

# Effect of different drying methods on phenolic content, antioxidant, antidiabetic, anti-obesity, and inhibition kinetic properties of selective green leafy vegetables

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**Abstract.** Maser WH, Karnjanapratum S, Kingwascharapong P, Venkatachalam K, Ali AMM, Bavisetty SCB. 2023. Effect of different drying methods on phenolic content, antioxidant, antidiabetic, anti-obesity, and inhibition kinetic properties of selective green leafy vegetables. *Biodiversitas* 24: 4896-4909. The present study assessed the impact of dehydration drying, oven drying, and freeze drying on Total Phenolic Content (TPC), antioxidant activities (DPPH, metal chelating, and FRAP), and the inhibition activity of  $\alpha$ -amylase,  $\alpha$ -glucosidase, and lipase in selected green leafy vegetables, namely celery (*Apium graveolens* L.), coriander (*Coriandrum sativum* L.), parsley (*Petroselinum crispum* (Mill.) Fuss), and spring onion (*Allium cepa* L.). FTIR analysis was conducted to examine the functional groups, GC-MS was utilized to characterize the compounds, and a kinetic study of  $\alpha$ -glucosidase inhibition was undertaken to assess its inhibitory mode as an antidiabetic agent. Freeze drying exhibited the highest extraction yield (17.89% to 26.15%). However, freeze-drying showed lower drying efficiency, TPC, and bioactivities. Oven drying showed the highest bioactivities, including antioxidant and enzyme inhibitory activities. Spring onion demonstrated the highest bioactivity with dehydration drying, particularly in  $\alpha$ -glucosidase inhibitory activity. The results notified that oven drying can be highly suitable for green leafy vegetables with thinner structures, facilitating an efficient drying process. On the other hand, dehydration drying is better suited for thicker green leafy vegetables with the appropriate heat pump intensity. The results for bioactivities were aligned with the observed inhibition mechanism, functional groups, and bioactive compounds in the samples analyzed via FTIR and GC-MS analysis. Therefore, selecting the drying method for a specific sample should consider its unique characteristics to ensure optimal bioactivity.

**Keywords:**  $\alpha$ -Amylase,  $\alpha$ -glucosidase, drying methods, GC-MS, green leafy vegetables, inhibition mechanism, lipase

## INTRODUCTION

Drying is among the most ancient and fundamental processing methods; it entails applying heat to a substance, which affects the transport of water content within the substance to its surface and then the water loss from the substance to the environment (Onwude et al. 2016). The most effective drying method is determined by several factors, including product kinds, drying conditions, drying efficiency, drying operational expenses, and other critical selection parameters such as energy usage and product quality (Onwude et al. 2017). Drying can be achieved through different techniques, such as sun drying, oven drying, dehydration drying, and in freeze drying. Each method possesses distinct characteristics that can influence the preservation of bioactive compounds, potentially altering these vegetables' nutritional quality and health benefits (Ademiluyi et al. 2018). The specific drying method employed can significantly impact the bioactivity of plant materials. Different drying methods involve varying temperature

conditions (Abd Rahman et al. 2018). High temperatures in specific drying techniques, such as microwave or oven drying, may cause thermal degradation of sensitive bioactive compounds (Abd Rahman et al. 2018). Prolonged exposure to high temperatures leads to the loss of heat-sensitive vitamins, enzymes, and phytochemicals, thus reducing the overall bioactivity (Ademiluyi et al. 2018). In addition, the drying process can expose plant materials to oxygen, thus promoting oxidation reactions (Abd Rahman et al. 2018). Oxidation can result in the degradation of certain bioactive compounds, particularly those with antioxidant properties (Amorim et al. 2020). Antioxidants are known to protect cells from damage caused by free radicals; therefore, minimizing oxidation during drying is crucial to preserving the bioactivity of plants (Amorim et al. 2020).

Some drying methods, such as freeze drying, may involve longer drying, allowing for prolonged enzymatic activity (Ho et al. 2018). Enzymes naturally present in plants can catalyze reactions that affect the bioactivity of specific compounds. For example, enzymes like polyphenol

oxidase can cause the degradation of phenolic compounds, reducing their bioavailability and potential health benefits (García et al. 2021). Moreover, drying removes water from plant materials, altering their physical and chemical properties (Abd Rahman et al. 2018). Removing water can increase the concentration of bioactive compounds, potentially enhancing their bioactivity (Ademiluyi et al. 2018). However, excessive drying or over-drying can result in the loss of essential water-soluble nutrients and affect the overall bioactivity of the dried product (Amorim et al. 2020). Thus, evaluating drying conditions and techniques is crucial to minimize the negative impacts on bioactivity and maximize the retention of beneficial compounds.

Green Leafy Vegetables (GLVs) have long been recognized as a significant component of a healthy diet due to their rich nutritional content and potential health benefits (Natesh et al. 2017). These vegetables are a valuable source of various bioactive compounds, including vitamins, minerals, fiber, and phytochemicals, which have been associated with numerous positive effects on human health (Natesh et al. 2017), such as antioxidant activities (Vinholes and Vizzotto 2017), antidiabetic (Maser et al. 2023), anti-obesity (Paul and Majumdar 2022), antimicrobial (Erugur et al. 2019), anti-Alzheimer's disease (Erugur et al. 2019), anti-tyrosinase activity (Blahova et al. 2021), etc. Understanding the effects of drying methods on the bioactivity of GLVs is crucial for retaining the maximum bioactivity and health benefits. For example, in plants, Shonte et al. (2020) reported that freeze-drying exhibited lower total antioxidant activity and phenolic content values than oven-dried in stinging nettle leaves. García et al. (2021) reported that the process of dehydration drying led to a substantial increase in the total phenolic content and antioxidant activity, along with notable levels of specific phenolic compounds such as caffeoylmalic acid and caffeoylmalic acid dimer in *U. dioica* L. leaves. In addition, the application of freeze-drying methods resulted in elevated levels of phytoconstituents, antioxidant capacity, and enzyme ( $\alpha$ -amylase and  $\alpha$ -glucosidase) inhibitory effect in moringa leaf (Ademiluyi et al. 2018). Therefore, comparing the effect of drying on GLVs would be of great interest. A comparison of the antioxidant and enzyme inhibitory activity of various drying methods in celery (*Apium graveolens*), coriander (*Coriandrum sativum*), parsley (*Petroselinum crispum*), and spring onion (*Allium cepa*) has not been reported in the literature. This study, therefore, investigates the inhibitory effects of  $\alpha$ -amylase,  $\alpha$ -glucosidase, and lipase and antioxidant activities of celery, coriander, parsley, and spring onion extracts from various drying methods. Several techniques, such as Fourier Transform Infrared spectroscopy (FTIR), Gas Chromatography-Mass Spectrometry (GC-MS), and kinetic analysis of  $\alpha$ -glucosidase inhibition, were employed to evaluate its inhibitory mechanism as an antidiabetic agent.

## MATERIALS AND METHODS

### Reagents and chemicals

$\alpha$ -Glucosidase enzyme, p-nitrophenyl- $\alpha$ -D-glucopyranoside (p-NPG),  $\alpha$ -amylase enzyme (Type IV-B), acarbose, 4-

methylumbelliferyl oleate (4MUO), pancreatic lipase enzyme (Type II), methoxyamineHCl, N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) with 1% trimethylchlorosilane and pyridine were purchased from Sigma-Aldrich (MO, USA). Gallic acid, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), L-Ascorbic acid, Folin-Ciocalteu reagent, Ethylenediaminetetraacetic Acid (EDTA), and 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p-disulfonic acid monosodium salt hydrate (Ferrozine) were bought from Acros Organics (NJ, USA). 2,2-Diphenyl-1-picrylhydrazil was acquired from Thermo Fisher Scientific (Ward Hill, USA). The chemicals utilized were of analytical grade.

### Materials

Celery, coriander, parsley, and spring onion aerial parts were obtained from Hua Takhe market in Bangkok. The gathering and experimental investigations of the samples followed the guidelines set forth by the national authorities of Thailand (NIH 2023). Dr. Ali Muhammed Moula Ali identified the GLVs using LeafSnap, a pioneering mobile application for automated GLV species identification. Each GLV was carefully packed from Hua Takhe market and transported to KMITL, Bangkok. The GLVs were obtained fresh, promptly washed, and determined for moisture content.

### Preparation of samples

The cleaned GLVs samples were dried using Progress Type 016010 oven drier at 45°C (Bangkok, Thailand), IKE WRH-100 dehydration dryer at 45°C (Guangdong, China), and Kinetic Model LD0.5 freeze drier at -50°C (Bangkok, Thailand). The complete drying process was conducted until it reached a consistent weight. Before extraction, the samples underwent grinding with a Philips HR 2222 (Best, Netherlands) to form a powdered consistency and were stored in airtight plastic bags (PP). The moisture content of dried samples was determined. The drying efficiency (%) was calculated using the equation below (Kaveh et al. 2021):

$$\text{The drying efficiency (\%)} = \left( \frac{E_{\text{evap}} + E_{\text{heating}}}{\text{EU}} \right) \quad (1)$$

$$E_{\text{evap}} = h_{f,g} \times M_w \quad (2)$$

$$E_{\text{heating}} = W_d \times C_m \times \Delta T \quad (3)$$

$$C_m = 1465 + 3560 \left[ \frac{M_p}{(1 + M_p)} \right] \quad (4)$$

$$M_p = \frac{(W_w - W_d)}{W_d} \quad (5)$$

Where:

$E_{\text{evap}}$ : Energy consumed to evaporate moisture from drying samples (kJ)

$E_{\text{heating}}$ : Energy for the material heating (kJ)

EU: Total energy consumption

$h_{f,g}$ : Vaporization latent heat (kJ/kg)

$C_m$ : Specific heat of material (kJ/kg K)

$\Delta T$ : Temperature difference (°C)

$M_w$ : Evaporated water mass from the samples (kg)

$W_d$ : Dry matter mass of samples(kg)

$W_w$ : Mass of the initial samples (kg)

$M_p$ : Dry basis particle moisture content (kg water/kg solid)

### Extract preparation

The GLVs (100 g) were combined with 80% ethanol in a ratio of 1:10 (w/v) and then homogenized using IKA T25 Ultra-Turrax homogenizer (Staufen, Germany) at  $28 \pm 2^\circ\text{C}$  with a rotation speed of 13,000 rpm for 2 min. After macerating the samples for 24 h at  $4^\circ\text{C}$ , they were centrifuged using Eppendorf 5910 R centrifuge (Hamburg, Germany) at 4,000 rpm at  $25^\circ\text{C}$  for 15 min. The extracts underwent filtration utilizing Whatman no. 1 filter paper (Maidstone, UK) and then concentrated at  $40^\circ\text{C}$  under vacuum using a Buchi Rotavapor R-300 rotary evaporator (Flawil, Switzerland). The obtained extracts were vacuum freeze-dried at  $-50^\circ\text{C}$  for 48 h using a Kinetic Model LD0.5 freeze drier (Bangkok, Thailand). These extracts were used for analysis.

### Moisture content

The AOAC (2010) Method 930.04 was used to determine the moisture content of fresh and dried GLVs samples.

### Extraction yield

The extraction yield (%) of the sample extract was defined as the dry weight of the extract in comparison to the initial dry weight of GLVs. The yield of the extraction was determined using the given formula:

$$\text{Yield of the extraction (\%)} = \frac{W_1}{W_2} \times 100\% \quad (6)$$

Where:

$w_1$ : the dry weight of the obtained extract

$w_2$ : dry weight of initial GLVs samples.

### Total Phenolic Content (TPC)

TPC analysis was carried out following the method outlined by Bavisetty and Venkatachalam (2021) with minor modifications. The dissolved extract had a 1 mg/mL concentration of 50% methanol. The samples (100  $\mu\text{L}$ ) were mixed with 200  $\mu\text{L}$  of 10% Folin-Ciocalteu reagent and then added 800  $\mu\text{L}$  of 700 mM sodium carbonate. The mixtures were left to incubate in the dark at ambient temperature for 2 h. The samples' absorbance was recorded at a wavelength of 765 nm using a PerkinElmer EnSight microplate reader (MA, USA). A calibration curve was generated utilizing gallic acid. The TPC was quantified as milligrams of Gallic Acid Equivalent (GAE) per gram of the extract.

### DPPH radical scavenging analysis

The DPPH radical scavenging assay was performed with some modifications, as described by Ali et al. (2021). The extracts were dissolved in methanol (1 mg/mL). These samples (100  $\mu\text{L}$ ) were mixed with 100  $\mu\text{L}$  of 0.2 mM DPPH. The mixture was left in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. A standard curve was constructed using L-ascorbic acid. DPPH radical scavenging activity was calculated as L-ascorbic acid equivalent antioxidant capacity (mg AEAC/g extract).

### Metal chelating analysis

The assay was measured following the procedure outlined by Maser et al. (2023). The extracts were

dissolved in methanol at 1 mg/mL concentrations. 100  $\mu\text{L}$  of the samples were combined with 25  $\mu\text{L}$  of  $\text{FeCl}_2$  (0.6 mM) and 25  $\mu\text{L}$  of Ferrozine (5 mM). The mixture was allowed to stand for 10 min at room temperature. The measurement of absorbance was conducted at a wavelength of 562 nm. A standard curve was constructed using Ethylenediaminetetraacetic Acid (EDTA). The metal chelating activity was calculated as milligrams of EDTA Equivalent Chelating Capacity (EECC) per gram of the extract.

### Ferric reducing antioxidant power (FRAP) analysis

The FRAP analysis was carried out as described by Ali et al. (2021). The extracts were dissolved in methanol (1 mg/mL). The samples (25  $\mu\text{L}$ ) were mixed with 175  $\mu\text{L}$  of FRAP reagent. The mixture was left in the dark at room temperature for 30 min. The absorbance was measured at 593 nm. A standard curve was constructed using L-ascorbic acid. FRAP was calculated as L-ascorbic acid equivalent antioxidant capacity (mg AEAC/g extract).

### $\alpha$ -Amylase inhibitory assay

The  $\alpha$ -amylase inhibitory assay was determined as described by Maser et al. (2023). The samples (20  $\mu\text{L}$  at a concentration of 1 mg/mL) were combined with 20  $\mu\text{L}$  of  $\alpha$ -amylase solution (20 U/mL) and kept in a BINDER Model BD 56 incubator (Tuttlingen, Germany) for 10 min at  $37 \pm 2^\circ\text{C}$ . The mixture was added with 30  $\mu\text{L}$  of starch solution (0.5 %) and then incubated for 8 min at  $37 \pm 2^\circ\text{C}$ . The reaction was halted by adding 20  $\mu\text{L}$  of HCl (1 M) and 100  $\mu\text{L}$  of iodine solution (0.25 mM). The measurement of absorbance was performed at a wavelength of 565 nm. A calibration curve was established using acarbose. The inhibitory activity was determined as millimoles of Acarbose Equivalent (ACE) per gram of the extract.

### $\alpha$ -Glucosidase inhibitory assay

The  $\alpha$ -glucosidase inhibitory assay was conducted following the method outlined by Maser et al. (2023). The samples (10  $\mu\text{L}$  at a concentration of 10 mg sample per mL DMSO) were combined with 50  $\mu\text{L}$  of phosphate buffer (0.1 M, pH 6.9), 25  $\mu\text{L}$  of 0.1 U/mL  $\alpha$ -glucosidase, and 25  $\mu\text{L}$  of 10 mM p-NPG. Subsequently, the mixture was kept in a BINDER Model BD 56 incubator (Tuttlingen, Germany) at  $37 \pm 2^\circ\text{C}$  for 30 min. The reaction was stopped by adding 100  $\mu\text{L}$  of sodium carbonate (0.2 M), and absorbance was measured at a wavelength of 410 nm. A calibration curve was created using acarbose. The activity was determined as millimoles of Acarbose Equivalent (ACE) per gram of the extract.

### Lipase inhibitory assay

The lipase inhibitory assay followed the procedure Maser et al. (2023) outlined. The prepared samples (25  $\mu\text{L}$ ) at concentrations of 1 mg/mL were combined with 50  $\mu\text{L}$  of 4MUO (0.5 mM) and 25  $\mu\text{L}$  of lipase solution (50 U/mL). Afterward, the combination was left to incubate at room temperature for 30 minutes. The reaction was stopped by adding 100  $\mu\text{L}$  of sodium citrate (0.1 M, pH 4.2). The fluorescence intensity was recorded at 355 nm during excitation and 460 nm during emission. The percentage

inhibition of the activity was determined using the provided formula:

$$\text{Inhibition (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100\% \quad (7)$$

Where:

$A_0$ : intensity of the fluorescence in the absence of the extract

$A_1$ : intensity of the fluorescence in the presence of the extract.

### FTIR analysis

The analysis was measured following the method outlined by Ali et al. (2019). The GLVs extracts (5 mg) were analyzed using a Bruker Invenio-S FTIR spectrophotometer (Ettlingen, Germany). The FTIR spectra were captured in the 4000–400  $\text{cm}^{-1}$  range using 32 scans per minute in absorbance mode, with a resolution of 4  $\text{cm}^{-1}$ . The data were analyzed using Bruker OPUS software ver. 4.2 (Ettlingen, Germany).

### GC-MS analysis

The extracts were derivatized following a description by Maser et al. (2023) using various chemicals, including pyridine, methoxyamine HCl, and MSTFA (N-Methyl-N-(trimethylsilyl)trifluoroacetamide) with 1% trimethylchlorosilane. A 1  $\mu\text{L}$  volume of the derivatized sample was injected into the Agilent Technologies 6890-GC-MS system (CA, USA) with a mass-selective detector (HP-5973). A 5% DB-5MS phenyl methyl siloxane column was used for the GC analysis, and helium was employed as the carrier gas at a 1 mL/min rate. The analysis process in the GC-MS system followed the one previously described by Maser et al. (2023). For the identification of metabolites, the retention time and chromatogram peak of each compound were compared with the information available in Wiley7n.l database. The results were expressed in peak abundance, and the similarity between the compounds and the library data was reported as a percentage (%).

### Kinetic study of the $\alpha$ -glucosidase inhibition

The inhibition of the  $\alpha$ -glucosidase enzyme was carried out as previously mentioned. The kinetic analysis, which revealed the mode of inhibition against  $\alpha$ -glucosidase, was conducted using different levels of samples (spring onion and parsley from dehydration drying) at concentrations of 0, 5, and 10 mg/mL and varying p-NPG concentrations (0, 1.25, 2.5, 5, and 10 mM). The initial reaction rate ( $v$ ) was calculated for different substrate and sample concentrations. The models employed were as outlined by Mittal et al. (2023), as detailed below:

Lineweaver-Burk equation:

$$\frac{1}{v} = \left[ \left( \frac{K_m}{V_{max}} \right) \times \left( \frac{1}{S} \right) \right] + \frac{1}{V_{max}} \quad (8)$$

Where:

$v$ : the initial reaction rate of the substrate at varying concentrations

$v_{max}$ : the maximum reaction rate

$K_m$ : the Michaelis inhibition constants

$S$ : the substrate concentration.

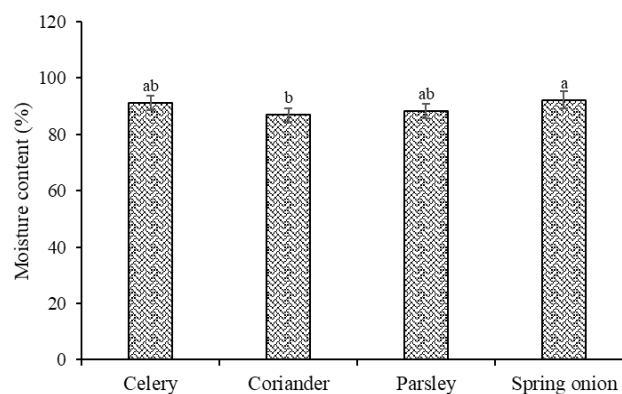
### Statistical analysis

All experiments were expressed as means  $\pm$  standard deviations in triplicates for each sample. A one-way ANOVA and Duncan Multiple Comparison, significance levels tests, were conducted at a significance level of  $p < 0.05$ . The data analysis was performed using SPSS software for Windows 28.0 (IL, USA).

## RESULTS AND DISCUSSION

### The moisture content and efficiency of drying

Figure 1 illustrates the moisture levels of four fresh GLVs. The moisture content varied between 86.86% and 92.23%, suggesting minimal variation among the samples. Fresh GLVs were dried using three different drying processes, i.e., oven, dehydration, and freeze-drying. After drying, the dried samples' remanent moisture content was measured and presented in Table 1. Spring onion exhibited higher water content than the other GLVs, likely due to its denser stems and thicker leaves (Babu et al. 2018). There were no significant variations in moisture content between oven and dehydration drying for the four GLVs ( $p \geq 0.05$ ). The exact temperature at 45°C in both drying methods resulted in similar moisture content. The freeze-drying yields lower levels of moisture content than oven-drying. This study shows the yield of moisture content in freeze-drying leaves at an average of 8.29%. It is lower than oven drying at an average of 10.77% and dehydration drying at 11.45%. Freeze drying proved to be more effective in reducing moisture content, aligning with the findings by Ghasemzadeh et al. (2016) and Tan et al. (2021), which stated that freeze drying yields lower levels of moisture content than oven drying. Freeze drying involves eliminating water through sublimation from frozen substances in an environment characterized by low pressure and temperature. This lyophilization process facilitates efficient water removal (Tang and Pikal 2004).



**Figure 1.** The moisture content of fresh leaves of selected green leafy vegetables ( $n=3$ , SD). Different lowercase letters indicate significant differences ( $p < 0.05$ )

**Table 1.** The moisture content of dehydrated leaves, drying efficiency, and extraction yield of selected green leafy vegetables

Parameters	Drying process	Celery	Coriander	Parsley	Spring onion
The moisture content of dried leaves (%)	Oven drying	11.77 ± 0.75 <sup>aA</sup>	8.98 ± 0.58 <sup>abB</sup>	10.09 ± 0.50 <sup>aB</sup>	12.19 ± 1.02 <sup>aA</sup>
	Dehydration drying	12.10 ± 0.98 <sup>aA</sup>	9.84 ± 0.77 <sup>aB</sup>	10.24 ± 0.58 <sup>aB</sup>	13.62 ± 1.06 <sup>aA</sup>
	Freeze drying	10.02 ± 0.59 <sup>bA</sup>	8.61 ± 0.22 <sup>bB</sup>	6.29 ± 0.51 <sup>bC</sup>	8.25 ± 0.45 <sup>bB</sup>
Drying efficiency (%)	Oven drying	7.37 ± 0.72 <sup>bC</sup>	10.24 ± 0.94 <sup>bB</sup>	15.45 ± 1.34 <sup>aA</sup>	9.57 ± 0.87 <sup>bB</sup>
	Dehydration drying	10.14 ± 0.86 <sup>aC</sup>	12.24 ± 0.95 <sup>aB</sup>	15.64 ± 0.99 <sup>aA</sup>	11.36 ± 0.71 <sup>aBC</sup>
	Freeze drying	3.70 ± 0.24 <sup>cC</sup>	4.62 ± 0.28 <sup>cB</sup>	7.09 ± 0.28 <sup>bA</sup>	4.37 ± 0.36 <sup>aB</sup>
Extraction yield (%)	Oven drying	17.48 ± 1.17 <sup>aB</sup>	10.98 ± 0.81 <sup>bC</sup>	17.61 ± 1.06 <sup>abB</sup>	20.82 ± 1.48 <sup>bA</sup>
	Dehydration drying	16.90 ± 0.45 <sup>aA</sup>	10.95 ± 0.77 <sup>bB</sup>	16.87 ± 1.06 <sup>bA</sup>	17.15 ± 1.30 <sup>cA</sup>
	Freeze drying	17.89 ± 0.87 <sup>aB</sup>	12.75 ± 0.70 <sup>aC</sup>	19.05 ± 0.81 <sup>aB</sup>	26.15 ± 2.44 <sup>aA</sup>

Notes: Mean ± SD from triplicate determinations. Different lowercase letters in the same column indicate significant differences ( $p < 0.05$ ). Different uppercase letters in the row under the parameters tested indicate significant differences ( $p < 0.05$ ).

The drying efficiencies of the four GLVs are shown in Table 1. The GLVs from dehydration drying exhibited high drying efficiency (10.14% to 15.64%). This can be attributed to the constant airflow passage in a sealed environment, which results in more efficient energy utilization (García et al. 2021). On the other hand, the GLVs from freeze drying exhibited the lowest drying efficiency (3.70% to 7.09%). This aligns with a study by Boateng et al. (2021) wherein it is reported that freeze drying requires a more considerable amount of energy to maintain the vacuum, sublimation, and circulation. Additionally, parsley showed the highest drying efficiency compared to other GLVs. This phenomenon can be attributed to the stems' hollow structure with extensive vascular tissue and the presence of pinnate flat leaves in the samples, which have been shown to exhibit high drying efficiency (Agyare et al. 2017).

### Extraction yields

Table 1 displays the extraction yield of GLVs extracts. The samples obtained from freeze drying exhibited higher extraction yields than the others. This can be attributed to the high extraction efficiency of freeze drying, which occurs due to the formation of ice crystals within the plant matrix (Babu et al. 2018). These ice crystals can rupture the cell structure, allowing the release of cellular components and facilitating solvent access, thereby leading to improved extraction (Babu et al. 2018; Liu et al. 2022). On the other hand, oven drying and dehydration drying showed comparable extraction yields, likely due to their similar moisture content (Table 1). Moisture content plays a role in the mass transfer and extraction kinetics (Ameer et al. 2017). A high moisture content in the plant structure promotes a strong hydrolyzation tendency. Therefore, employing a polar solvent such as 80% ethanol helps penetrate the solvent into the plant matrix, resulting in enhanced mass transfer of solutes and an accelerated rate of compound diffusion from plant samples during the extraction process (Ameer et al. 2017). Overall, the four GLVs demonstrated varying extraction yields. The extraction yields are influenced by factors such as the composition of the plant matrix, moisture content, and the constituents' varying chemical properties and polarities (Ameer et al. 2017; Stramarkou et al. 2017). Overall, spring onion exhibited the highest extraction yield, likely attributed to the effectiveness of the 80% ethanol solvent in

recovering compounds from spring onion in comparison to other GLVs. Similarly, Maser et al. (2023) also reported that spring onion yielded high extraction yields when employing polar solvents, such as 80% ethanol, absolute ethanol, absolute methanol, and hot water.

### Total Phenolic Content (TPC)

Except for the spring onion, the other GLVs obtained from oven drying exhibited the highest TPC (Figure 2). This variation in the drying method effect can be attributed to different herbs responding differently, depending on their species and the type of metabolites they contain (Ghasemzadeh et al. 2016). On the other hand, GLVs from freeze-drying showed low TPC, which could be due to the potential cell disruption caused by freeze-drying. This may lead to the release of certain enzymes and activators that cause the degradation of phenolic compounds (Tan et al. 2021). Moreover, Khoo et al. (2015) reported that the low TPC in freeze-dried material could also be attributed to the strong dehydrating ability of the freeze-drying method, which decreases the extraction of hydrophilic compounds from the plant compared to other drying methods. In addition, the preservation properties of freeze drying may have impeded the effective release of phenolic compounds during the extraction process due to its protective effect on the plant structure (Khoo et al. 2015). Among the four GLVs, celery generally demonstrates high TPC ranging from 17.96 to 28.22 mg GAE/g extract in 80% ethanol. In a study by Chandrashekharaiah (2013), who reported that celery exhibited varying levels of TPC can be recovered based on different solvent types, i.e., ethyl acetate, butanol, and methanol extracts of 18.21, 19.45, and 49.77 mg GAE/g extract, respectively. Mouhoubi et al. (2022) also reported that celery leaves from microwave drying had a higher TPC than parsley leaf extracts.

### DPPH

Table 2 displays the DPPH activity of the four GLVs with three different drying variations. Similar to TPC results, the DPPH activity of oven drying demonstrated the highest activity in celery, coriander, and parsley. Spring onion, on the other hand, showed the highest DPPH activity from dehydration drying. GLVs from freeze drying exhibited the lowest activity, possibly due to the low TPC (Figure 2). The increased antioxidant activity observed in

samples dried at higher temperatures can be attributed to several factors (García et al. 2021).

Firstly, disrupting the cell structure during drying may lead to the release of bound antioxidant substances, such as phenolic compounds, thereby facilitating their natural extraction for analysis (García et al. 2021). Secondly, thermal chemical reactions, precisely the Maillard reaction, can form new antioxidants, such as melanoidins (García et al. 2021). Lastly, the heat-induced inactivation of oxidative enzymes, such as polyphenol oxidase, can suppress oxidation (García et al. 2021). Furthermore, Ho et al. (2018) suggested that the duration of drying time is a critical factor influencing the concentration of antioxidant activity. It is thus essential to minimize the drying time to maximize antioxidant activity (Ho et al. 2018). In this study, the oven and dehydration dried samples were subjected to an overnight drying process, whereas the freeze-dried samples required two days. This observation suggested that as the duration of drying time increases, the antioxidant activity tends to decrease. This phenomenon can be attributed to extended exposure to oxygen, which results in heightened redox activity and the breakdown of phenolic compounds, consequently diminishing the antioxidant activity (Ho et al. 2018).

Coriander exhibited the highest DPPH activity among all the GLVs, except for dehydration drying. Similarly, Palmieri et al. (2020) reported that the activity of DPPH in coriander seeds was higher compared to *T. vulgaris* L. and *Cannabis sativa* L. This activity did not align with the TPC of GLVs, indicating that other compounds may possess potential DPPH activity. Sathishkumar et al. (2016) reported that coriander leaves were rich in alkaloids, glycosides, phenols, saponins, steroids, tannins, and terpenoids. It has been reported that these compounds exhibit antioxidant activity (Zhang et al. 2015).

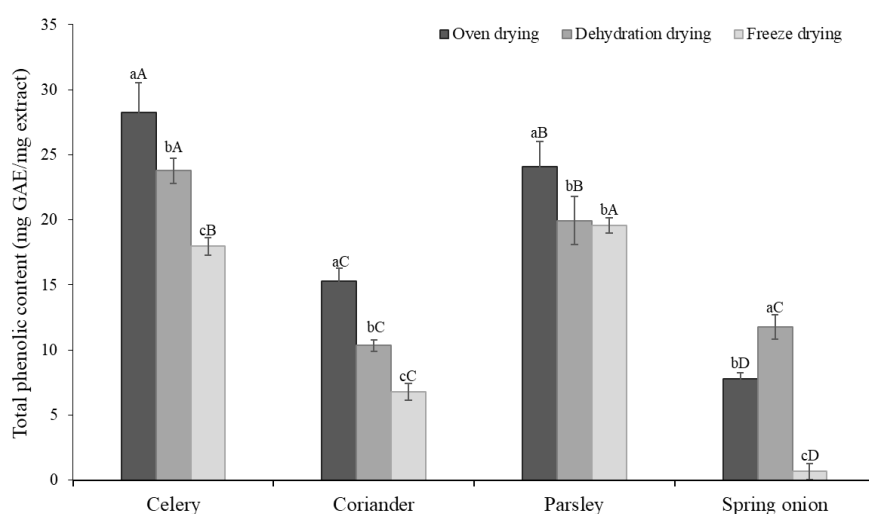
### Metal chelating

The activity of the four GLVs followed a similar pattern to the DPPH activity, with GLVs from oven drying

exhibiting the highest activity (Table 2). Similarly, Amorim et al. (2020) reported that *G. tenuifrons* and *P. capillacea* showed higher metal chelating activity from oven drying than freeze drying. Additionally, coriander demonstrated the highest metal chelating activity compared to other GLVs. The metal chelating activity did not correlate with the TPC (Figure 2). The bioactive compounds such as alkaloids, tannins, terpenoids, steroids, and saponins may be implicated in the metal-chelating activity observed in the coriander (Sathishkumar et al. 2016; Adusei et al. 2019). These compounds can bind with toxic metal ions to form complex structures that neutralize these free radicals and prevent or slow down the damage caused by these free radicals (Adusei et al. 2019).

### FRAP

The Fe (III) reduction is commonly employed in the FRAP assay to assess the electron-donating activity, a valuable indicator of antioxidant activity. In the process of antioxidative action, reducing agents disrupt free radical chains by offering a hydrogen atom, thereby stabilizing them (Mustafa et al. 2019). The FRAP activity of GLVs from oven drying exhibited the highest activity, which followed a similar pattern to other antioxidant activities (Table 2). This can be attributed to various factors, including releasing antioxidant compounds through the thermal destruction of cell walls and subcellular compartments (Shonte et al. 2020). Additionally, thermal chemical reactions can lead to the formation of antioxidants, and the oxidative enzymes responsible for oxidation can be deactivated by heat, thus suppressing the oxidation of antioxidants (Shonte et al. 2020). The increase in total antioxidant activity following heat treatment might be attributed to the enhanced release of phytochemicals from the matrix (Shonte et al. 2020). Furthermore, several researchers have reported that samples obtained from oven drying exhibited higher FRAP activity than freeze drying (Ling et al. 2015; Ghasemzadeh et al. 2016; Abd Rahman et al. 2018; Mustafa et al. 2019).



**Figure 2.** The total phenolic content of selected green leafy vegetables ( $n=3$ , SD). Different lowercase superscripts in the samples indicate significant differences ( $p<0.05$ ). Different uppercase superscripts in the same drying process show significant differences ( $p<0.05$ ).

### $\alpha$ -Amylase inhibitory activity

Table 3 presents the  $\alpha$ -amylase inhibitory activity of the four GLVs using different drying methods. Except for spring onion, GLVs obtained from oven drying generally exhibited the highest activity, followed by dehydration and freeze-drying. This aligns with the findings of Kittibunchakul et al. (2022), who reported that Sacha inchi young leaves from oven drying demonstrated higher inhibitory activity than freeze drying. In general, enzyme inhibition by plant extracts is attributed to the existence of phenolic compounds (Kittibunchakul et al. 2022). The inhibition activity in this study correlated with the TPC (Figure 2), except for celery. This could be possibly because various mechanisms have been suggested to elucidate the enzyme inhibition caused by bioactive compounds derived from plants, primarily emphasizing the specificity of compounds and inhibition mechanism characteristics in influencing the observed effects (Kittibunchakul et al. 2022). In crude biological extracts, numerous enzyme inhibitors have the potential to engage with metal ions and large molecules like proteins and polysaccharides, consequently altering their ability to inhibit enzymes. Thus, the interaction of phenolics may lead to synergistic or antagonistic effects, resulting in notable changes in the overall inhibitory capacity (Kittibunchakul et al. 2022). In addition, parsley generally exhibited the highest activity compared to other GLVs. Similarly, Bashkin et al. (2021) reported that parsley displayed higher activity than *O. basilicum*, *A. sativa*, and *C. cyminum*. Apart from its high TPC, parsley is also rich in essential oils and fatty acids (Stan et al. 2015;

Agyare 2017), which have antidiabetic properties (Masood 2021; Yoon 2021).

### $\alpha$ -Glucosidase inhibitory activity

In contrast to  $\alpha$ -amylase inhibitory activity,  $\alpha$ -glucosidase inhibitory activity in spring onion generally exhibited the highest activity (Table 3), which did not correlate with TPC (Figure 2). Similarly, Kongstad et al. (2015) reported that the  $\alpha$ -glucosidase inhibitory activity derived from spring onion peel was found to be the highest among 25 different plant species, including *R. palmatum* roots, *C. zeylanicum* bark, *B. juncea* leaves, *C. frutescens* fruits, *A. sativum* bulbs, *A. deliciosa* peels, and *G. max* beans. It is likely that the responsible compounds were not phenolic but natural organosulfur compounds, abundant in spring onion and accountable for the antidiabetic activity (Ahmad et al. 2021). Furthermore, dehydration and oven-drying samples exhibited the highest activity, followed by freeze-drying. This aligns with Khoo et al. (2015) report that the inhibition activity in the leaves and stems of *C. nutans* was higher in oven drying than freeze drying. It has been suggested that the drying process enhances the fragility of plant cell walls, making them more susceptible to breakdown and facilitating the release of a higher number of bioactive compounds during the extraction (Khoo et al. 2015). In the case of thermal treatment, such as oven drying, the intercellular spaces may collapse within the plant cell wall's structure, releasing an increased quantity of phytochemicals derived from the plant matrix (Khoo et al. 2015).

**Table 2.** DPPH, metal chelating, and FRAP activities of selected green leafy vegetables

Parameters	Drying process	Celery	Coriander	Parsley	Spring onion
DPPH (mg AEAC/g extract)	Oven drying	130.15 $\pm$ 7.68 <sup>aB</sup>	141.56 $\pm$ 2.22 <sup>aA</sup>	29.19 $\pm$ 1.37 <sup>aC</sup>	11.00 $\pm$ 1.06 <sup>bD</sup>
	Dehydration drying	125.57 $\pm$ 4.27 <sup>aA</sup>	119.42 $\pm$ 6.42 <sup>bA</sup>	21.04 $\pm$ 0.87 <sup>bB</sup>	12.67 $\pm$ 0.58 <sup>aC</sup>
	Freeze drying	59.31 $\pm$ 1.48 <sup>bB</sup>	104.01 $\pm$ 1.88 <sup>cA</sup>	18.41 $\pm$ 1.13 <sup>cC</sup>	9.23 $\pm$ 0.74 <sup>cD</sup>
Metal chelating (mg EECC/g extract)	Oven drying	78.22 $\pm$ 7.59 <sup>aC</sup>	122.56 $\pm$ 2.02 <sup>aA</sup>	92.99 $\pm$ 7.06 <sup>aB</sup>	8.25 $\pm$ 0.50 <sup>bD</sup>
	Dehydration drying	70.33 $\pm$ 5.14 <sup>aC</sup>	92.85 $\pm$ 5.37 <sup>bA</sup>	77.96 $\pm$ 3.02 <sup>bB</sup>	11.07 $\pm$ 1.08 <sup>aD</sup>
	Freeze drying	58.03 $\pm$ 1.52 <sup>bB</sup>	75.66 $\pm$ 4.80 <sup>cA</sup>	45.82 $\pm$ 3.25 <sup>cC</sup>	5.71 $\pm$ 0.42 <sup>cD</sup>
FRAP (mg AEAC/g extract)	Oven drying	35.54 $\pm$ 2.73 <sup>aB</sup>	56.27 $\pm$ 4.11 <sup>aA</sup>	21.60 $\pm$ 1.09 <sup>aC</sup>	18.26 $\pm$ 1.69 <sup>bC</sup>
	Dehydration drying	34.78 $\pm$ 2.03 <sup>aB</sup>	46.98 $\pm$ 3.09 <sup>bA</sup>	19.25 $\pm$ 1.34 <sup>bC</sup>	21.62 $\pm$ 1.36 <sup>aC</sup>
	Freeze drying	13.58 $\pm$ 1.16 <sup>bB</sup>	40.98 $\pm$ 1.85 <sup>bA</sup>	9.77 $\pm$ 0.63 <sup>cC</sup>	6.82 $\pm$ 0.42 <sup>cD</sup>

Notes: Mean  $\pm$  SD from triplicate determinations. Different lowercase letters in the same column indicate significant differences ( $p < 0.05$ ). Different uppercase letters in the row under the parameters tested indicate significant differences ( $p < 0.05$ ).

**Table 3.**  $\alpha$ -Amylase,  $\alpha$ -glucosidase, and lipase inhibitory activities of selected green leafy vegetables

Parameters	Drying process	Celery	Coriander	Parsley	Spring onion
$\alpha$ -Amylase inhibitory activity (mg ACE/g extract)	Oven drying	8.87 $\pm$ 0.81 <sup>aC</sup>	29.84 $\pm$ 0.84 <sup>aB</sup>	32.20 $\pm$ 0.84 <sup>aA</sup>	7.51 $\pm$ 0.40 <sup>bC</sup>
	Dehydration drying	7.27 $\pm$ 0.49 <sup>bD</sup>	26.06 $\pm$ 2.35 <sup>bB</sup>	30.61 $\pm$ 1.10 <sup>aA</sup>	10.16 $\pm$ 1.00 <sup>aC</sup>
	Freeze drying	6.74 $\pm$ 0.32 <sup>bC</sup>	22.65 $\pm$ 0.63 <sup>cB</sup>	24.76 $\pm$ 1.02 <sup>bA</sup>	3.98 $\pm$ 0.27 <sup>cD</sup>
$\alpha$ -Glucosidase inhibitory activity (mg ACE/g extract)	Oven drying	144.95 $\pm$ 9.92 <sup>aC</sup>	162.37 $\pm$ 11.90 <sup>aBC</sup>	181.14 $\pm$ 4.55 <sup>aB</sup>	572.10 $\pm$ 23.48 <sup>aA</sup>
	Dehydration drying	130.50 $\pm$ 6.09 <sup>bB</sup>	131.55 $\pm$ 6.01 <sup>bB</sup>	111.57 $\pm$ 6.91 <sup>bB</sup>	595.28 $\pm$ 27.26 <sup>aA</sup>
	Freeze drying	92.75 $\pm$ 4.19 <sup>cB</sup>	102.11 $\pm$ 6.81 <sup>cB</sup>	84.01 $\pm$ 6.02 <sup>cB</sup>	396.02 $\pm$ 30.62 <sup>bA</sup>
Lipase inhibitory activity (%)	Oven drying	30.81 $\pm$ 1.05 <sup>aA</sup>	14.14 $\pm$ 1.06 <sup>aB</sup>	31.60 $\pm$ 1.30 <sup>aA</sup>	14.53 $\pm$ 0.96 <sup>bB</sup>
	Dehydration drying	27.45 $\pm$ 0.64 <sup>bB</sup>	13.33 $\pm$ 0.97 <sup>aD</sup>	31.07 $\pm$ 0.82 <sup>aA</sup>	17.74 $\pm$ 0.98 <sup>aC</sup>
	Freeze drying	16.51 $\pm$ 0.43 <sup>cB</sup>	6.52 $\pm$ 0.41 <sup>bD</sup>	29.54 $\pm$ 1.63 <sup>aA</sup>	10.48 $\pm$ 0.65 <sup>cC</sup>

Notes: Mean  $\pm$  SD from triplicate determinations. Different lowercase letters in the same column indicate significant differences ( $p < 0.05$ ). Different uppercase letters in the row under the parameters tested indicate significant differences ( $p < 0.05$ ).

### Lipase inhibitory activity

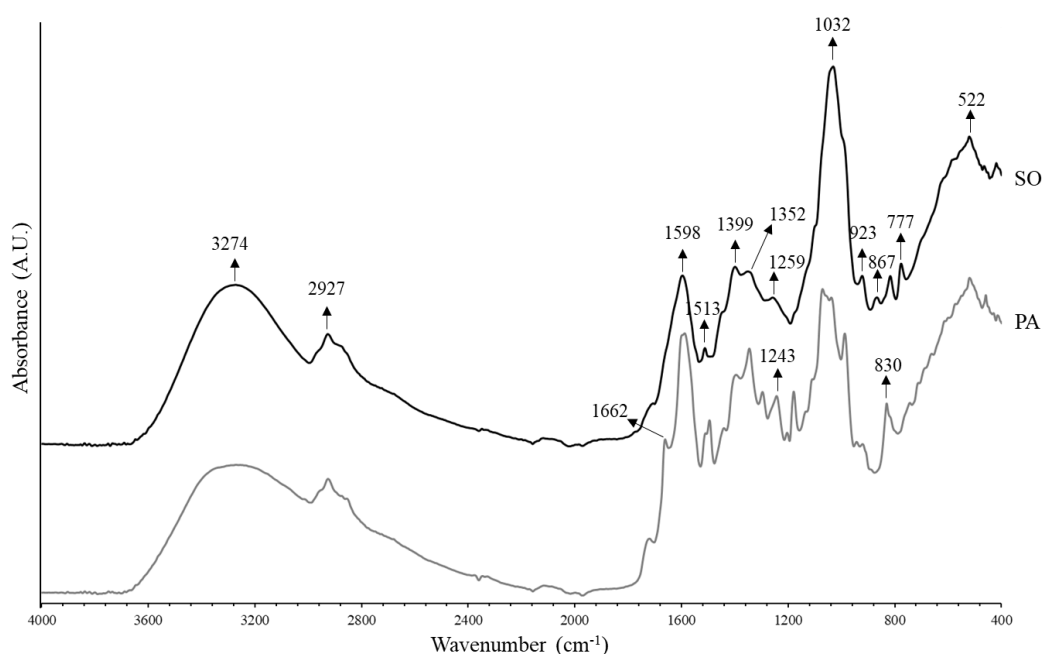
Table 3 presents the lipase inhibitory activity of GLVs. There are significant variations observed in the results for each GLV. Various factors influence the qualitative and quantitative composition of bioactive food compounds. In the case of plant-based foods, these factors include growth and cultivation conditions (Jakubczyk et al. 2021). Overall, GLVs from oven drying exhibited the highest activity, which slightly differs from those from dehydration drying. This finding aligns with Sirichai et al. (2022) report that ten plant extracts from oven drying displayed higher lipase inhibition activity than freeze drying. Furthermore, parsley demonstrated the most increased activity among the other GLVs, which correlated with its TPC (Figure 2). Sirichai et al. (2022) reported that phenolic compounds possess lipase inhibition properties that can act competitively or in mix-type inhibition.

### FTIR

Upon noting the substantial  $\alpha$ -glucosidase inhibitory potential in spring onion from dehydration drying, the highest and lowest activity extracts in dehydration drying were then analyzed using FTIR to identify variations in functional groups. Figure 3 presents the FTIR spectra of spring onion and parsley from dehydration drying, covering a range of 4000 to 400  $\text{cm}^{-1}$ , which have been utilized to gather details regarding the functional groups. Characterization of spring onion revealed several peaks in the spectra, including bands at 3274, 2927, 1598, 1513, 1399, 1352, 1259, 1032, 923, 867, 777, and 522  $\text{cm}^{-1}$ , which have previously been reported in spring onion roots and bulbs (Stan et al. 2015; Taranath et al. 2015; Sachdev et al. 2016; Ahmed et al. 2017). In contrast to spring onion, several peaks at 1662, 1243, and 830  $\text{cm}^{-1}$  were seen in

parsley, which have also been reported previously by Roy et al. (2015) and Maser et al. (2023).

The peak at 3274  $\text{cm}^{-1}$  was attributed to the bending of C-H bonds and the stretching of O-H bonds in proteins, polysaccharides, and water (Ahmed et al. 2017; Nair and Mukne 2017). The peak at 2927  $\text{cm}^{-1}$  was due to the asymmetric stretching of the C-H and C=H bonds (Ahmed et al. 2017; Nair and Mukne 2017). The peak at 1662  $\text{cm}^{-1}$  was associated with the tertiary amides C=O stretching (Roy et al. 2015; Rafi et al. 2021). The peaks at 1598 and 1513  $\text{cm}^{-1}$  were assigned to stretching C=N and C=C bonds in the  $\alpha$ -helix and  $\beta$ -sheet structures of proteins (amide I and amide II, respectively) (Ahmed et al. 2017). The peak at 1513  $\text{cm}^{-1}$  was also related to the stretching of C=O bonds in the polyphenols (Stan et al. 2015). The peak at 1399  $\text{cm}^{-1}$  indicated the presence of C-O bonds in COO<sup>-</sup> groups (Ahmed et al. 2017). The peak at 1352  $\text{cm}^{-1}$  represented the symmetrical stretching of aliphatic nitro compounds (Taranath et al. 2015). Peaks at 1259  $\text{cm}^{-1}$  were associated with the asymmetric and symmetric stretching of P=O bonds in the phosphodiesteres (Ahmed et al. 2017). The peak at 1234  $\text{cm}^{-1}$  was related to the C-O stretching (Roy et al. 2015; Saragih et al. 2021). A strong band at 1032  $\text{cm}^{-1}$  was attributed to the S-O bond and C-N stretching in aliphatic amines (Stan et al. 2015; Taranath et al. 2015). In addition, a band at 923  $\text{cm}^{-1}$  and a lower frequency band at 867  $\text{cm}^{-1}$  corresponded to the stretching of S=O bonds and the absorption of S-C bonds in organosulfur compounds (Stan et al. 2015; Sachdev et al. 2016). A band at 830  $\text{cm}^{-1}$  was associated with bending C-H bonds (Maser et al. 2023). A sharp band at 777  $\text{cm}^{-1}$  was observed, arising from stretching C-Cl bonds in alkyl halides and C-S bonds (Taranath et al. 2015; Sachdev et al. 2016). The peak at 522  $\text{cm}^{-1}$  was assigned to the S-S bonds (Nair and Mukne 2017).



**Figure 3.** Fourier transform infrared spectroscopy spectra of Spring Onion (SO) and Parsley (PA) from dehydration drying

The several peaks in spring onion exhibited higher intensity than parsley, particularly at 3274 (C-H) and 1032  $\text{cm}^{-1}$  (S-O), suggesting the existence of organosulfur compounds. These compounds had strong  $\alpha$ -glucosidase inhibitory activity (Table 3). The presence of functional groups such as C-H bending, C-H stretching, C=CH stretching, C=C stretching, S=O stretching, C-S stretching, and S-S bonds also confirmed the presence of organosulfur compounds in spring onion, such as diallyl disulfide, allyl methyl trisulfide, and allicin, which may be responsible for its higher  $\alpha$ -glucosidase inhibitory activity (Nair and Mukne 2017; Ahmad et al. 2021). Moreover, the high TPC found in parsley is associated with the presence of peaks at 1662 (C=O), 1243 (C-O), and 830  $\text{cm}^{-1}$  (C-H), which indicated the presence of apigenin, of which parsley is recognized as one of the primary sources (Poureini et al. 2020). The FTIR results also correlate with the GC-MS results, further explained in the GC-MS section.

### GC-MS

GC-MS chromatograms of spring onion and parsley extracts from dehydration drying and mass spectra of several compounds from both are shown in Figure 4. Table 4 presents the metabolites analyzed of spring onion and parsley extracts from dehydration drying, arranged in order of decreasing abundance per compound group. The analysis results indicated that the chemical composition similarity exceeds 86%, suggesting that each sample exhibited relatively good consistency. The retention times of identical spring onions and parsley compounds were similar. The metabolites identified in both samples belonged to various groups, including sugars, fatty acids, organic acids, amino acids, and other compounds. In contrast to spring onion, parsley (Apiacea family) shows the presence of sugar alcohol, including mannitol, which has previously been reported to be abundant in Apiacea (Bianco and Avellone 2014). In both samples, sugars were a prominent group of compounds. In addition, fatty and organic acids were also abundant in both samples.

The prominent compounds found in spring onion were fructose, glucose, fructose oxime, glucose oxime, propanoic acid, myoinositol, and succinic acid. The high presence of sugar compounds correlated with the results of FTIR analysis, which showed elevated abundance in bands at 3274  $\text{cm}^{-1}$  (Figure 3), indicating the bending of C-H bonds and stretching of O-H bonds. Additionally, the presence of fatty acids and organic acids was supported by bands observed at 2927  $\text{cm}^{-1}$ , attributed to asymmetric stretching of C-H bonds, and 1399  $\text{cm}^{-1}$ , indicating the presence of C-O bonds in  $\text{COO}^-$  groups. The identification of amino acids aligns with the presence of functional groups of C=N and C=C bonds in  $\alpha$ -helix and  $\beta$ -sheet structures of proteins at bands 1598 and 1513  $\text{cm}^{-1}$ , as well as C-N stretching in aliphatic amines at the peak 1032  $\text{cm}^{-1}$ . Several identified compounds have been reported for antidiabetic activities, which correlated with the high

inhibitory activity of spring onion against  $\alpha$ -glucosidase (Table 3). These compounds included propanoic acid (Heimann et al. 2015), myoinositol (Chhetri 2019), succinic acid (Ives et al. 2020), hexadecanoic acid (Gori et al. 2020), and various amino acids, such as proline, valine, threonine, and isoleucine (Srinivasan et al. 2019).

**Table 4.** The metabolites of spring onion and parsley from dehydration drying by GC-MS analysis

Group	Compound name	Abund. <sup>1</sup>	Sim. (%)	Ret. time (min)
<b>Spring onion</b>				
Sugars	Fructose	63.34	91	18.88
	Glucose	57.26	95	25.49
	Fructose oxime	52.47	90	18.97
	Glucose oxime	37.16	90	19.13
	Myo-inositol	11.32	86	21.00
	Sorbopyranose	8.10	87	18.30
	Galactose oxime	7.49	90	19.32
	Ribofuranose	0.75	87	17.76
Fatty acids	Galactose	0.61	80	22.96
	Propanoic acid	13.70	99	12.53
Organic acids	Hexadecanoic acid	0.87	99	20.69
	Succinic acid	9.00	98	14.61
	2,3,4-Trihydroxy-butyric acid	4.40	86	15.46
Amino acids	Gluconic acid	1.45	90	20.13
	Proline	3.32	90	15.04
	Valine	1.43	87	10.89
	Threonine	0.78	91	13.27
	Isoleucine	0.62	86	12.02
Others	Glycerol	7.61	91	11.74
<b>Parsley</b>				
Sugars	Glucose	44.01	90	25.49
	Fructose	5.68	94	18.87
	Fructose oxime	4.80	91	18.97
	Myo-inositol	4.26	86	20.99
	Sorbopyranose	0.44	86	18.30
Sugar alcohols	Mannitol	17.15	91	19.44
Fatty acids	Propanedioic acid	2.35	92	10.74
	Hexadecanoic acid	1.17	99	20.69
Organic acids	Linolenic acid	1.04	99	22.52
	Propanoic acid	0.83	91	12.53
	Linoleic acid	0.81	95	22.20
	Succinic acid	14.41	99	14.62
	Citric acid	0.95	91	18.38
	Gluconic acid	0.92	93	20.13
	Phosphoric acid	0.69	87	21.84
Amino acids	Valine	6.72	87	10.89
	Isoleucine	4.84	90	12.02
	Asparagine	4.71	98	16.77
	Phenylalanine	3.89	90	16.28
	Threonine	3.66	90	13.27
	Alanine	2.19	90	9.12
	Proline	1.74	90	15.04
	Aspartic acid	1.43	86	15.01
	Glutamine	0.58	87	16.21
Others	Glycerol	5.90	91	11.74

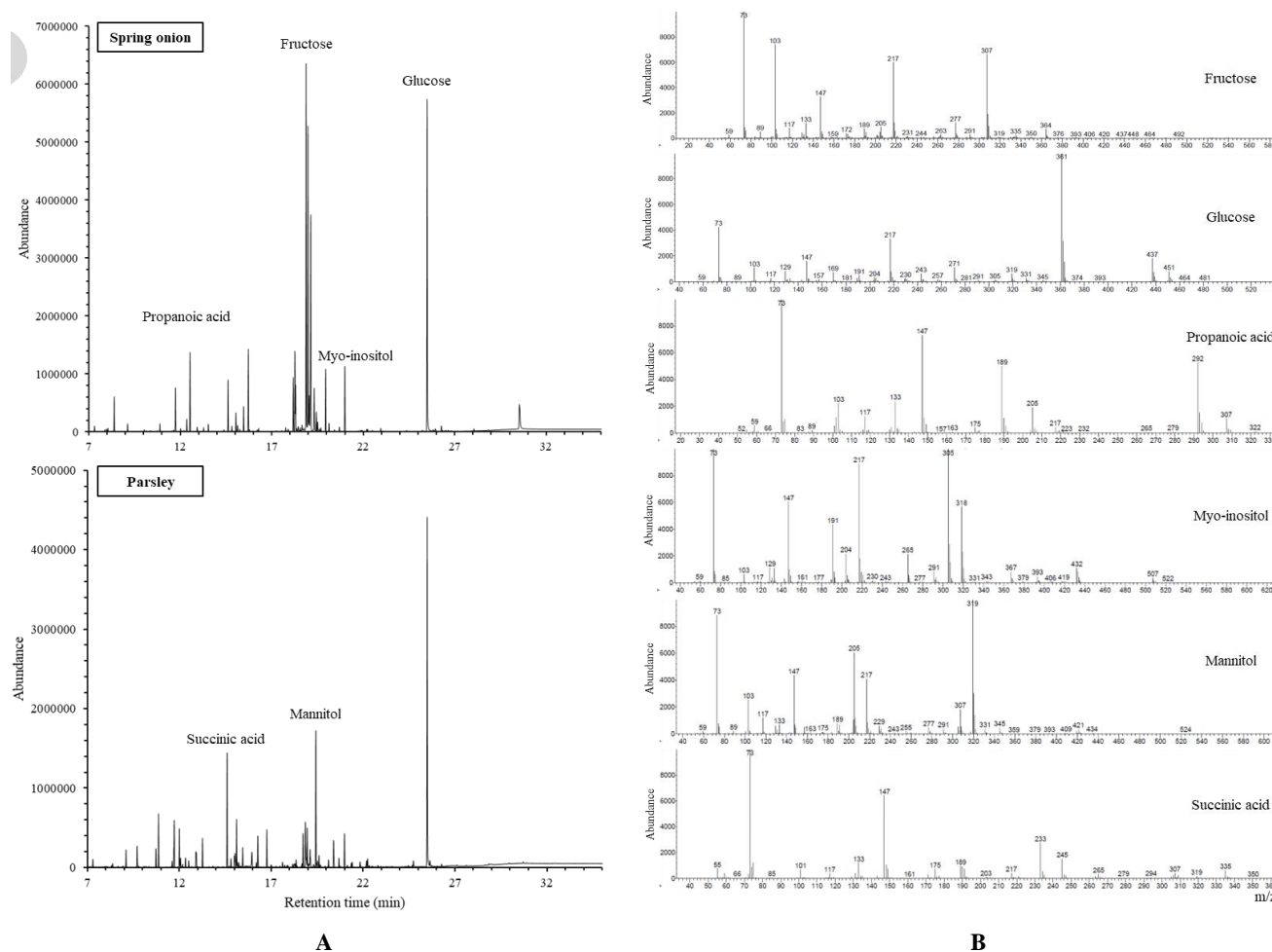
Note: <sup>1</sup>Values are represented as abundance ( $\times 10^5$ )

In parsley, the dominant compounds were glucose, mannitol, succinic acid, valine, glycerol, and fructose. The highest inhibition activities against  $\alpha$ -amylase and lipase in parsley were observed (Table 3), which could be attributed to the significant amount of mannitol indicated by the GC-MS analysis results (Table 4). Mannitol has been reported to possess antidiabetic (Wu et al. 2021) and anti-obesity properties (Jeon et al. 2021). Minor compounds can also contribute and synergize to generate high bioactivity (Yang et al. 2014). Several minor identified compounds in parsley have been reported to exhibit antidiabetic activity, such as succinic acid (Ives et al. 2020), valine, isoleucine, asparagine, phenylalanine, threonine, alanine, proline, aspartic acid, glutamine (Srinivasan et al. 2019), myoinositol (Chhetri 2019), hexadecanoic acid (Gori et al. 2020), linolenic acid (Jovanovski et al. 2017), citric acid (Mustafa et al. 2018), propanoic acid (Heimann et al. 2015), and linoleic acid (Yoon et al. 2021). In addition, certain minor compounds identified in parsley have been documented to exhibit anti-obesity properties, including

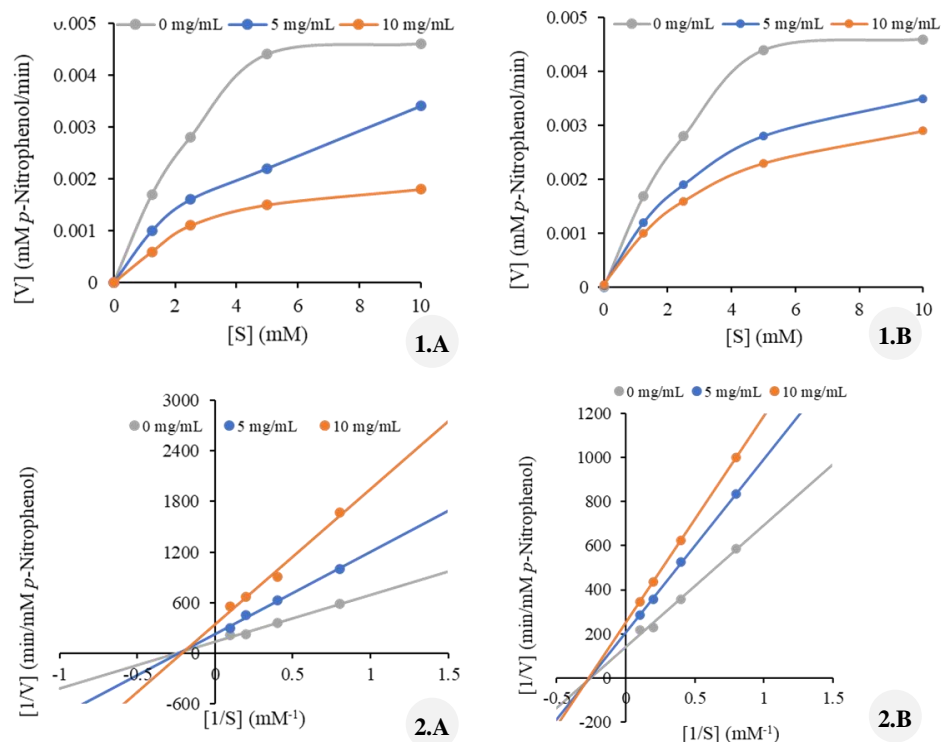
valine, isoleucine, asparagine, phenylalanine, threonine, alanine, proline, aspartic acid, glutamine (Srinivasan et al. 2019), myoinositol (Chhetri 2019), citric acid (Mustafa et al. 2018), and propanoic acid (Heimann et al. 2015).

### Kinetic assessment of $\alpha$ -glucosidase inhibition

The inhibitory effects of spring onion and parsley from dehydration drying at different concentrations on  $\alpha$ -glucosidase were examined by measuring the initial reaction rate through p-NPG digestion over varying digestion times (0–30 min) in the presence of  $\alpha$ -glucosidase. The reaction rate ( $v$ ) decreased with increasing spring onion and parsley concentrations, indicating a concentration-dependent inhibitory effect (Figure 5). This trend was consistent regardless of the p-NPG concentration (Figures 5, 1A and 1B). These findings indicated that the inhibitory effect on  $\alpha$ -glucosidase enzymes was reversible, and there were noncovalent interactions between the inhibitors and  $\alpha$ -glucosidase (Dong et al. 2021).



**Figure 4.** Gas chromatography-mass spectrometry (GC-MS) chromatograms of spring onion and parsley from dehydration drying (A) and mass spectra of some compounds from these two samples (B)



**Figure 5.** Initial reaction velocity in the presence of p-NPG at different concentrations (1) and Lineweaver-Burk plots (2) for  $\alpha$ -glucosidase inhibition. Plots with a and b correspond to spring onion and parsley from dehydration drying, respectively

**Table 5.** The inhibitory action and the comprehensive kinetics of  $\alpha$ -glucosidase inhibition by spring onion and parsley

Samples		Concentration (mg/mL)			Inhibition type
		0	5	10	
Spring onion	$v_{max}^*$	0.0072	0.0044	0.0029	Mixed (non-competitive)
	$K_m^{**}$	3.9701	4.2757	4.6219	
Parsley	$v_{max}^*$	0.0072	0.0049	0.0040	Mixed (non-competitive)
	$K_m^{**}$	3.9701	3.8294	3.7621	

Note:  $*v_{max}$  and  $**K_m$  were expressed in mM *p*-Nitrophenol/min and mg/mL, respectively

Figure 5 (2A and 2B) displays the Lineweaver-Burk plots showing the evaluation of inhibition type on  $\alpha$ -glucosidase caused by various samples at different concentrations. Table 5 provides the calculated values of  $K_m$  and  $v_{max}$  of various samples using the Michaelis-Menten plot. The extension lines resulting from linear fitting were intersected at a close point near the x-axis with the increase in spring onion concentration (Figures 5, 2A). It was observed that  $v_{max}$  and  $K_m$  values were decreased and relatively unchanged, respectively (Table 5), indicating that uncompetitive inhibition was equivalent to competitive inhibition, which can be considered a specific case of mixed-type inhibition, referred to as non-competitive inhibition. A similar pattern was observed in parsley, wherein elevating the parsley concentration reduced the  $v_{max}$  value and a slightly unchanged  $K_m$  value (Table 5). Non-competitive inhibition occurs through binding the inhibitor to the enzyme in its free form, which causes

changes in the active site that interfere with substrate binding (Mittal et al. 2023). Despite  $K_m$  showing no apparent change, there seems to be a rising tendency in spring onion (approaching competitiveness) and a declining trend in parsley (approaching uncompetitiveness). Competitive action is the most relevant type of inhibition. It is typically optimized as an antidiabetic medication, such as acarbose, which exhibits competitive inhibition due to its ability to bind directly to the enzyme's active site (Proença et al. 2017). It possibly explains why the  $\alpha$ -glucosidase inhibitory activity in spring onions is higher than that in parsley from dehydration drying (Table 3).

In general, various drying methods have influenced active compounds' recovery in celery, coriander, parsley, and spring onion. Freeze drying exhibited high extraction results but did not show superior TPC and bioactivity compared to other drying methods. Additionally, freeze-drying also demonstrated lower efficiency drying. Using

heat in the oven and dehydration drying positively impacted and yielded better results in terms of TPC and bioactivity for all four GLVs. Interestingly, the findings indicate that oven drying outperformed the others for celery, coriander, and parsley among the investigated methods. However, for spring onions, dehydration drying proved to be the most effective. Notably, the  $\alpha$ -glucosidase inhibitory activity of spring onion extracts from dehydration drying stood out as the highest, a conclusion corroborated by the outcomes of characterization using FTIR and GC-MS, and the kinetics of inhibition. Thus, both oven drying and dehydration methods have the potential to generate extracts with better bioactivity and drying efficiency, opening possibilities for their utilization in diverse functional food and health-related applications for disease treatment.

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