

Stability of Phytochemicals in Pumpkin Seeds and Sunflower Seeds Using Ethanol Extraction

Azmi Nurul-Umirah¹, Aida F. M. N. A¹, Razif Dasiman², Nazimah Hamid³ and Siva Raseetha¹

¹Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia

²Faculty of Health Sciences, Universiti Teknologi MARA Selangor, 42300 Puncak Alam, Selangor, Malaysia

³Department of Food Science and Microbiology, Auckland University of Technology, New Zealand

Pumpkin seeds (*Cucurbita moschata*) and sunflower seeds (*Helianthus annuus*) are underutilised as food in Malaysia. Benefits of seeds are often overlooked. This research showed that 95% ethanol extract of pumpkin seeds exhibited greenish colour, while 95% ethanol extract of sunflower seeds extract exhibited yellowish colour. Crude fat analysis (Soxhlet) contained $29.53 \pm 0.58\%$ in pumpkin seeds and $27.74 \pm 0.31\%$ in sunflower seeds. Crude protein was significantly higher in pumpkin seeds ($36.69 \pm 1.26\%$) compared to sunflower seeds ($12.03 \pm 0.14\%$). FTIR spectroscopy revealed functional groups such as hydroxyl, nitrile, nitric oxide in 95% ethanol extract for both seeds compared to 50% ethanol extracts and powdered forms. ICP-OES indicated that potassium was abundant (4408.20 ± 0.00 mg/100g) and copper least (3.42 ± 0.00 mg/100g) in pumpkin seeds. Sunflower seeds contained 3665.85 ± 47.46 mg/100g potassium and 6.78 ± 0.03 mg/100g copper. Alkaloid content was significantly higher in 95% ethanol extract of sunflower seeds (5.01 ± 0.10 mg/g) compared to 50% ethanol extract (4.60 ± 0.16 mg/g). Pumpkin seeds, 95% and 50% ethanol extracts contained 3.16 ± 0.10 mg/g and 3.04 ± 0.10 mg/g of alkaloids, respectively. The 95% ethanol extracts had higher tannin content, with pumpkin seeds containing 0.91 ± 0.008 mg CE/mL for pumpkin seeds and sunflower seeds (0.81 ± 0.003 mg CE/mL), compared to 50% ethanol extracts. This research aligns with sustainability approach and SDG 12 framework.

Keywords: *Cucurbita moschata*; *Helianthus annuus*; ethanol extraction; minerals; FTIR; colorimetry

I. INTRODUCTION

The pumpkin seeds market in Malaysia is valued at about 70 million and is projected to rise to USD 110 million by 2028 (ScopeGrid Analytics, 2025). Pumpkin is widely known for its fruit and seed, which can be eaten raw or processed to make baked goods, soups, purees, jams and pastries (Yang *et al.*, 2022). Singh and Kumar (2023) stated that every part of the pumpkin can be used. The edible part of a pumpkin consists approximately 78.69% flesh, 17.95% peel and 3.63% seeds. According to Mandal and Mishra (2020), pumpkin and sunflowers seeds are a nutrient-dense powerhouse. Super seeds are a plant-based source of important minerals and amino acids in vegetarian and vegan diet such as

calcium, zinc, copper and magnesium. Super seeds include pumpkin and sunflower seeds. The seeds have a rich, nutty flavour and chewy texture. Pumpkin seeds are consumed in roasted and salted forms, used in bakery industries and are gaining popularity as a healthier alternative to fried snacks. Pumpkin seeds also have been utilised for extracting edible oil since they contain a lot of minerals and bioactive compounds. They have a natural protein content of 25-37% and an oil content of 37-45%. Pumpkin seed is a good source of fibre and amino acids. Pumpkin seeds were proven to alleviate anaemia due to rich source of nonheme iron, especially among adults (Ray & Singh, 2023).

*Corresponding author's e-mail: raseetha@uitm.edu.my

Meanwhile, sunflower seeds have become more well-known as they are popular snack by Malaysians. In 2024, the revenue for sunflower seeds in Malaysia is USD 9.2 million (Data Bridge Market Research, 2024). The seeds are gaining increasing importance as an edible oil and protein source, with comparable nutritional quality to soybeans and other oilseed proteins (Tenyang *et al.*, 2021). Ray and Singh (2023) stated that sunflower seeds are high in polyunsaturated fatty acids (PUFAs) which can help lower the total cholesterol hence reducing the risk of cardiovascular disease. Rasheed *et al.* (2023) found that the combination of seeds (pumpkin and sunflower), contains high levels of trace minerals, antioxidants, omega 3 and omega 6 fatty acids, protein and carbohydrates and these nutrients support normal progesterone in females.

There is a need to utilise natural food sources that contain essential nutrients and bioactive compounds to promote a healthy lifestyle particularly in line with United Nation Sustainable Development Goals (SDGs) of Good Health and Well-Being (SDG3). COVID-19 have impacted daily lives of global population. Cross-sectional survey conducted in year 2020 indicated that 759 Malaysians adopted healthier eating habits (Ahmad *et al.*, 2022). Yuan *et al.* (2023) stated that older individuals consume plant-based diets can lower their risk of developing chronic illnesses such as diabetes, heart disease and stroke as well as reduce overall death rates.

Mounes *et al.* (2024) stated that pumpkin seed cake possesses strong antioxidative qualities and increased enzyme activity such as superoxide dismutase, catalase and glutathione peroxidase. According to Oh *et al.* (2024), pumpkin seed extract contributes an antioxidative role in cardiovascular disease, triacylglycerol accumulation in the body and dysfunction in energy generation by reducing oxidative stress brought on by menopause and aging. In cookie production, the combination of mocaflour and pumpkin flour (77.5%:22.5%) respectively, show greatest antioxidant activity by using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay (Anastasia Wheni Indrianingsih *et al.*, 2024). According to Gao *et al.* (2022), pumpkin seed meal is effective in creating a sturdy, flexible and edible texture with antioxidant properties. Pumpkin seeds are not only used in bakery products but can also be used in meat as preservatives. Improved sensory qualities was noted when 2%

crude pumpkin seed extract added into minced meat (Gadekar *et al.*, 2025). According to Abbas *et al.* (2024), sunflower sprouts have higher antioxidant activity than sunflower seeds due to the increased levels of total phenolic, melatonin and total isoflavones during the sprouting process.

Various health risks in Malaysia such as non-communicable disease as well as malnutrition could be addressed by implementing the plant seeds in daily diet (Aziz *et al.*, 2023; Patel *et al.*, 2024; Cheenam & Leena, 2016). Although pumpkin seeds and sunflower seeds are underutilised as food ingredients and functional food in Malaysia, they could offer solutions for these problems. However, there is very little research using these seeds in terms of supporting public health in Malaysia. This study contributes to promoting the consumption of underutilised seeds in Malaysia with their nutritional composition and potential therapeutic effects. These seeds can be further developed into functional food products or commercialisation of pharmaceutical products that can act as immunity booster for public health. This paper aims to determine the physical characteristics of pumpkin and sunflower seeds, their nutritional composition such as fat, protein, minerals analysed using ICP-OES and chemical characteristics such as alkaloids and tannins as well as functional groups using FTIR method. The efficiency of solvent extraction was studied using 95% and 50% ethanol as some studies stated mixture of alcohol and aqueous could improve the efficiency of extraction.

II. MATERIALS AND METHOD

A. Materials

Pumpkins (6 kg) were purchased from a local supermarket located at Seksyen 7, Shah Alam, Selangor, Malaysia. The fruits were peeled and the seeds were detached from its flesh. As for sunflower seeds, 1 kg of sunflower seeds were purchased from Jalan Timur Laut, Georgetown, Pulau Pinang, Malaysia.

B. Sample Preparation and Extraction

Firstly, the seeds were cleaned under running tap water to avoid surface contamination and rinsed. Then, the pumpkin and sunflower seeds were oven-dried at 60 °C for

approximately 4 hours. The dried pumpkin and sunflower seeds were ground into fine powder using an electric grinder (Khind, model BL1012, Malaysia) for 10 minutes and kept in an airtight plastic container for further analysis (Danbature *et al.*, 2013). Solvent extraction has been carried out using the maceration method described by Gowtham *et al.* (2022). Seed powder (5 g) extracted with 100 mL 50% ethanol and 95% ethanol using a shaker set at 200 rpm, at 30 °C for 24 hours. The extracts were filtered using Whatman No.1 filter paper and concentrated using a rotary evaporator at 40 °C to obtain ethanolic extracts. The extracts were stored at 4 °C for further analysis (Jane Monica *et al.*, 2022).

C. Physical Analysis

1. Colour measurements

Colour measurement of pumpkin seeds and sunflower seeds followed the method proposed by Rana *et al.* (2020) as mentioned by Razzak *et al.* (2023). The colour of samples was determined by using a colorimeter. The colorimeter was calibrated before measurement was taken. Ground seed samples were placed on a glass plate to test colour values (H, L*, a*, b* and C*). Same procedures were repeated using the solvent-extracted samples. Colour in terms of lightness or darkness was represented by the L* value. Black is denoted by L*=0 and white by L*=100. On the other hand, redness or greenness was indicated by its a* value, where a* > 0 denotes redness and a* < 0 denotes greenness. Yellowness or blueness was represented by its b* value, where b* > 0 denotes yellowness and b* < 0 denotes blueness. Each measurement was done three times and average of the result for each sample was calculated.

D. Chemical Analysis

1. Determination of fat

The method to estimate the fat content in the seeds followed the method described by Danbature *et al.* (2013) with a slight modification. 2 g of pumpkin seeds and sunflower seeds samples, (W₁) were placed on a filter paper and put into the Soxhlet extractor for extraction into a pre-weighed round-bottom flask with low-boiling petroleum ether (40 °C - 60 °C) using a Soxhlet extractor for 8 hours. The flask and its content were placed in a steam bath at 95 °C until no

steam is observed. Finally, the flask was heated at 105 °C in an oven for 1 hour and cooled in a desiccator and weighed. The process of heating and cooling was repeated until a constant weight was obtained.

$$\frac{\text{Fat content (\%)} = \frac{\text{Weight of fat materials (g)}}{\text{Weight of samples (g)}} \times 100\%}$$

$$\text{Weight of fat materials} = \text{Weight of (fat + flask)} - \text{Weight of flask}$$

2. Determination of protein

The determination of protein in the seeds followed the method by Magomya *et al.* (2014). 0.6-0.8 g of the powdered sample was digested in a Kjeldahl digestion flask by boiling it with 20 mL of concentrated H₂SO₄ and 2 pills of Kjeldahl digestion tablet (selenium catalyst) until the mixture becomes clear. The samples were allowed to cool. Before the distillation, the pH meter was calibrated. In the distillation unit, 20 mL of 2% boric acid was added into the flask. The tube was placed securely. 50 mL of deionised water was added to the digestion tube and 80 mL of 2% of NaOH added to the digestion tube by pressing the NaOH button. The blank (sulfuric acid + catalyst) was placed in the distillation unit and titrated with 0.1 M HCl followed by the samples. The volume of HCl used for sample and blank was recorded. The amount of protein has been calculated as follows:

$$\text{Total nitrogen (g) per 100 g of food} = \frac{(\text{Titre} - \text{Blank}) \times 1.4 \times 100}{1000 \times \text{sample weight}}$$

Crude protein (g/100 g) = Total nitrogen × conversion factor for food sample analysed

Conversion factor:

Pumpkin = 6.25 (Danbature *et al.*, 2013)

Sunflower = 5.30 (Li *et al.*, 2024)

3. Determination of functional groups by using FTIR

The determination of functional groups in pumpkin seeds and sunflower seeds was carried out using Fourier-Transformation Infrared spectroscopy (FTIR) (Perkin Elmer, Spectrum One) following the method described by Gowtham *et al.* (2022) using the 50% ethanol and 100% ethanol seed extracts. Solid samples were crushed into a thin pallet using an agate mortar, followed by milling with potassium

bromide (KBr). The samples were analysed between 4000-450 cm⁻¹.

4. Determination of minerals by using ICP-OES

The dried sample was placed in a muffle furnace at 550 °C for 3 hours. After that, the ash samples were taken from the furnace and allowed to cool in desiccators provided with a desiccant agent. The acid digestion method was used to determine elemental constituents. The ash samples were digested with 20 mL of 65% of HNO₃ for 24 hours at room temperature (25 °C) followed by boiling at 100 °C until the red fumes disappeared. After that, 2 mL of 35% hydrogen peroxide, H₂O₂ was added. After cooling, the samples were filtered using ashless filter paper until a clear solution is obtained. The solution was subsequently transferred to a 100 mL plastic volumetric flask and diluted with deionised water by taking 4.61 mL of the stock solution. The elements analysed using an ICP-OES were Na, K, Ca, Mg, Fe, Zn and Cu according to Shajan *et al.* (2024) and Liu *et al.* (2013) for pumpkin seeds and sunflower seeds respectively. The multi-element standard (Sigma Aldrich, St Louise, MO, USA) was prepared by taking 10 mL of each element and mixing it in 100 mL of deionised water.

E. Phytochemical Composition

1. Determination of alkaloids content

This was done by the alkaline precipitation gravimetric method according to Enujiugha *et al.* (2014). A volume of 0.2 mL extracts was dispersed into 50 mL of 10% glacial acetic acid solution in 95% ethanol. 10 drops of 30% ammonium hydroxide solution were added into it, and it was allowed to stay for 4 minutes. It was then placed on the hot plate to concentrate the solution at 50 °C. 10 drops of 30% ammonium hydroxide were added again and filtered using a weighted filter paper, W₀. The weight of filter paper after filtration was recorded, W₁. The filter paper with the residue was dried in the oven at 80 °C for 10 minutes and then it was allowed to cool in the desiccator for 10 minutes. The weight of filter paper after drying was recorded, W₂. The alkaloid content was calculated and expressed as a concentration (g/mL) of the volume of sample analysed.

$$\text{Alkaloid (mg/g)} = \frac{\text{Weight of wet residue (W}_3\text{)} - \text{Weight of dried residue (W}_4\text{)}}{\text{Volume of sample (mL)}} \times 100\%$$

$$W_3 = (\text{Weight of filter paper} + \text{wet residue}, W_1) - \text{Weight of filter paper}, W_0$$

$$W_4 = (\text{Weight of filter paper} + \text{dried residue}, W_1) - \text{Weight of filter paper}, W_0$$

2. Determination of tannin content

Tannin content was measured using the method described by Akullo *et al.* (2023) with slight modifications. Tannins were measured using a modified vanillin-hydrochloric acid method. Catechin hydrate was used to make standards at concentrations of 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL (R²= 0.9650). 1 mL of each sample extracts and standards were placed in test tubes. To the blank, 5 mL of 4% HCl in methanol without the reagent (vanillin) was added. Sample and standards were treated for 20 minutes with 5 mL of vanillin-HCl reagent (made by mixing the equal volumes of 4% HCl in methanol and 1% vanillin in methanol). The absorbance of the blank, standard and sample were measured at 500 nm and tannin content was estimated as concentration catechin equivalent (CE) using a standard calibration curve derived from catechin absorbance at various concentrations.

F. Statistical Analysis

Statistical Package for the Social Sciences (SPSS) 29.0 version was used for the analysis of data. The p-value of less than 0.05 was considered as significant. An independent t-test was applied to compare pumpkin seeds and sunflower seeds without the use of two different concentrations of seeds extract as well as to compare the concentration of extract within the same seeds. In order to analyse the differences in colour characteristics, one-way Analysis of Variance (ANOVA) was applied followed by the Duncan post-hoc test (Shoeb, 2016).

III. RESULT AND DISCUSSION

A. Physical Analysis

1. Extraction method efficiency

The yield of each solvent extraction is shown in Table 1. Pumpkin seeds that were extracted with 95% ethanol resulted higher yield (45.69%) compared to 50% ethanol

extract (23.88%). Similarly, sunflower seeds extracted with 95% ethanol was significantly higher (40.33%) compared to 50% ethanol extract (20.12%). Conventional solvent extraction method uses organic solvent but varying many factors such as temperature, stirring, time, pH and heat. However, this method utilises large amount of solvent and time consuming. Several studies have reported differences in yield according to extraction method. Pumpkin seed extracted using chloroform and methanol (2:1) resulted in 440-524 g/kg fresh weight (Kim *et al.*, 2012). Pumpkin seeds extracted using microwave assisted extraction using hexane (1:10) for 5-30 minutes (200 W) resulted in 51-62.5% (Hernandez-Santos *et al.*, 2016). The extraction yields of pumpkin seed oil were 48.99% for UAE (50 °C, 100 W) and 30.28% for pressurised ethanol extraction (50 °C, 6 mL/min, 50 bar) (Amarante *et al.*, 2025). UAE offers extraction of desired constituents by varying ultrasonic power, time, solvent to solid ratio, temperature and sample size. These variations may influence the depth of cavitation and disruption of pumpkin seed cell walls (Amarante *et al.*, 2025). Meanwhile, pumpkin seeds extracted using microwave assisted extraction using ethanol water (100-150 °C, 20 min, 2.45 GHz) resulted phenolic compounds 35-84 mg GAE/g dry weight (Stupar *et al.*, 2021). Microwave assisted extraction for pumpkin seed oil using hexane (5-30 min, 200 W, 1:10 g/mL) yielded 51-62.5% (Hernandez-Santos *et al.*, 2016). Microwave energy combined with effective magnetic stirring allows swift extraction to below 30 minutes while minimising solvent usage. Meanwhile, microwave roasted sunflower seeds resulted in higher phenolic compounds (895.6 mg/100 g) compared to boiling method (598.5 mg/100 g) (Kiczorowska *et al.*, 2019). Various extraction techniques are employed to plant seeds; however, extraction efficiency depends on many factors including analyte to be extracted and instrumental parameters.

Table 1. Extraction yield for pumpkin seeds and sunflower seeds

Samples	Extract	Yield (%)
Pumpkin seeds	50% ethanol	23.88 ^a
	95% ethanol	45.69 ^a
Sunflower seeds	50% ethanol	20.12 ^b
	95% ethanol	40.33 ^a

Note: Values are the means $\pm$ standard deviation of three replicate analysis. Means values with different superscripts are significantly different at $p < 0.05$.

2. Colour measurement

As indicated in Table 2, the colour for both seeds was expressed as L^* , a^* and b^* values. Based on the data, the powdered pumpkin seeds had significantly higher lightness (L^* value) compared to the fresh pumpkin seeds as well as extracts of 50% and 95% ethanol respectively. This indicated that the powder and fresh seeds were lighter, while the pumpkin seeds extracts were darker as shown in Figure 1. Both pumpkin seeds and sunflower seeds with 95% ethanol extract had the lowest value of a^* , indicating a greenish colour. Pumpkin seed extract had a higher $-a^*$ value compared to sunflower seeds. As for the sunflower seeds, powdered seeds had the highest b^* value followed by 95% of ethanol extract, 50% ethanol extract and fresh seeds. Sunflower seed extracts had a higher value of b^* , indicating that they are towards the yellow colour. The 95% of ethanol extract is effectively extracting the colour and pigments (Dan *et al.*, 2009). Pigments from coloured plants can be useful for many applications in food, pharmaceutical and textile industries. Artificial colours in the food business have increased as they are more stable and have versatile colours (Nor-Aleesya *et al.*, 2023). However, despite current studies into synthetic chemicals, natural pigments have emerged in response to recent findings about the toxicity and adverse consequences of several synthetic dyes (Novais *et al.*, 2022). Food industries have begun replacing artificial colours with natural colourants because they have both pigmentation and health benefits. Plant pigments considered to be valuable as colourants (Ebrahimi *et al.*, 2023). Sunflower seeds might be associated with yellow pigments as it exhibits a higher b^* value in their extract compared to pumpkin seeds. Xanthophylls and carotenes contributes to the yellow pigment in sunflower seeds (Kiczorowska *et al.*, 2019). Chloroplasts mainly responsible to contribute for greenish

colour in pumpkin seeds (Boujemaa *et al.*, 2020). (Cvitković *et al.* (2021) found that the highest concentration of chlorophyll a and chlorophyll b were found in ethanol extracts.

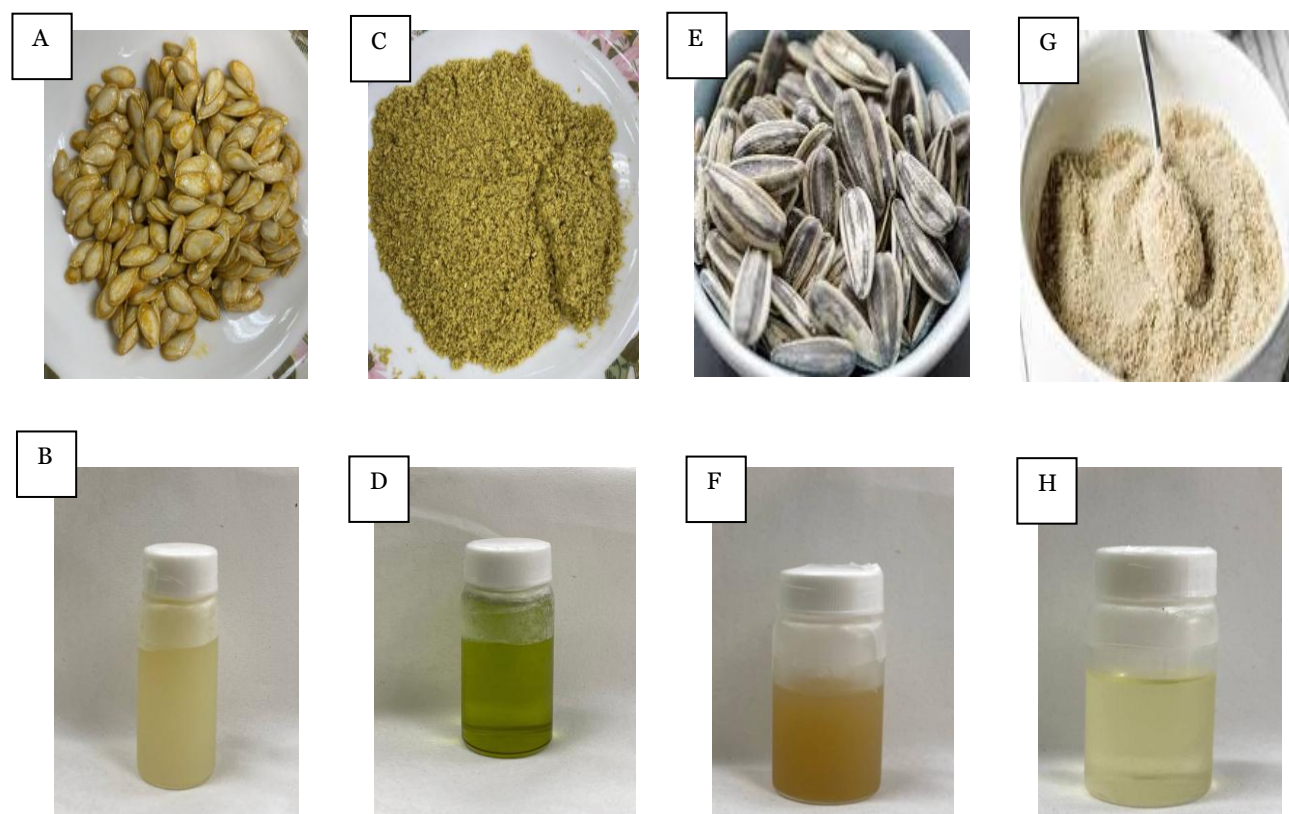


Figure 1. Images indicating (a) pumpkin seeds, (b) pumpkin seeds 50% ethanol extract, (c) pumpkin seeds powder, (d) pumpkin seeds 95% ethanol extract, (e) sunflower seeds, (f) sunflower seeds 50% ethanol extract, (g) sunflower seeds powder and (h) sunflower seeds 95% ethanol extract.

Table 2. Colour measurements for pumpkin seeds and sunflower seeds

Samples		L*	a*	b*
Pumpkin seeds	Fresh seeds	65.46±0.36 ^{ba}	3.37±0.35 ^{aA}	17.82±0.13 ^{ba}
	Powdered seeds	69.17±0.40 ^{aA}	-0.92±0.03 ^{bB}	26.46±0.31 ^{aA}
	50% ethanol extract	39.62±0.14 ^{cA}	-1.90±0.16 ^{cB}	1.94±0.17 ^{dB}
	95% ethanol extract	30.15±0.36 ^{dB}	-3.48±0.15 ^{dA}	8.66±0.24 ^{cB}
Sunflower seeds	Fresh seeds	56.75±0.50 ^{ba}	1.52±0.35 ^{bB}	6.80±0.51 ^{cB}
	Powdered seeds	69.10±0.10 ^{aA}	2.92±0.03 ^{aA}	15.40±0.14 ^{aB}
	50% ethanol extract	30.76±0.57 ^{dB}	0.43±0.07 ^{cA}	4.80±0.12 ^{dA}
	95% ethanol extract	42.38±0.78 ^{cA}	-3.63±0.14 ^{dA}	14.08±0.48 ^{ba}

Note: Values are the means ± standard deviation of three replicate analysis. Means values with different superscripts are significantly different at $p < 0.05$. Capital letter indicates the effect of seeds at the specific conditions on the colour (A-B). Small letters indicate the effect of seed conditions on the colour (a-d).

B. Chemical Analysis

1. Determination of fat

The results of fat analysis of pumpkin seeds and sunflower seeds are presented in Table 3. The crude fat content was determined using Soxhlet extraction method for both plant seeds. The fat content analysis revealed that pumpkin seeds had a significantly higher amount of crude fat (29.53%) compared to sunflower seeds (27.74%). Different species of pumpkin seed oils contains high levels of unsaturated fatty acids (Boujemaa *et al.*, 2020). Infrared radiation resulted in higher fat content compared to boiled and microwave roasted sunflower seed (Kiczorowska *et al.*, 2019). Pumpkin seeds are rich in bioactive compounds including unsaturated fatty acid, promoting its health benefits towards cardiovascular disease. The confectionary sunflower seeds are widely applied in bakery products. Sunflower seed extract shows a positive effect on reducing body fat in adults with obesity. Kim *et al.* (2024) mentioned that supplementation with sunflower seeds extract not only resulted in a significant reduction in body weight gain and adipose tissue, it also inhibited adipogenesis and promoted lipolysis and thermogenesis. This result differs with studies from Polyzos *et al.* (2024) that found 42.74 g/100g dw fat in pumpkin seeds. Meanwhile, Petraru *et al.* (2021) reported that sunflower seed contained 65.42% fat, 32.50% fat in kernel and 8.81% in its hull. Their nutritional values are influenced by various factors such as genotype, soil characteristics, agricultural practices, climatic conditions and processing conditions.

Table 3. Crude fat content of pumpkin seeds and sunflower seeds

Samples	Crude fat content (%)
Pumpkin seeds	29.53±0.58 ^a
Sunflower seeds	27.74±0.31 ^b

Note: Values are the means ± standard deviation of three replicate analysis. Means values with different superscripts are significantly different at $p < 0.05$.

2. Determination of protein content

The protein content of protein seeds and sunflower seeds are presented in Table 4. The Kjeldahl method was used to calculate the crude protein content of pumpkin seeds and sunflower seeds using a conversion factor 6.25 for pumpkin

seeds and 5.30 for sunflower seeds according to the Food Regulations 1985, Regulations 18B. Although the amount of protein in plant seeds is not comparable to the other source of protein such as fish, eggs, poultry or meat, research has shown that they have a high protein content among fruits and vegetables.

From Table 3, pumpkin seeds had a significantly higher crude protein content compared to sunflower seeds. The crude protein in pumpkin seeds was 36.69%. Findings from Sinkovič *et al.* (2021) reported the protein content in pumpkin seeds ranged from 38.3% to 39.5% among different pumpkin cultivars. The difference due to geographic location, cultivars could also contribute to differences in protein content. Sunflower seeds had a protein content of 12.03%. Protein content in raw sunflower seed was higher (244.5 g/kg) compared to boiling, infrared and microwave roasted sunflower seeds (Kiczorowska *et al.*, 2019). Previously, Petraru *et al.* (2021) found 33.85% protein content in sunflower seeds, 23.73% in kernel and 7.82% in its hull. Barsby *et al.* (2021) reported that sunflower seeds contained 21% of protein using the Qubit Fluorometric Protein Assay Kit and used aqueous extract of sunflower seeds. Nzotta and Onabanjo (2021) found 29.83% protein in pumpkin seed. Nicoleta *et al.* (2024) stated that the pumpkin by-products, especially pumpkin seeds, are rich in protein. This bioactive compound exhibits diverse biological activities such as antihypertensive, antioxidant, antibacterial and immunomodulatory effects. Protein as a primary metabolite of pumpkin seeds play a crucial role in disease prevention and improvement in human health (Patel *et al.*, 2023). A study from Sá *et al.* (2021) revealed that pumpkin seeds contain numerous essential amino acids such as histidine, isoleucine, leucine, lysine, threonine, valine, methionine as well as cysteine. Each amino acid is the building block for a human body that makes up the enzymes which initiates many chemical reactions for usual body homeostasis. Proteins originating from plant-based food contains lower total and LDL cholesterol compared to diet with red meat (Mounes *et al.*, 2024).

One of the health benefits of pumpkin seeds is its antidiabetic property. The hypoglycaemic effects of protein isolate from various pumpkin seeds varieties within the Cucurbitaceae family including *Cucurbita moschata* cultivar

have been reported (Syed, 2019). In addition, Batool *et al.* (2022) stated that pumpkin seeds protein isolate had the potential to reduce the level of aspartate transaminase (AST), alkaline phosphatase (ALP), alanine transaminase (ALT), and lactate dehydrogenase (LD), indicating its potential benefits in managing liver dysregulation, as well as enhancing antioxidant activities. Furthermore, protein-rich food also contains tryptophan that acts as a precursor of serotonin and melatonin. Arora *et al.* (2023) stated that pumpkin seeds are a natural source of tryptophan. Although serotonin cannot cross the blood-brain barrier, the synthesis of serotonin in the central nervous system can be influenced by a diet rich in tryptophan. This kind of diet may serve as a protective factor against depression and is positively associated with improving social cognition (Storz, 2021).

Although the protein content of sunflower seeds is comparatively lower than pumpkin seeds, sunflower seeds is widely utilised in food products especially bakery products such as bread, pancakes, cookies as well as brownies. According to de Oliveira Filho and Egea (2021), sunflower protein isolates are free from toxic substances and possess lower levels of antinutritional factors compared to other protein-rich sources. Thus, utilising agro-industrial byproducts for protein production represents a sustainable approach that enhances the commercial value of these byproducts. The advantages include producing valuable chemicals at cheaper cost, biomass reuse, bioproducts, wastewater treatment and reduced agricultural waste management cost (Benelia *et al.*, 2025; Wan-Mohtar *et al.*, 2023).

Table 4. Crude protein content of pumpkin seeds and sunflower seeds

Samples	Crude protein (%)
Pumpkin seeds	36.69±1.26 ^a
Sunflower seeds	12.03±0.14 ^b

Note: Values are the means ± standard deviation of two replicate analyses. Means values with different superscripts are significantly different at $p < 0.05$.

3. Determination of functional group using FTIR

FTIR results demonstrate how the ethanol extraction enhances the functional groups linked to the bioactive compound indicating that processed extracts can have even

more therapeutic potential. This highlights the value of including sunflower seeds and pumpkin seeds in a balanced diet due to their potential to prevent disease and enhance general health in addition to their nutritional advantages. The functional groups in the powder and ethanol extracts of pumpkin seeds and sunflower seeds were characterised based on absorption using an infrared spectrum and the results are displayed in Figure 2 and Figure 3. The results of the FTIR spectrum assisted in identifying the active components listed in Table 5 and Table 6. From the results obtained, 95% of ethanol extract had the most functional group and the highest transmittance for both seeds compared to the powder and 50% of ethanol extract samples. Rajhard *et al.* (2021) stated that polar solvent and mixture of alcohol and water can extract polar compounds. Hence, since 95% of ethanol extract is less polar, it was able to extract less polar and nonpolar compounds, such as hydrocarbons.

In pumpkin seeds, the prominent peak at 1745.86 cm^{-1} , indicated the presence of aromatic rings, pointing to the possibility of polyphenols such as flavonoids and tannins being present. Tannins, which are well-known for their cardioprotective, anti-inflammatory and antioxidant benefits have been associated with a lower chance of developing chronic illnesses like diabetes, cancer and cardiovascular disease (Cosme *et al.*, 2025). The presence of hydrocarbon-based molecules is further supported by the C-H stretching ($3000\text{--}2840\text{ cm}^{-1}$), which validates the existence of lipid structures that support metabolic health and energy storage. Bikmurzin *et al.* (2022) stated that the lipid region is linked to C-H stretching modes around $3000\text{--}2840\text{ cm}^{-1}$. The presence of alcohols and phenols is shown by the broad O-H stretching ($3200\text{--}3550\text{ cm}^{-1}$), which supports pumpkin seeds' antioxidant capacity. Phenols with one or more aromatic rings and hydroxyl groups serve as antioxidants, disease inhibiting agents as well as colourants (Peng *et al.*, 2021). Both the N-O stretching ($1500\text{--}1550\text{ cm}^{-1}$) and the C-N stretching ($1266\text{--}1342\text{ cm}^{-1}$) indicate the presence of proteins and nitrogen-containing substances. Bioactive peptides and proteins (BAPPs) are functional molecules with distinct amino acid sequences that have a role in a wide range of biological activities such as immunomodulation, biocatalysis, signalling pathway

activation and inhibition and the control of cellular purpose and behaviour. As a result, they exhibit an excellent potential as treatment agents for a number of illnesses including diabetes, tissue damage, cancer, infection and chronic pain (Hao *et al.*, 2024).

In sunflower seeds, the existence of phenolic and flavonoid compounds is shown by the prominent peak at 1745.26 cm^{-1} , which corresponds to C-H stretching in aromatic compounds. Flavonoids which are present in natural dyes, cosmetics and skin care products as well as anti-wrinkle agents have important medical uses such as anticancer, antibacterial, antiviral, antimalarial, antioxidant, neuroprotective, antitumor anti proliferative (Ullah *et al.*, 2020). The C-H stretching ($2840\text{-}3000\text{ cm}^{-1}$) further confirmed the existence of lipid molecules, indicating the presence of important fatty acids such as linoleic acid. Linoleic acid lowers total and low-density lipoprotein

cholesterol when compared with saturated fatty acids and carbohydrates (Jackson *et al.*, 2024). The O-H stretching ($3200\text{--}3550\text{ cm}^{-1}$) indicated a high concentration of phenolic substances. Numerous scientific studies have proposed that substances produced from plants have biological effects such as preventing disease linked to oxidative stress as these actions have been associated with the polyphenol compounds found in plants (Wongsa *et al.*, 2022). The presence of proteins and polyphenols in the seeds is confirmed by the C=C stretching ($1626\text{--}1662\text{ cm}^{-1}$) and C-N stretching ($1266\text{--}1342\text{ cm}^{-1}$) which aids in muscle repair, enzyme activity as well as anti-inflammatory properties. Furthermore, distinct functional groups like nitrile ($\text{C}\equiv\text{N}$ at $2222\text{--}2260\text{ cm}^{-1}$) and isothiocyanate ($\text{N}=\text{C}=\text{S}$ at $1990\text{--}2140\text{ cm}^{-1}$) are seen in sunflower seeds, indicating the presence of sulphur-containing bioactive compounds with detoxifying characteristics and cancer-protective properties.

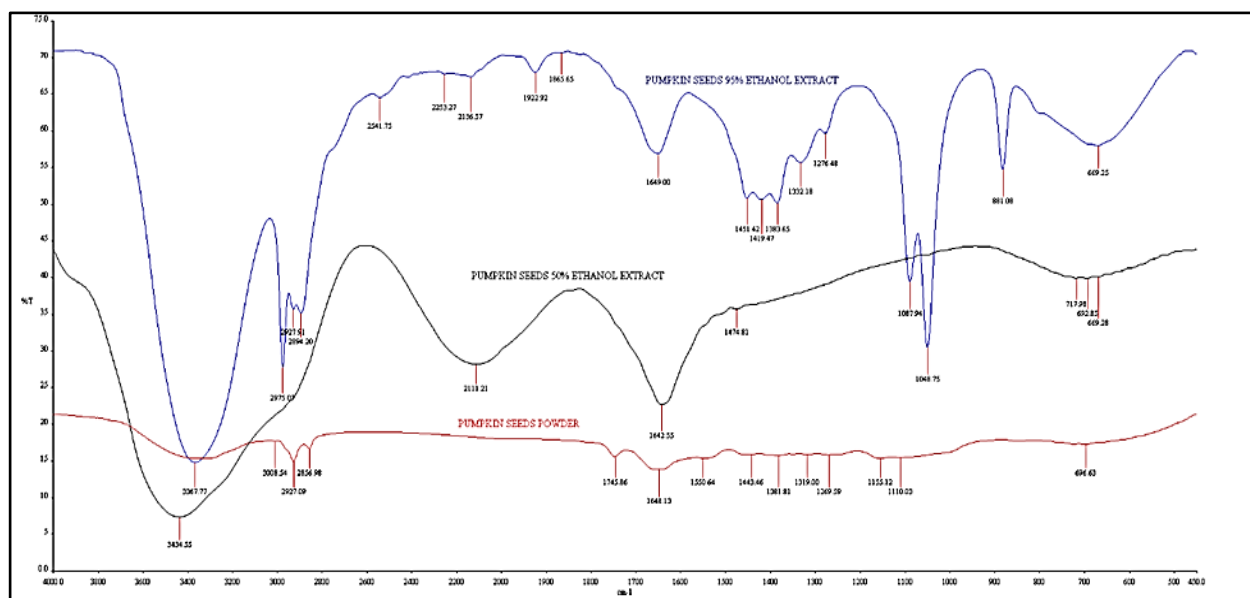


Figure 2. FTIR spectrum of pumpkin seeds.

Red line: Powdered seeds, Blue line: 50% ethanol extract, Black line: 95% ethanol extract

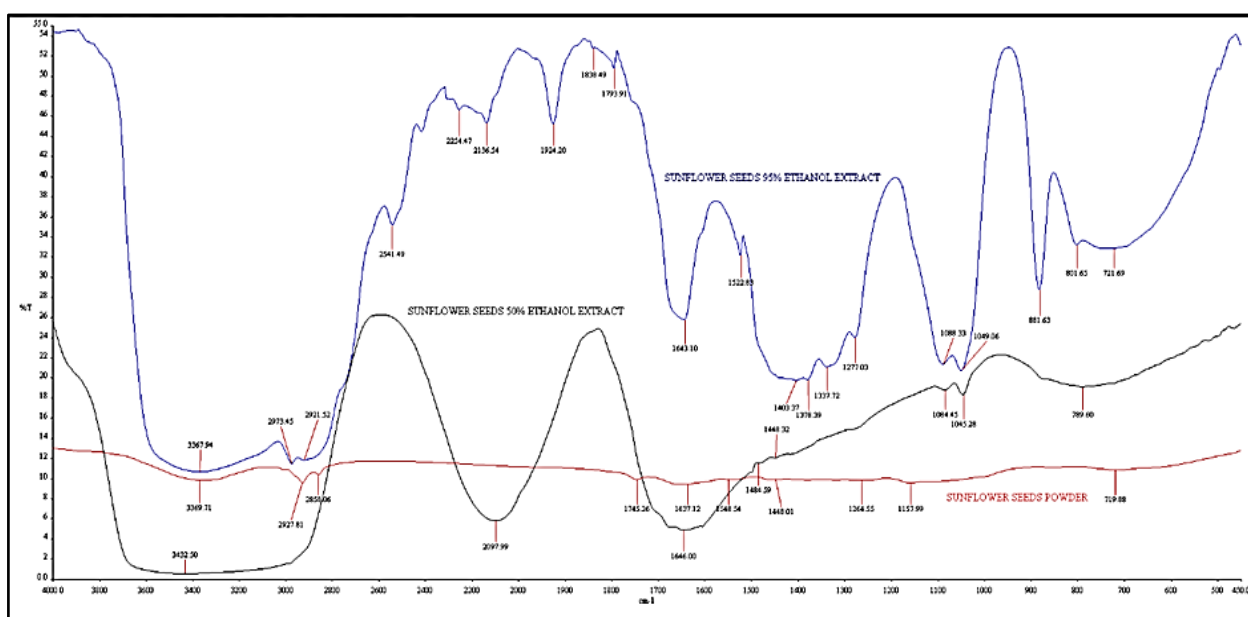


Figure 3. FTIR spectrum of sunflower seeds.
Red line: Powdered seeds, Blue line: 50% ethanol extract, Black line: 95% ethanol extract

Table 5. Peak value, functional group and class for FTIR spectra of powdered and extracts of pumpkin seeds

Wavenumber (cm ⁻¹)	Pumpkin seeds			Functional group	Class
	Powdered seeds	50% ethanol extract	95% ethanol extract		
3300-3400			3367.77	N-H	Aliphatic 1° amine
3200-3550		3434.55		O-H	Alcohol
2840-3000			2975.07	C-H	Alkane
			2927.91	C-H	Alkane
			2894.20	C-H	Alkane
2700-3200	3008.54			O-H	Alcohol
2500-3300	2927.09		2541.75	O-H	Carboxylic acid
	2856.98				
2190-2260			2253.27	C≡C	Alkyne
			2136.57	C≡C	Alkyne
2100-2140		2111.21		C≡C	Alkyne
1900-2000			1922.92	C=C=C	Allene
1650-2000	1745.86		1865.65	C-H	Aromatic compound
1648-1658	1648.13		1649.00	C=C	Alkene
1626-1662		1642.55		C=C	Alkene
1500-1550	1550.64			N-O	Nitro compounds
1450	1443.46	1474.81	1451.42	C-H	Alkane
1380-1390	1381.81			C-H	Phenol
1330-1420			1419.47	O-H	Alcohol
1310-1390	1319.00		1383.65	O-H	Phenol
			1332.18		
1266-1342	1269.59		1276.48	C-N	Aromatic amine
1087-1124			1087.94	C-O	2° alcohol

1030-1070			1048.75	S=O	Sulfoxide
1020-1250	1155.12			C-N	Amine
	1110.03				
860-900			881.08	C-H	1,2,4-trisubstituted, 1,3-disubstituted
680-720	696.63	717.98			Benzene derivative
		692.85			
665-730		669.28	669.25	C=C	Alkene

Table 6. Peak value, functional group and class for FTIR spectra of powdered and extracts of sunflower seeds

Wavenumber (cm ⁻¹)	Sunflower seeds			Functional group	Class
	Powdered seeds	50% ethanol extract	95% ethanol extract		
3200-3550	3369.71	3432.50	3367.94	O-H	Alcohol
2840-3000	2927.81		2973.45	C-H	Alkane
	2858.06				
2700-3200			2921.52	O-H	Alcohol
2500-3300			2541.49	O-H	Carboxylic acid
2222-2260			2254.47	C≡N	Nitrile
2100-2140			2136.54	C≡C	Alkyne
1990-2140		2097.99		N=C=S	Isothiocyanate
1900-2000			1924.20	C=C=C	Allene
1650-2000	1745.26		1838.49	C-H	Aromatic compound
			1793.91		
1626-1662	1637.12	1646.00		C=C	Alkene
1580-1650			1643.10	C=C	Alkene
1500-1550	1548.54		1522.88	N-O	Nitro compound
1450	1448.01	1484.59		C-H	Alkane
		1448.32			
1395-1440			1403.37	O-H	Carboxylic acid
1330-1420			1378.39	O-H	Alcohol
			1337.72		
1266-342			1277.03	C-N	Aromatic amine
1250-1310	1264.55			C-O	Aromatic ester
1020-1250	1157.99	1084.45	1088.33	C-N	Amine
		1045.28	1049.06		
860-900				C-H	1,2,4- trisubstituted, 1,3-disubstituted
			881.63		
790-840			801.65	C=C	Alkene
760-800		789.60		C-H	1,2,3- trisubstituted

680-720	719.88			Benzene
				derivative
665-730		721.69	C=C	Alkene

4. Determination of mineral using ICP-OES

The minerals composition of pumpkin and sunflower seeds is presented in Table 7. Minerals are inorganic nutrients that are vital for sustaining life and supporting various physicochemical processes within the body. Minerals can be classified into two categories: macro elements and mineral elements. Macro elements are essential nutrients required by the body in relatively large quantities compared to other minerals as they play a critical role in maintaining overall health and physiological functions (Siddiqui *et al.*, 2014). In pumpkin seeds, potassium exhibited the highest concentration followed by magnesium, calcium and sodium. The amount of concentration of minerals obtained contradicts findings from Elinge *et al.* (2012), however they still found that potassium has the highest concentration in the pumpkin seeds. The slight difference in the mineral content might be due to the pumpkin varieties chosen as well as soil characteristics and climate conditions in certain countries. On the other hand, sunflower seeds contain potassium as the highest amount of mineral, followed by magnesium, calcium and sodium respectively. Petraru *et al.* (2021) in their study found a slightly different amount of mineral content with this study.

From the result obtained, potassium is the most abundant mineral in both pumpkin and sunflower seeds. Hypokalaemia is one of the consequences of electrolyte imbalance due to low potassium levels in the blood and supplemental potassium has been recommended for treating hypokalaemia (Alfano *et al.*, 2021). Aside from potassium, both plant seeds are also rich in magnesium. The magnesium content of the samples was found 2112.99 mg/100g and 1052 mg/100g for pumpkin seeds and sunflower seeds respectively. Storz (2021) stated that magnesium-containing food could enhance sleep quality, prolonged sleep duration. The iron and zinc content in pumpkin seeds were significantly higher than in sunflower seeds. Pumpkin seeds can be processed into pumpkin seed flour through the drying process, this nutrient-rich flour has potential applications as a supplement in food products,

notably in bakery items. Hussain (2023) found that biscuits formulated with pumpkin seeds flour, which is rich in iron and zinc could serve as an effective means of enhancing immunity in children. Zinc plays a crucial role for the immune system, contributing to both antiviral and antibacterial properties (Younes, 2024).

Table 7. Mineral composition of pumpkin seeds and sunflower seeds

Concentration (mg/100g)	Pumpkin seeds	Sunflower seeds
Calcium, Ca	518.99±0.31 ^b	594.12±0.60 ^a
Sodium, Na	114.02±0.31 ^a	77.99±2.57 ^b
Potassium, K	4408.20±0.00 ^a	3665.85±47.46 ^b
Magnesium, Mg	2112.99±2.14 ^a	1052.92±6.43 ^b
Iron, Fe	46.53±0.30 ^a	21.02±0.03 ^b
Copper, Cu	3.42±0.00 ^b	6.78±0.03 ^a
Zinc, Zn	41.77±0.09 ^a	22.32±0.21 ^b

Note: Values are the means ± standard deviation of two replicate analysis. Means values with different superscripts are significantly different at $p < 0.05$

C. Phytochemical Analysis

1. Determination of alkaloid content

The alkaloid contents of sunflower seeds were significantly higher compared to pumpkin seeds (Table 8). In addition, the alkaloid content of sunflower seeds treated with 95% ethanol is significantly higher compared to 50% ethanol. A study from Krisridwany *et al.* (2023) reported that the alkaloid content in pumpkin seeds to be 1.77% using the UV-Vis Spectrophotometer method. A study by Prakash *et al.* (2024) reported that alkaloids were abundant in sunflower seeds through phytochemical screening using the Mayer's test, Wagner's test and Hager's test. Agubosi *et al.* (2022) further reported that sunflower seeds contain 3.86% alkaloids. These differences might be due to the different extraction methods and solvent used. Yan *et al.* (2021) mentioned that alkaloids exhibit antibacterial activity through multiple mechanisms such as suppressing bacterial nucleic acid and protein synthesis, altering cell membrane permeability, causing damage in cell membrane and cell wall,

disrupting bacterial metabolism. The presence of small amount of alkaloids in edible plant parts is indicative for their potential to exhibit various activities such as antimalarial properties, anticancer activity and antimicrobial effects (Aziz *et al.*, 2021). Alkaloids also shown to portray the ability to target multiple therapeutic pathways and exhibit strong antiviral activity (Majnooni *et al.*, 2021).

Table 8. Alkaloid content in pumpkin seeds and sunflower seeds in ethanolic extract

Sample	Solvent	Alkaloid
		concentration (mg/g extract)
Pumpkin seeds	50% ethanol	3.04±0.10 ^{aB}
	95% ethanol	3.16±0.10 ^{aB}
Sunflower seeds	50% ethanol	4.60±0.16 ^{bA}
	95% ethanol	5.01±0.10 ^{aA}

Note: Values are the means ± standard deviation of three replicate analysis. Means values with different superscripts are significantly different at $p < 0.05$. Capital letters indicate the effect of seeds at the specific concentration on the alkaloid content (A-B). Small letter indicates the effect of ethanol concentration on the alkaloid content (a-b)

2. Determination of tannin content

The results for the tannin contents of both extracts of the two plant seeds are presented in Table 9. Tannin content in pumpkin seed extract ranged from 0.16 to 0.91 mg CE/mL extract, while sunflower seeds extract ranged from 0.21 to 0.81 mg/mL (Table 8). It is worthwhile to note that the tannin content of pumpkin seed and sunflower seed extracts using 95% ethanol were significantly higher compared to 50% ethanol. The tannin content in the pumpkin seeds contradicts with the finding from Nzotta and Onabanjo (2021) that reported 0.01%. However, for sunflower seeds, the total tannin content is supported by the study from Muttagi and Joshi (2020) that reported 30.91 mg/100g tannic acid for sunflower seed variety Confectionery-1 from Bengaluru, India. The differences in results might be due to the different extraction method and the standard used to measure the tannin. Nzotta and Onabanjo (2021) used a mixture of acetone and glacial acetic acid (80:20) to extract the tannin and used tannic acid to estimate the total tannin content, while this study used ethanol at 50% and 95% concentration to extract the tannin compound and catechin

hydrate as a standard used. Tannins are categorised into two main types; hydrolysable tannins and condensed tannins or also known as catechin tannins or proanthocyanidins (Smeriglio *et al.*, 2016).

Table 9. Tannin content in pumpkin seeds and sunflower seeds in ethanolic extract

Sample	Solvent	Tannin
		concentration (mg CE/mL extract)
Pumpkin seeds	50% ethanol	0.16±0.002 ^{bB}
	95% ethanol	0.91±0.008 ^{aA}
Sunflower seeds	50% ethanol	0.21±0.002 ^{bA}
	95% ethanol	0.81±0.003 ^{aB}

Note: Values are the means ± standard deviation of three replicate analysis. Means values with different superscripts are significantly different at $p < 0.05$. Capital letters indicate the effect of seeds at the specific concentration on the tannins content (A-B). Small letter indicates the effect of ethanol concentration on the tannins content (a-b).

IV. CONCLUSION

The colour, nutritional compositions such as fat, protein, minerals analysed using ICP-OES and chemical characteristics such as alkaloids and tannins as well as functional groups using FTIR method of pumpkin seeds and sunflower seeds extracted with 50% and 95% ethanol were determined. From the analysis conducted, the colour of 95% ethanol pumpkin seed extract was greenish and yellowish colour. Pumpkin seeds had significantly higher amount of fat, protein and potassium compared to sunflower seeds. Analysis of the functional group using FTIR revealed that the 95% ethanol extract exhibited the most spectra of the functional group compared to 50% ethanol. Potassium, magnesium, sodium, iron and zinc was found significantly higher in pumpkin seeds compared to sunflower seeds. In terms of alkaloids, 95% ethanol extract of sunflower seeds had the highest alkaloid content compared to the 50% ethanol extract. For tannin analysis, the 95% ethanol extract pumpkin seeds had significantly the highest condensed tannin content compared to 50% ethanol extract.

V. ACKNOWLEDGEMENTS

This research was funded by the Fundamental Research Grant Scheme: FRGS/1/2023/WAB13/UITM/O2/1 from the

Ministry of Higher Education Malaysia. The authors thank the lab staff at Universiti Teknologi MARA for the facilities and assistance provided during this research.

VI. REFERENCES

- Abbas, MS, Doweidar, MMM & Saleh, SAA 2024, 'Evaluation of healthy pan bread enriched with sesame, peanut and sun flower seeds', *Egyptian Journal of Chemistry*, vol. 67, no. 5, pp. 89-108.
- Agubosi, OCP, James, A & Alagbe, JO 2022, 'Influence of dietary inclusion of sunflower (*Helianthus annuus*) oil on growth performance and oxidative status of broiler chicks', *American Journal of Interdisciplinary Research and Development*, vol. 1, pp. 1-11.
- Akullo, JO, Kiage-Mokua, BN, Nakimbugwe, D, Ng'ang'a, J & Kinyuru, J 2023, 'Phytochemical profile and antioxidant activity of various solvent extracts of two varieties of ginger and garlic', *Heliyon*, vol. 9, no. 2003, e18806, pp. 1-13.
- Alfano, G, Ferrari, A, Fontana, F, Perrone, R, Mori, G, Ascione, E, Magistroni, R, Venturi, G, Pederzoli, S, Margiotta, G, Romeo, M, Piccinini, F, Franceschi, G, Volpi, S, Faltoni, M, Ciusa, G, Bacca, E, Tutone, M, Raimondi, A & Menozzi, M 2021, 'Hypokalemia in patients with COVID-19', *Clinical and Experimental Nephrology*, vol. 25, pp. 1-9.
- Amarante, BA, Saccomori, E, Bilibio, D, Bender, JP, Silva, GBD, Manica, D, Bagatini, MD, Bruno, AN & Priamo WL 2025, 'Targeting metastatic melanoma: Pumpkin seed extracts as potential antineoplastic agents', *The Journal of Supercritical Fluids*, vol. 223, no. 106643, pp. 1-15.
- Arora, A, Sharma, L, Sharma, D, Ghangale, G, Bidkar, J & Tare, H 2023, 'The nutraceutical role of pumpkin seed and its health effect: A review', *International Journal of Pharmaceutical Quality Assurance*, vol. 14, no. 1, pp. 233-238.
- Ahmad, A Shahril, MR, Wan-Arfah, N, Bakar, WAMA, Piernas, C & Lua, PL 2022, 'Changes in health-related lifestyles and food insecurity and its association with quality of life during the COVID-19 lockdown in Malaysia', *BMC Public Health*, vol. 22, no. 1150, pp. 1-9.
- Aziz, AA, Noreen, S, Khalid, W, Ejaz, A, Rasool, IFU, Maham, Munir, A., Farwa, Javed, M, Ercisli, S, Okcu, Z, Marc, RA, Nayik, GA, Ramniwas, S & Uddin, J 2023, 'Pumpkin and pumpkin byproducts: phytochemical constitutes, food application and health benefits', *ACS Omega*, vol. 8, no. 26, pp. 23346-23357.
- Barsby, JP, Cowley, JM, Leemaqz, SY, Grieger, JA, McKeating, DR, Perkins, AV, Bastian, SEP, Burton, RA & Bianco-Miotto, T 2021, 'Nutritional properties of selected superfood extracts and their potential health benefits', *PeerJ*, vol. 9, no. e12525.
- Batool, M, Ranjha, MMAN, Roobab, U, Manzoor, MF, Farooq, U, Nadeem, HR, Nadeem, M, Kanwal, R, AbdElgawad, H, Al Jaouni, SK, Selim, S & Ibrahim, SA 2022, 'Nutritional value, phytochemical potential, and therapeutic benefits of pumpkin (*Cucurbita sp.*)', *Plants*, vol. 11, no. 11, no. 1394, pp. 1-23.
- Benelia, A, Baatache, O, Zerguine, KE, Khediri, A, Derbal, K, Ferroudj, N, Khalfaoio, A & Pizzi, A 2025, 'Wastewater treatment using a combination of pumpkin seed waste after extraction of essential oils (bio-coagulant) and ferric chloride (chemical coagulant): Optimization and modelling using a Box-Behnken design', *Applied Sciences*, vol. 15, no. 5439, pp. 1-20.
- Bikmurzin, R, Bandzevičiūtė, R, Maršalka, A, Maneikis, A & Kalėdienė, L 2022, 'FT-IR method limitations for β -Glucan analysis', *Molecules*, vol. 27, no. 4616, pp. 1-14.
- Boujemaa, I, Bernoussi, SE, Harhar, H & Tabyaoui, M 2020, 'The influence of the species on the quality, chemical composition and antioxidant activity of pumpkin seed oil', *Oilseeds & Fats Crops and Lipids*, vol. 27, no. 40, pp. 1-7.
- Cheenam, B & Leena, P 2016, 'Effects of sunflower seeds on fasting blood glucose in diabetes mellitus type 2 patients', *Journal of Chemical and Pharmaceutical Research*, vol. 8, no. 4, pp. 1211-1217.
- Cosme, F, Aires, A, Pinto, T, Oliveira, I, Vilela, A & Gonçalves, B 2025, 'A comprehensive review of bioactive tannins in foods and beverages: Functional properties, health benefits, and sensory qualities', *Molecules*, vol. 30, issue 4, no. 800, pp. 1-28.
- Cvitković, D, Lisica, P, Zorić, Z, Repajić, M, Pedisić, S, Dragović-Uzelac, V & Balbino, S 2021, 'Composition and

- antioxidant properties of pigments of Mediterranean herbs and spices as affected by different extraction methods', *Foods*, vol. 10, no. 2477, pp. 1-16.
- Dan, G, Helikhm A, Duan, Z, Liu, Y & Shang, F 2022, 'Development of pumpkin seed meal biscuits', *Eastern-European Journal of Enterprise Technologies*, vol. 2, no. 11, issue 116, pp. 46-42.
- Danbature, WL, Yahaya, A & Muhammad, UY 2013, 'Proximate and some mineral analysis of pumpkin (*Cucurbita maxima*) leaf', *Journal of Physical Sciences and Environmental Safety*, vol. 3, issue 1.
- Ebrahimi, P, Shokramraji, Z, Tavakkoli, S, Mihaylova, D & Lante, A 2023, 'Chlorophylls as natural bioactive compounds existing in food by-products: A critical review', *Plants*, vol. 12, issue 7, pp. 1533-1533.
- Elinge, CM, Muhammad, A, Atiku, FA, Itodo, AU, Peni, IJ, Sanni, OM & Mbongo, AN 2012, 'Proximate, mineral and anti-nutrient composition of pumpkin (*Cucurbitapepo* L.) seeds extract' *International Journal of Plant Research*, vol. 2, issue 5, pp. 146-150.
- Enujiugha, VN, Oluwole, TF, Talabi, JY & Okunlola, AI 2014, 'Selected bioactive components in fluted pumpkin (*Telfairia occidentalis*) and amaranth (*Amaranthus caudatus*) leaves', *American Journal of Experimental Agriculture*, vol. 4, issue 9, pp. 996-1006.
- Filho, JGDO & Egea & MB 2021, 'Sunflower seed byproduct and its fractions for food application: An attempt to improve the sustainability of the oil process', *Journal of Food Science*, vol. 86, issue 5, pp. 1497-1510.
- Gadekar, YP, Jairath, G, Soni, A, Bhatt, RS, Yashavanth, BS & Eric, NP 2025, 'Utilisation of pumpkin seed (*Cucurbita maxima*) as a meat matrix preservative: Influence on colour and lipid stabilities', *Meat Science*, vol. 223, p. 109769.
- Gao, D, Helikh, A, Duan, Z, Liu, Y & Shang, F 2022, 'Development of pumpkin seed meal biscuits', *Eastern-European Journal of Enterprise Technologies*, vol 2/11, issue 116, pp. 36-42.
- Gowtham, K, Saruniyadevi, M, Thenmozhi, M, Khanna, VG & Jayanthi, M 2022, 'Bio characterization via FTIR and GCMS analysis of cucurbita variety (yellow and white pumpkin)', *Journal of Experimental Biology and Agricultural Sciences*, vol. 10, issue 5, pp. 1076-1092.
- Hao, ZW, Zhang, ZY, Wang, ZP, Wang, Y, Chen, JY, Chen, TH, Shi, G, Li, HK, Wang, JW, Dong, MC, Hong, L & Li, JF 2024, 'Bioactive peptides and proteins for tissue repair: Microenvironment modulation, rational delivery, and clinical potential' *Military Medical Research*, vol. 11, no. 75, pp. 1-62.
- Hernández-Santos, B, Martínez-Sánchez, CE, Torruco-Uco, JG, Rodríguez-Miranda, J, Ruiz-López, II, Vajando-Anaya, ES, Carmona-García, R & Herman-Lara, E 2016, 'Evaluation of physical and chemical properties of carrots dried by refractance window drying', *Drying Technology*, vol. 34, no. 12, pp. 1414-1422.
- Hussain, A 2023, 'Development of nutritional biscuits for children, rich in Fe and Zn, by incorporation of pumpkin (*Cucurbita maxima*) seeds powder; a healthy pharma food in current post COVID 19 period', *Pure and Applied Biology*, vol. 12, no. 1, pp. 392-403.
- Indrianingsih, AW, Rosyida, VT, Darsih, C, Apriyana, W, Iwansyah, AC, Khasanah, Y, Kusumaningrum, A, Windarsih, A, Herawati, ERV, Muzdalifah, D & Sulistyowaty, MI 2024 'Physicochemical properties, antioxidant activities, β -carotene content, and sensory properties of cookies from pumpkin (*Cucurbita moschata*) and modified cassava flour (*Manihot esculenta*)', *Bioactive Carbohydrates and Dietary Fibre*, vol. 31, p. 100398.
- Jackson, KH, Harris, WS, Belury, MA, Kris-Etherton, PM & Calder, PC 2024, 'Beneficial effects of linoleic acid on cardiometabolic health: an update', *Lipids in Health and Disease*, vol. 23, no. 296, pp. 1-8.
- Kiczorowska, B, Samolinska, W, Andrejko, D, Kiczorowski, P, Antoszkiewicz, Z, Zajac, M, Winiarska-Mieczan, A & Bakowski, M 2019, 'Comparative analysis of selected bioactive components (fatty acids, tocopherols, xanthophyll, lycopene, phenols) and basic nutrients in raw and thermally processed camelina, sunflower and flax seeds (*Camelina sativa* L. Crantz, *Helianthus* L., and *Linum* L.)', *Journal of Food Science and Technology*, vol. 56, no. 9, pp. 4296-4310.
- Kim, HN, Lee, YA & Song, SW 2024, 'Sunflower seed extract supplementation reduces body fat in adults with obesity: a double-blind, randomized, placebo-controlled trial', *Nutrition Research*, vol. 122, pp. 113-122.
- Kim, MY, Kim, EJ, Kim, YN, Choi C & Lee, BH 2012, 'Comparison of the chemical compositions and nutritive values of various pumpkin (*Cucurbitaceae*) species and parts', *Nutrition Research and Practice*, vol. 6, no. 1, pp. 21-27.
- Krisridwany, A, Ningrum, DWC, Susilowati, F, Octavia, R & Winanta, A 2023, 'The cytotoxic activities of n-Hexane extract of pumpkin seed (*Cucurbita moschata*) on widr colon cancer cell and hela cervical cancer cell', *Asian*

- Journal of Pharmaceutical Research and Development, vol. 11, no. 5, pp. 1–6.
- Li, Z, Xiang, F, Huang, X, Liang, M, Ma, S, Gafurov, K, Gu, F, Guo, Q & Wang, Q 2024, 'Properties and characterization of sunflower seeds from different varieties of edible and oil sunflower seeds', *Foods*, vol. 13, no. 1188, pp. 1-18.
- Liu, HW, Qin, ZH, Xie, HL & Cao, S 2013, 'Study on the determination of 28 inorganic elements in sunflower seeds by ICP-OES/ICP-MS', *Guang Pu Xue Yu Guang Pu Fen Xi*, vol. 33, no. 1, pp. 224–227.
- Majnooni, MB, Fakhri, S, Bahrami, G, Naseri, M, Farzaei, MH & Echeverría, J 2021, 'Alkaloids as potential phytochemicals against SARS-CoV-2: Approaches to the associated pivotal mechanisms', *Evidence-Based Complementary and Alternative Medicine*, vol. 2021, no. 6632623, pp. 1–21.
- Mandal, M & Mishra, S 2020, 'Nutritional value of product and its enrichment using super seeds (pumpkin seeds, chia seeds, sunflower seeds and almond) in application of ready to eat upma', *Annals, Food Science and Technology*, vol. 21, no. 1, pp. 48-54
- Mesquita, LMS, Martins, M, Pisani, LP, Ventura, SPM & Rosso, VV 2020, 'Insights on the use of alternative solvents and technologies to recover bio-based food pigments', *Comprehensive Reviews in Food Science and Food Safety*, vol 20, issue 1, pp. 787-818.
- Monica, SJ, John, S, Madhanagopal, R, Sivaraj, C, Khusro, A, Arumugam, P, Gajdács, M, Lydia, DE, Sahibzada, MUK, Alghamdi, S, Almeahadi, M & Emran, TB 2022, 'Chemical composition of pumpkin (*Cucurbita maxima*) seeds and its supplemental effect on Indian women with metabolic syndrome', *Arabian Journal of Chemistry*, vol. 15, no. 103985, pp. 1-14.
- Mounes, HAM, Zeinab, MA, Azeem, AE, El-Bary, DAA, Al-Sagheer, AA, Abd-Elhakim, YM, Hassan, BA, Sadek, SS & Ahmed, KM 2024, 'Effect of substituting soybean meal in oreochromis niloticus diets with pumpkin (*Cucurbita maxima*) seed cake on water quality, growth, antioxidant capacity, immunity, and carcass composition', *Animals (Basel)*, vol. 14, no. 195, pp. 1-15.
- Muttagi, GC & Joshi, N 2020, 'Physico-chemical composition of selected sunflower seed cultivars', *International Journal of Chemical Studies*, vol. 8, issue 4, pp. 2095–2100.
- Nor-Aleesya, MN, Wan-Mohtar, WAAQI, Inshirah, I, Farhana, R, Nazimah, H, Mohamad, YNS, Aida, FMNA & Raseetha, S 2023, 'Stability of pigments from *Termitomyces* sp (termite mushroom), *Pleurotus citrinopileatus* (yellow oyster mushroom) and *Pleurotus djamor* (pink oyster mushroom) as natural food colouring', *Food Research*, vol. 7, issue Suppl. 4, pp. 84-92.
- Novais, C, Molina, AK, Abreu, RMV, Santo-Buelga, C, Ferreira, ICFR, Pereira, C & Barros, L 2022, 'Natural food colorants and preservatives: A review, a demand, and a challenge', *Journal of Agricultural and Food Chemistry*, vol. 70, issue 9, pp. 2789–2805.
- Nzotta, AO & Onabanjo, RS 2021, 'Evaluation of flaxseed, sesame and pumpkin seeds as an alternative source of functional feed ingredients', *Nigerian Journal of Animal Science*, vol. 23, issue 1, pp. 116–125.
- Oh, J, Hong, S, Ko, SH & Kim, HS 2024, 'Evaluation of antioxidant effects of pumpkin (*Cucurbita pepo* L.) seed extract on aging- and menopause-related diseases using saos-2 cells and ovariectomized rats', *Antioxidants*, vol. 13, issue 2, pp. 241–241.
- Patel, DM, Rani, KS & Patani, P 2024, 'A comprehensive review of botanical ingredients like pumpkin seeds, black cumin seeds and burdock roots and their efficacy for healthy hair and preventing premature graying of hairs', *Journal of Population Therapeutics and Clinical Pharmacology*, vol. 31, issue 1, pp. 2171–2888.
- Patel, K, Soni, A & Tripathi, R 2023, 'Pumpkin seed: Nutritional composition, health benefits', *International Journal of Home Science*, vol. 9, issue 3, pp. 93–98.
- Petraru, A, Ursachi, F & Amariei, S 2021, 'Nutritional characteristics assessment of sunflower seeds, oil and cake. perspective of using sunflower oilcakes as a functional ingredient', *Plants*, vol. 10, no. 2487, pp.1-22.
- Polyzos, N, Fernandes, Â, Calhelha, RC, Petrović, J, Soković, M, Isabel, Barros, L & Petropoulos, SA 2024, 'Biochemical composition of pumpkin seeds and seed by-products', *Plants*, vol. 13, issue 17, pp. 2395–2395.
- Prakash, V, Kumar, A, Chaudhary, Y, Joshi, OP & Kumar, R 2024, 'Investigating the anxiolytic potential of *Helianthus annuus* L. seeds: A comprehensive study', *Educational Administration: Theory and Practice*, vol. 30, issue 1, pp. 793–801.
- Rajhard, S, Hladnik, L, Vicente, FA, Srčić, S, Grilc, M & Likozar, B 2021, 'Solubility of luteolin and other polyphenolic compounds in water, nonpolar, polar aprotic and protic solvents by applying FTIR/HPLC', *Processes*, vol. 9, issue 11, no. 1952, pp. 1-15.
- Rasheed, N, Ahmed, A, Nosheen, F, Imran, A, Islam, F, Noreen, R, Chauhan, A, Shah, MA & Amer Ali, Y 2023,

- 'Effectiveness of combined seeds (pumpkin, sunflower, sesame, flaxseed): As adjacent therapy to treat polycystic ovary syndrome in females', *Food Science & Nutrition*, vol. 11, issue 6, pp. 3385-3393.
- Ray, V & Singh, SP 2023, 'Calcium and magnesium rich cookies, fortified with pumpkin (*Cucurbita moschata*) and sunflower seed (*Helianthus annuus* L.)', *Current Journal of Applied Science and Technology*, vol. 42, issue 18, pp. 7-15.
- Razzak, A, Mahjabin, T, Khan, MR, Hossain, M, Sadia, U & Zzaman, W 2023, 'Effect of cooking methods on the nutritional quality of selected vegetables at Sylhet City', *Heliyon*, vol. 9, issue 11, no. e21709, pp. 1-9.
- Sá, AGA, Silva, DC, Pacheco, MTB, Moreno, YMF & Carciofi, BAM 2021, 'Oilseed by-products as plant-based protein sources: Amino acid profile and digestibility', *Future Foods*, vol. 3, issue 2021, no. 100023, pp. 1-7.
- Saini, RK, Prasad, P, Lokesh, V, Shang, X, Shin, J, Keum, YS & Lee, JH 2022, 'Carotenoids: Dietary sources, extraction, encapsulation, bioavailability, and health benefits—A review of recent advancements', *Antioxidants (Basel)*, vol. 11, issue 4, no. 795, pp. 1-34.
- Shajan, AE, Dash, KK, Hamid, N, Bashir, O & Shams, R 2024, 'Comprehensive comparative insights on physico-chemical characteristics, bioactive components, and therapeutic potential of pumpkin fruit', *Future Foods*, vol. 9, issue 2024, no. 100312, pp. 1-17. doi: 10.1016/j.fufo.2024.100312
- Siddiqui, K, Bawazeer, N & Joy, SS, 2014, 'Variation in macro and trace elements in progression of type 2 diabetes', *The Scientific World Journal*, vol. 2014, issue 1, pp. 1-9.
- Singh, A & Kumar, V 2023, 'Pumpkin seeds as nutraceutical and functional food ingredient for future: A review', *Grain & Oil Science and Technology*, vol. 7, issue 1, pp. 12-29.
- Sinkovič, L & Kolmanič, A 2021, 'Elemental composition and nutritional characteristics of *Cucurbita pepo* subsp. *pepo* seeds, oil cake and pumpkin oil', *Journal of Elementology*, vol. 26, issue 1, pp. 97-107.
- Smeriglio, A, Barreca, D, Bellocco, E & Trombetta, D 2016, 'Proanthocyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects', *British Journal of Pharmacology*, vol. 174, issue 11, pp. 1244-1262.
- Storz, MA 2021, 'Lifestyle adjustments in long-COVID management: Potential benefits of plant-based diets. *Current Nutrition Reports*, vol. 10, issue 4, pp. 352-363.
- Stupar, A, Seregelj, V, Ribeiro, BD, Pezo, L, Cvetanovic, A, Misan, A & Marrucho, I 2021, 'Recovery of β -carotene from pumpkin using switchable natural deep eutectic solvents', *Ultrasonics Sonochemistry*, vol. 76, p. 105638.
- Syed, QA, Akram, M & Shukat, R 2019, 'Nutritional and therapeutic importance of the pumpkin seeds', *Biomedical Journal of Scientific & Technical Research*, vol. 21, issue 2, pp. 15798-15803.
- Tenyang, N, Ponka, R, Tiencheu, B, Djikeng, FT & Womeni, HM 2021, 'Effect of boiling and oven roasting on some physicochemical properties of sunflower seeds produced in Far North, Cameroon' *Food Science & Nutrition*, vol 10, issue 2, pp. 402-411.
- Ullah, A, Munir, S, Badshah, SL, Khan, N, Ghani, L, Poulson, BG, Emwas, AH & Jaremko, M 2020, 'Important flavonoids and their role as a therapeutic agent', *Molecules*, vol. 25, issue 22, no. 5243, pp. 1-39.
- Wan-Mohtar, WAAQI, Khalid, NI, Rahim, MHA, Luthfi, AAI, Zaini, NSM, Din, NAS & Zaini, NAM 2023, 'Underutilised Malaysian agro-industrial wastes as sustainable carbon sources for lactic acid production', *Fermentation*, vol 9, issue 905, pp. 1-19.
- Wongsa, P, Phatikulrungsun, P & Prathumthong, S 2022, 'FT-IR characteristics, phenolic profiles and inhibitory potential against digestive enzymes of 25 herbal infusions', *Scientific Reports*, vol. 12, issue 1, no. 6631.
- Yan, Y, Li, X, Zhang, C, Lv, L, Gao, B & Li, M 2021, 'Research progress on antibacterial activities and mechanisms of natural alkaloids: A review', *Antibiotics (Basel)*, vol. 10, issue 3, no. 318, pp. 1-30.
- Yang, Z, Amrit, BK, Zhao, W, Shi, L, Wu, H, Barrow, C, Dunshea, F & Suleria, HAR 2022, 'Bioaccessibility and bioavailability changes of phenolic compounds in pumpkins (*Cucurbita moschata*): A review', *Food Bioscience*, vol. 47, no. 101753.
- Younes, S 2024, 'The role of nutrition on the treatment of Covid 19', *Human Nutrition & Metabolism*, vol. 36, no. 200255, pp. 1-16.
- Yuan, L, Jiang, Q, Zhai, Y, Zhao, Z, Liu, Y, Hu, F, Qian, Y & Sun, J 2023, 'Association between plant-based diet and risk of chronic diseases and all-cause mortality in centenarians in China: A cohort study', *Current Developments in Nutrition*, vol. 8, issue 1, no. 102065, pp. 1-10.