acetate buffer + TPTZ + $FeCl_3$. $6H_2O$ (at a volume ratio of 10:1:1) in a tube. The mixture was kept in the dark for 8 min, after which absorbance was measured at 595 nm using a spectrophotometer. The results were expressed as milligrams of Trolox equivalence /mL of extract.

MTT proliferation assay

Cell viability was determined with MTT assay as described by Mosmann (1983), with a slight modification. Human cervical adenocarcinoma (Hela), human hepatocellular carcinoma (HepG2), human oral cavity carcinoma (KB), and murine leukemia (P388) were seeded separately in 96-well plates, each at a density of 1 x 10^5 cells/well, and were incubated at 37°C in a 5% CO_2 incubator for 24 hr. The extract was put in each well at a final concentration of 2,000 µg/mL. Cells treated with 1% DMSO were used as a negative control. Untreated cell cultures (control) and blank wells without cells containing 100 µL of medium were incubated for 20 hr. Thereafter, 10 µL of MTT reagent (5 mg/mL) was added to each well and incubated for 4 hr, after which 100 µL of a mixture of 100% DMSO and 10% sodium dodecyl sulfate (SDS; volume ratio: 9:1) was added to the wells, followed by shaking to solubilize the formazan crystal formed. Absorbance was measured at 570 nm. Percentage cell viability was calculated as an index of cytotoxicity. The percentage cytotoxicity was determined as shown in Equation 4.

Equation 4:

$$\text{Cell cytotoxicity (\%)} = \frac{100 - As - Ab}{Ac - Ab} \times 100$$

Where *As*, *Ab,* and *Ac* are the absorbance values of the treated sample, blank sample, and control sample, respectively.

Quality evaluation

Texture analysis

Plant-based meat was cut to width and height of about 20 and 10 mm, respectively. The trigger was 2 g, the deformation was 4 mm, and the test speed was 0.5 mm/sec. A cylinder probe was used. Texture profile analysis (TPA) was done with Texture Analyzer (Brookfield Ametek, CTX). The following parameters were determined: hardness (g), cohesiveness, adhesiveness (mJ), springiness, chewiness (g), and gumminess (g).

Water absorption

Water absorption was measured according to the AACC (2010) methods 66-50 with some modifications. The plant-based meat was boiled (5 g with distilled water 100 mL) at about 85°C for 30 sec, cooled for 1 min in cold water, drained for 1 min, and weighed. The percentage (%) of water absorption was calculated from the weights of samples before and after boiling, as shown in Equation 5.

Equation 5:

$$\text{Water absorption (\%)} = \frac{\text{Weight of sample after boiling - weight of sample before boiling}}{\text{Weight of sample before boiling}} \times 100$$

Morphological analysis

The microstructure of dried samples was analyzed using scanning electron microscopy (SEM). Samples of about 1 × 1 cm in size were placed on aluminum stubs containing carbon tape. The samples were analyzed with SEM (Quanta250, FEI) in high vacuum mode using a secondary electron detector: SED). The SEM was set at an ETV target of about 10-15 keV, the spot size of about 2-4, and a working distance of about 5-10 mm, with magnifications of 150x and 1000x.

Sensory evaluation

The samples were prepared about 0.5 × 1 cm. The elderly people were tested only on plant-based meat first. Then, it was added to seaweed-flavored porridge to asses overall preference. Each formulation which was assigned a three-digit code was served in random order to a panel of 30 untrained elderly people aged 60-70 years who were required to assess differences in taste. Acceptance of the meat by the elderly was assessed using a 9-point hedonic scale, with 1 standing for 'least liked', and 9 as 'liked most', concerning 5 characteristics such as color, odor, flavor, texture, and overall liking. Thereafter, the best formulation was chosen for use in a study for acceptable levels of suitable stabilizers (xanthan gum and sodium alginate) which were incorporated at levels of 0.1% and 0.2%. Sensory tests in the textural aspect were re-evaluated. Finally, plant extracts were added to the samples at the level of 0.25%, 0.5%, and 0.75%, and the final sensory test was performed.

Statistical analysis

Data are reported as mean ± standard deviation of duplicate determinations. A completely randomized experimental and randomized complete block design was used. Analysis of variance (ANOVA) was used for statistical analysis with IBM SPSS software, version 24 (IBM Singapore Pte. Ltd., Changi, Singapore). Values of $p<0.05$ indicated statistically significant differences.

RESULTS AND DISCUSSION

Protein contents of plant samples

The plant samples were analyzed for protein content, and the results are shown in Table 1. The highest protein content was seen in sacha inchi, followed by soybean, perilla, wheat flour, and quinoa. Kyaw et al. (2019) reported that sacha inchi contained high levels of protein (24.8g) and fat (42.30g). It is rich in omega-3 and omega-6 fatty acids. These fatty acids prevent various diseases such as cardiovascular disease, stroke, Alzheimer's disease, rheumatoid arthritis, inflammation, and cancer (Wakdikar, 2004). Quinoa seeds have high protein content (10 -17%), and the contents of essential amino acids such as lysine, methionine, and cysteine are higher than those of common cereals and legumes (Reyes *et al*., 2006). Apart from its high content of protein, quinoa is popular for its high protein quality (Repo-Carrasco-Valencia & Serna, 2011). Perilla seed is nutritious due to its high content of fat, protein, vitamins, minerals, and phytochemicals. Studies have shown that perilla seeds contain crude protein and total lipids levels of 25.38 ± 0.10 % and 42.27 ± 1.69%, respectively (Sargi *et al*., 2013). Perilla seed oil contains essential fatty acids. The major fatty acids of perilla oil are α-linolenic acid (omega-3) and linoleic acid (omega-6) (Joshi *et al*., 2015). The results obtained in analyses of the plant samples showed high protein content. Therefore, the plant samples are suitable for use in the development of plant-based meat products, especially for the elderly.

The outcome of sensory analysis

Four formulations of plant protein were served to 30 elderly people aged 60 -70 years. Focused group discussions and sensory tests were evaluated. Each formulation was evaluated in terms of color, odor, flavor, texture, and overall acceptance. Formulations 1 to 4 had different levels of soybean and sacha inchi flours, whereas the levels of quinoa, perilla seed, and wheat flour were similar. Formula 1 had the highest content of sacha inchi, while it had the lowest content of soybean flour. Formula 4 had the most soybean flour and the lowest level of sacha inchi flour. The results are presented in Table 2. Almost all elders had a preference for formulation 2 in terms of flavor and texture, and it gave the highest score in overall acceptance. This formula contained soybean flour, sacha inchi flour, wheat flour, quinoa, and perilla seed at levels of 33, 40, 20,5, and 2%, respectively. The elderly participants felt that this formula had a more suitable texture than any of the other formulations. In a study conducted by Sreeitthiyawet *et* al. (2019), it was found the most suitable formula for producing meat analog was 37.5% soybean flour, 40% white kidney bean, and 22.5% Jerusalem artichoke flour. Increasing the protein content to a higher level resulted in a hard texture and reduced elasticity, while too little protein content resulted in a very soft-textured product that did not form a fibrous network. It has been demonstrated that protein and carbohydrate contents of raw materials affect the fibrous structure and texture of the supplemented meat analog product because they are the critical components for the formation and mimicking of fibrous meat texture in the products (Sheard *et al*., 1984). In addition, soybean protein contains abundant sulfur amino acids which further contribute to the formation of disulfide bonds and enhancement of the formation of the fibrous network (Lee *et al*., 2022). Moreover, wheat protein forms a three-dimensional network structure maintained by disulfide bonds, which not only maintains the fibrous structure of meat but also increases the viscoelasticity and hardness of the product while improving juiciness, water retention, and color (Samard *et al*., 2019).

Quality analysis

There are several ways of mimicking the texture of meat. Food hydrocolloids are used as alternative materials for improving texture and achieving optimal binding properties to create a fibrous structure. The texture attributes of two different hydrocolloids (sodium alginate & xanthan gum) were evaluated by the elderly, and the results are shown in Table 3. It was found that most of the elderly people were satisfied with the plant-based meat containing 0.1% sodium alginate. They stated that the sample containing more xanthan gum made the texture too soft and gummy, making it stick to the tooth. In contrast, sodium alginate produced a firm texture that was easy to chew and swallow.

Plant-based proteins with different hydrocolloids were subjected to texture profile analysis using a texture analyzer. The results are shown in Table 4. The hardness, cohesiveness, adhesiveness, springiness, chewiness, and gumminess of the samples were determined. Hardness is a measure of the maximum force of the first compression. Cohesiveness refers to how well a food retains its form between the 1st and 2nd chews. Adhesiveness is the work/force necessary to overcome the attractive forces between the surface of the product and the surface of the material. Springiness shows the capacity of the product to return to its original structure after the first compression. Chewiness shows how much work is needed to chew the product to achieve a suitable texture before swallowing. Gumminess is the energy required to disintegrate a semi-solid food into a state ready for swallowing. An increase in hydrocolloid concentration increased the hardness of the samples. Arora *et al*. (2017) reported that as the level of the thickening agent increased, there were proportional increases in values of hardness, chewiness, gumminess, and compression. Texture profile analysis (TPA) is related to sensory characteristics. However, food for elderly individuals should not be too hard or adhesive. Instead, it should be soft and moist, making it easier to break down fibers with minimal chewing effort. For example, xanthan gum resulted in a formulation that was too soft for the elderly, when compared to sodium alginate which had higher hardness.

The structures of the plant-based meat formulations were studied using a scanning electron microscope at 150x and 1000x magnifications. The results are shown in Figure 1. In Figure 1A, the structure of the sample without hydrocolloid (control) showed adhesion of the mixture and some amount of porosity. In Figure 1B, the structure of the sample containing 0.1% xanthan gum was not different from that of the control but the cohesion of the mixture was more pronounced, and it had more adhesiveness and cohesiveness than that of the control. In Figure 1C, the structure of the sample containing 0.1% sodium alginate was firm and tightly packed together, making it look like a sheet. Thus, hydrocolloids were effective in binding the components and enhancing water absorption (Table 4). Sodium alginate is a widely used binding agent in food processing, and the structuring and water-binding properties (Mousa, 2016). Lee & Hong (2020) reported that sodium alginate modified textural and enhanced water-binding properties in soy-based meat analogs.

Table 2. Sensory characteristics of plant-based meat with different ingredients content

Formulations	Color	Odor	Flavor	Texture	Overall acceptance
1	7.50^{a}±0.12	7.45^{a}±0.13	7.05^{b}±0.92	7.15^{b}±0.53	7.62^{b}±0.98
2	6.00^{b}±0.09	6.00^{b}±0.25	8.00^{a}±0.19	8.00^{a}±0.44	8.29^{a}±1.10
3	6.45^{b}±0.20	6.15^{b}±0.14	6.60^{c}±1.10	7.00^{b}±0.63	7.55^{b}±1.15
4	7.00^{a}±0.54	7.00^{a}±0.32	7.00^{b}±1.04	8.00^{a}±0.47	7.19^{c}±1.34

$^{a-c}$ Different letters within the same column indicate significant differences (p<0.05)

Table 3. Sensory scores of the elderly on plant-based meat with different hydrocolloids contents

Formulations	Texture score
0.1 % xanthan gum	7.25^{b}±1.10
0.2% xanthan gum	6.50^{c}±1.67
0.1% sodium alginate	8.00^{a}±0.08
0.2% sodium alginate	7.69^{b}±1.35

$^{a-c}$ Different letters within the same column indicate significant differences (p<0.05)

Table 4. Texture profiles and water absorption of plant-based meat with different hydrocolloid contents

Samples	Hardness (g)	Cohesiveness	Adhesiveness (mJ)	Springiness	Chewiness (g)	Gumminess (g)	Water Absorption (%)
Control (without hydrocolloid)	$1944^{d}\pm102$	$0.10^{a}\pm0.01$	$0.04^{a}\pm0.02$	$0.50^{b}\pm0.01$	$292^{d}\pm2$	$278^{d}\pm5$	$45.93^{a}\pm0.25$
0.1% xanthan gum	$561^{a}\pm27$	$0.20^{b}\pm0.02$	$0.06^{b}\pm0.02$	$0.35^{a}\pm0.06$	$61^{a}\pm7$	$112^{a}\pm11$	$51.37^{b}\pm0.37$
0.2% xanthan gum	$598^{b}\pm22$	$0.30^{d}\pm0.06$	$0.09^{b}\pm0.01$	$0.70^{c}\pm0.14$	$129^{b}\pm18$	$177^{b}\pm17$	$52.52^{b}\pm0.16$
0.1% sodium alginate	$1003^{c}\pm114$	$0.25^{c}\pm0.06$	$0.17^{c}\pm0.02$	$0.45^{b}\pm0.05$	$110^{b}\pm15$	$222^{c}\pm15$	$51.10^{b}\pm0.45$
0.2% sodium alginate	$1154^{c}\pm117$	$0.20^{b}\pm0.02$	$0.23^{d}\pm0.01$	$0.55^{b}\pm0.12$	$126^{b}\pm20$	$245^{c}\pm20$	$51.93^{b}\pm0.51$

[a-d] Different letters within the same column indicate significant differences (p<0.05)

Phytochemical and antioxidant capacities

Results of the determination of phytochemical and antioxidant capacities are shown in Table 5. Red Amaranth extract was rich in anthocyanins, and it had high DPPH radical scavenging activity (458 mg/L & 62.7%). Nyonje *et* al. (2014) found that the methanol extracts of the amaranth varieties showed potential free radical scavenging activity against DPPH (57.7-64.2%.) Anthocyanin groups are responsible for the red or blue pigments in the leaves, flowers, fruits, stem, root and tubers of amaranth, and the color depends on pH: the color appears red in acidic pH, and blue in alkaline pH (Khoo *et al*., 2017). Anthocyanins are good antioxidants that prevent or reduce the risk of diseases. It is well-known that anthocyanins are strong antioxidants that effectively scavenge free radicals, thereby reducing oxidative stress. The health benefits of anthocyanins include antioxidative effects, anti-angiogenesis, CVD-preventing, anti-cancer, anti-diabetes, visual health-improving, anti-obesity, antimicrobial, and neuroprotective properties (Wang *et al*., 2009).

MTT proliferation assay

Four cell lines i.e., human cervical adenocarcinoma (Hela), human hepatocellular carcinoma (HepG2), human oral cavity carcinoma (KB), and murine leukemia (P388) were treated with the crude extracts. The cytotoxic effects of *Amaranthus dubius* extract at a concentration of 2000 µg/mL on these cell lines were similar (20.36, 21.32, 22.19, & 23.92%, respectively). Treatment of the cells with the extract resulted in the formation of smaller amounts of formazan crystals, when compared with the control to which no extract was added, indicating that it inhibited cell proliferation due to the toxicity produced by the phytochemicals in the extract (Figure 2). This effect may be due to the high levels of anthocyanins in the extract. Anthocyanins have been extracted and isolated from different plant sources, and their anticancer potential has been investigated. They have chemo-preventive potential, and they inhibit cell proliferation, inflammation, and angiogenesis while inducing apoptosis (Wang *et al*., 2009). However, the extract may have a strong inhibitory effect but this may be due to the reaction of the extract with media. Plant extracts rich in anthocyanins are sensitive to pH. After treating the cells with the extract, the medium changed to blue color, resulting in high optical density. However, MTT is the standard method for initial toxicity assessment. In subsequent studies, these results will be confirmed using other methods such as DNA fragmentation and apoptosis.

The study of the consumer acceptance of the supplemented *A. dubius* extract

Dried *A. dubius* extract was added to plant-based meat at levels of 0.25, 0.5, and 0.75%. Then, water was added, and the whole components were mixed and fed into a single screw extruder. The extrudates are shown in Figure 3. A high concentration of extract led to a high degree of pink color. Plant-based meat containing 0.25% extract had a very light pink color, while a sample containing 0.75% extract had a pronounced pink color.

Finally, plant-based meat containing different extract concentrations was assessed by the elderly. The results are shown in Table 6. The elderly gave the highest score to plant-based meat containing 0.5% extract. The elders said that the extract was not too strong or too light in color. Although an increase in food color indicates the presence of important substances that are good for health, if the color was too dark, the elders saw it as different from normal food. The elders need healthy food nutrients, but the food should not be too outstanding in color.

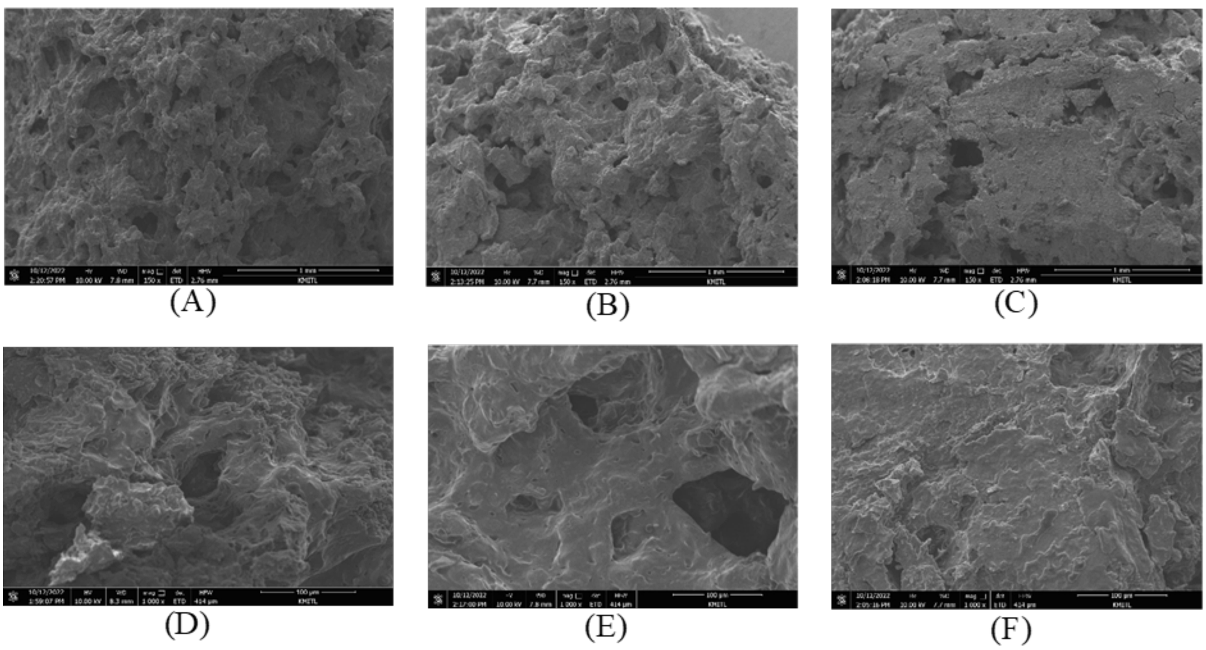

Fig. 1. Structures of plant-based meat formulations as seen using under scanning electron microscope (SEM) 150x; No adding stabilizer (A); Adding xanthan gum 0.1% (B); Adding sodium alginate 0.1% (C), 1000x: No adding stabilizer (D); Adding xanthan gum 0.1% (E); Adding sodium alginate 0.1% (F)

Table 5. Phytochemical and antioxidant capacities of Red Amaranth

Polyphenols (µg GAE/mL)	Anthocyanin (mg/L)	DPPH inhibition (%)	FRAP (µgTrolox/mL)
41.13±0.52	458±0.22	62.7±0.14	14.8±0.08

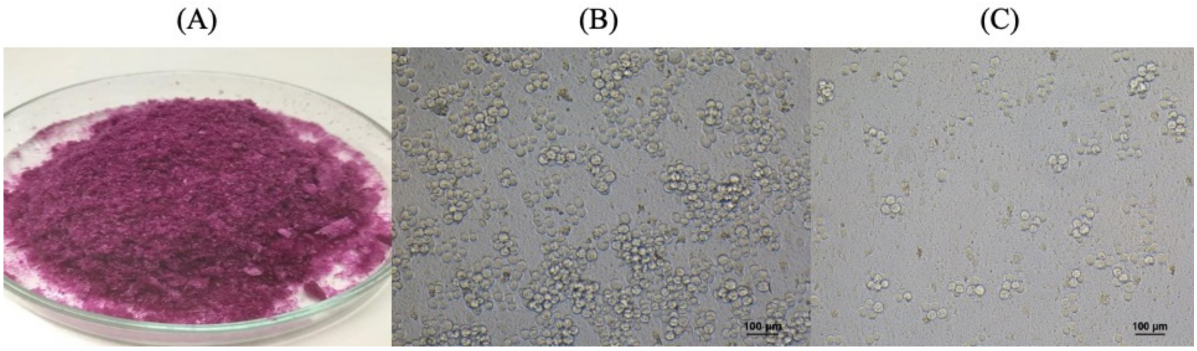

Fig. 2. *A. dubius* extract powder (A); P388 cells (Control) (B); P388 cells treated with the extract (C).

Chemical composition analysis

The chemical composition and energy of the plant-based meats containing red *Amaranth* were analyzed. The energy per 100 g of the plant meat sample was 247.95 kcal, while its contents of protein, fat, carbohydrate, ash, and dietary fiber were 24.71, 6.27, 23.17, 2.52, and 6.76%, respectively. The results show that the food for the elderly had high protein and dietary fiber content. In addition, Mithila & Khanum (2015) found that quinoa and *Amaranth* were effective in improving blood glucose response and maintaining plasma-free fatty acids (FFA) and general lipid profiles in rats. Additionally, they were effective in combating the growth in obesity and poverty, indicating that they were suitable for the elderly.

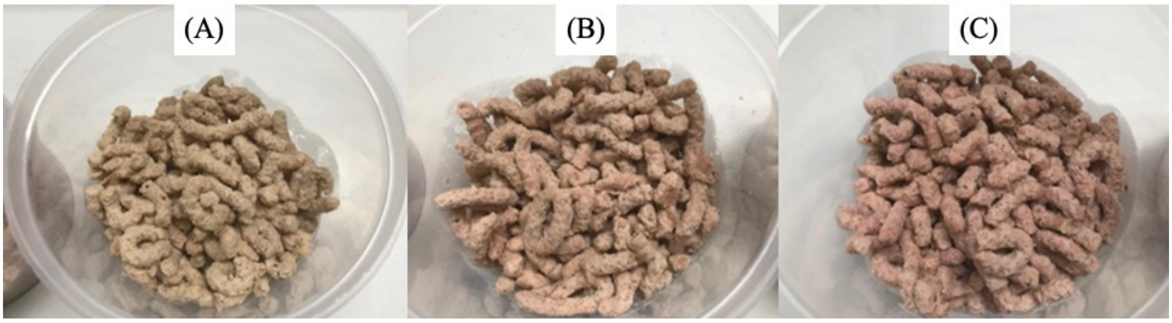

Fig. 3. Plant-based meat after extrusion with extruder machine. Meat containing 0.25% *A. dubius* extract (A); meat containing 0.5% *A. dubius* extract (B); and meat containing 0.75% *A. dubius* extract (C)

Table 6. Sensory score of elderly on plant-based meat with a different extract content

Formulations	Color	Odor	Flavor	Texture	Overall acceptance
0.25% Extract	7.50^{b}±0.26	7.45^{a}±0.18	8.05^{a}±0.51	7.65^{a}±0.23	8.10^{b}±0.67
0.5% Extract	8.50^{a}±0.14	7.60^{a}±0.24	8.20^{a}±0.34	7.87^{a}±0.46	8.85^{a}±0.02
0.75% Extract	7.00^{b}±0.18	7.40^{a}±0.33	7.60^{a}±0.28	7.55^{a}±0.37	7.70^{c}±0.22

$^{a-c}$ Different letters within the same column indicate significant differences (p<0.05)

CONCLUSION

The design of food for the elderly takes into account not only nutrient levels but also chewing and swallowing concerns at different ages. In this study, the levels of components of plant-based meat such as soybean flour, sacha inchi flour, wheat flour, quinoa, and perilla seed were 33, 40, 20, 5, and 2%, respectively. These levels were found suitable for designing food for the elderly. The addition of a low amount of sodium alginate had a positive effect on the structure of the plant-based meat. The plant-based meat had high protein and dietary fiber contents and contained high levels of bioactive compounds from *A. dubius* extract. This alternative food is considered suitable for the elderly and health-conscious consumers and vegetarians.

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ETHICAL STATEMENT

This study was approved by the ethical committee of Chiang Rai Rajabhat University approval number COA**.**P2**-**006**/**2565

CONFLICT OF INTEREST

The authors declare no conflict of interest**.**

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