

Integrated Biochemical and Biophysical Analyses Reveal Lipid Characteristics Associated with Orchid Seed Storability

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Abstract

Long-term conservation of orchid genetic resources depends on maintaining seed viability during storage, yet the biochemical and biophysical characteristics associated with differences in orchid seed storability remain poorly understood. This study investigated whether lipid composition and thermal behaviour are associated with species-specific differences in seed storability and can provide useful indicators for optimizing storage conditions. Seeds of four orchid species (*Dendrobium aphyllum*, *Phalaenopsis amabilis*, *Phalaenopsis cornu-cervi*, and *Vanda tricolor*) were stored at -20°C for 0, 3, 6, 9, and 12 months, followed by germination assessment, GCMS-based fatty acid methyl ester (FAME) profiling, and differential scanning calorimetry (DSC). *D. aphyllum* exhibited the most rapid decline in viability, whereas *P. amabilis* maintained the highest germination throughout the storage period. FAME analysis identified linoleic acid (32.50–44.60 mol%) as the predominant fatty acid in all species, followed by palmitic acid. *D. aphyllum* exhibited the lowest monounsaturated/polyunsaturated fatty acid (MUFA/PUFA) ratio (0.399) and the highest double bond index (DBI; 112.15) and peroxidizability index (PI; 47.94), indicating a lipid profile with greater theoretical susceptibility to oxidative damage. DSC analysis detected no measurable crystallization during cooling to -125°C but revealed three endothermic transitions at approximately -78°C , -37 to -33°C , and -19 to -17°C . These thermal transitions indicate distinct lipid phase behaviour and suggest that conventional -20°C storage may overlap with temperature ranges in which lipid phase transitions occur. Collectively, the findings show that species-specific differences in orchid seed storability are associated with differences in fatty acid composition and lipid thermal behaviour. Integrated biochemical and biophysical profiling therefore provides useful indicators for evaluating seed storability and may inform the optimization of storage temperatures, including the potential application of cryogenic storage for long-term conservation.

Introduction

The family Orchidaceae comprises flowering ornamental plants known as orchids. Indonesia is particularly famous for its diverse range of orchid species, with over 5,000 recorded species (Handini et al. 2021). There are approximately 30,381 species in 704 genera that exist worldwide, as reported in Fay et al. (2025). Research on orchids has made many significant advances so far. Research has shifted from initially focusing solely on taxonomy to the molecular biology of orchids, which aids in the conservation and propagation of orchids, particularly those at risk of extinction (Wraith et al. 2020). Maintaining biodiversity requires the protection and preservation of orchids. Methods of seed storage have been included in biodiversity conservation efforts (Handini and Handayani 2019). Protecting and preserving orchids in botanical garden collections in the form of living plants as well as seed bank collections is essential to ensure that extinction in the wild does not mean extinction on planet Earth (Vitt et al. 2023). The potential shelf life of seeds relies on the quality of the seeds at the storage's commencement, and maximising seed shelf longevity hinges on storage conditions (Kameswara et al. 2023), the seed variety's species, as well as the specified shelf life duration (Sundareswaran et al. 2023). Orchid seeds lose viability rapidly when stored at room temperature, which is between 21 – 22 degrees Celsius. Seeds are usually dried with a desiccant for five days to reduce moisture content, thereby

enhancing longevity, as was noted in Puspitaningtyas and Handini (2014). Research by Hay et al. (2010) found that seeds with a relative humidity (RH) of 5% can retain germination potential, but their germination rates dropped after two years of storage at -18°C. Not all orchid species have seeds that can withstand being stored at -20°C, according to Pence (2022).

The natural aging process and seed damage are the result of the interaction of cytological, physiological, biochemical, and physical processes in seeds, leading to a decrease in vigour, germination, and ultimately seed viability loss (Sundareswaran et al. 2023). Orchid embryos, made up of small, uniform parenchymal cells with lipid stores, enable successful cryopreservation because they can tolerate sufficient dehydration (Tammasiri and Soamkul 2007). Lipids act as key signal molecules in plant responses to abiotic stress (Cassim et al. 2018; Narayanan et al. 2020). Continuous degradation of proteins, lipids, and nucleic acids can disrupt the ability of seeds to germinate by damaging cell components and reducing their metabolic capacity (Nadarajan et al. 2023). Lipid stability is therefore an important consideration in understanding seed deterioration. Changes in lipid physical properties, including melting behaviour and the associated energy requirements, may influence the stability of cellular membranes and other lipid-containing structures during storage and, consequently, affect subsequent germination (Mira et al. 2019). These observations indicate that both the biochemical composition and physical behaviour of seed lipids may contribute to differences in seed longevity among orchid species.

The biochemical analysis of orchid seeds is difficult due to their tiny size, which restricts the amount of material that can be used in standard analytical techniques. Researchers led by Colville in 2015 showed that direct transesterification can enable fatty acid profiling from very small quantities of orchid seeds. Conventional fatty acid analysis typically involves an initial extraction with organic solvents like hexane, whereas direct transesterification skips the lipid-extraction step and offers a quicker and more efficient method that minimises the requirement for toxic chlorinated organic solvents. Gas chromatography-mass spectrometry (GC-MS) is a highly effective method for fatty acid profiling, allowing for the separation and identification of individual fatty acid derivatives based on their chromatographic and mass-spectral characteristics (Kazaz et al. 2022). Gas chromatography is particularly useful for separating volatile and thermally stable compounds, and mass spectrometry provides information that is useful for determining molecular structures, molecular formulas, and molecular weights (Hotmian et al. 2021). Fatty acids with carbon-carbon double bonds can be categorised into saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) (Kazaz et al. 2021). Profiling such provides a biochemical basis for evaluating differences in lipid composition among orchid species and may help explain differences in their responses to storage.

The physical behaviour of seed lipids during temperature changes is also relevant to storage stability in addition to biochemical composition. Differential scanning calorimetry (DSC) can characterise thermal transitions associated with lipid-containing seed tissues. During storage, lipids can undergo crystallization and melting across characteristic temperature ranges, with these transitions influencing their physical state (Mira et al. 2019). Storage at -20°C may intersect with lipid phase-transition ranges in

some seeds, potentially affecting the physical stability of lipid components as a result. Interpreting differences in seed storage behaviour and identifying suitable temperature ranges for long-term conservation requires a good understanding of these thermal characteristics.

Investigating thermodynamic properties of biological materials using differential scanning calorimetry is particularly useful because physical transitions involving measurable heat flow include melting and crystallization. These transitions involve heat absorption in endothermic processes, such as melting, and heat release in exothermic processes, such as crystallization, which can be expressed in joules per gram (J/g). DSC offers advantages in seed analysis, including analysing small sample quantities (approximately 1–10 mg), being applicable to both solid and liquid samples, having relatively short analysis times (approximately 10–30 min), and simple sample preparation (Thomas 2005). Consequently, DSC provides complementary information to fatty acid profiling by characterising the thermal behaviour of lipid-containing seed tissues.

The relationship between lipid composition and physical properties in seed ageing and species-specific storability in orchid seeds remains insufficiently characterised despite growing recognition of their importance. Characterisation of orchid seed lipids through biochemical and biophysical methods may offer complementary indicators of variations in seed longevity and assist in identifying storage conditions that reduce lipid degradation. This study examined the connection between seed durability, fatty acid composition, and lipid thermal properties in four orchid species by combining in vitro germination testing with fatty acid methyl ester (FAME) profiling via GCMS and differential scanning calorimetry (DSC). The study particularly examined alterations in seed germination during storage at -20°C, and identified species-specific differences in fatty acid composition and thermal transitions to establish whether these characteristics were linked to variation in seed storability.

Materials and methods

Materials preparation and seed storage

This study utilised seeds from four orchid species: *Dendrobium aphyllum* (Roxb.) C.E.C.Fisch., *Phalaenopsis amabilis* (L.) Blume, *P. cornu-cervi* (Breda) Blume & Rchb.f., and *Vanda tricolor* Lindl (Fig. 1). Conventional storage methods, which are usually at -20°C, involve removing seeds from the fruit with a spatula and then separating them from the fibres using a sieve with a mesh size of 40–80. Seeds were placed in a petri dish and stored in a desiccator for 3–4 days, as reported by Seaton and Ramsay in 2005. Silica gel was employed as a desiccant. For each sample, five milligrams of dried seeds were weighed, placed in a paper envelope, and then placed in a tube, with the relative humidity adjusted to approximately 50%. The tube, which had been previously wrapped in plastic wrap, was then stored in a glass bottle containing silica gel, tightly sealed and kept at a temperature of -20°C. Experiments were carried out to assess the percentage of seed germination via in vitro culture after storage periods of 0, 3, 6, 9, and 12 months at a temperature of -20°C. Samples were prepared additionally for fatty acid methyl ester (FAME) analysis and differential scanning calorimetry (DSC).

Direct seed viability test

Seed sterilisation was the first step in the in vitro germination test. The seed placed in sterilised aquadest with 0–1% Tween was then rinsed with a 10% Clorox solution (0.5% NaOCl) containing 0–1% Tween 20 and shaken for 10 minutes, followed by rinsing with a 5% Clorox solution (0.25% NaOCl) containing 0–1% Tween 20 and shaken for 5 minutes. The seeds were then rinsed three times with sterile distilled water (aquadest) (Puspitaningtyas and Handini 2021). The sterilized aquadest, after the third rinse, was used to distribute the seeds onto the optimal seedling medium for each orchid species, with *D. aphyllum*, *P. amabilis*, and *P. cornu-cervi* being cultured on Knudson C modified medium, and *V. tricolor* on a leaf fertilizer-modified medium (Handini and Puspitaningtyas 2020). We then cover the petri dish with plastic wrap and label it.

The cultures were stored in a culture room at a temperature below 25°C. The seeds started germinating roughly one month following sowing. A total of 100 seeds were evaluated per observation to assess seedling development in petri dishes by categorising seeds as germinated, swollen, or dead. Five repetitions of each seed sample were sown in petri dishes. Observation was conducted using a binocular microscope device, specifically the Olympus U-TV0.5XC-3.

Germination percentage was calculated based on the number of germinated seeds observed in one petri dish, with 5 repetitions taken from 5 petri dishes. The germination percentage was calculated using the formula provided by Hossain et al. (2013):

$\% \text{ Germination} = (\text{Number of germinated seeds} \times 100) / \text{Total number of seeds observed}.$

The germination percentages of seeds from each orchid species stored at -20°C statistically analyzed with a one-way ANOVA test using IBM SPSS Statistics 26 software.

P₅₀ calculation to find out the storage period when the germination ability of the seeds is only 50% left, using Curve Expert 1.3 software.

Fatty Acid Methyl Ester (FAME) profiling by Gas Chromatography-Mass Spectrometry (GC-MS)

In this study, two repetitions of fresh seed samples were used for analysis. Fatty Acid Methyl Esters were produced using a direct trans-esterification method based on the protocols of Conville et al. (2015), with modifications by Diantina et al. (2022). For each replicate, 10 mg of seed samples less than one year old were placed in 20 mL glass vials containing 1 mL of toluene. The toluene solution included 50 mg/L of butylated hydroxytoluene (BHT) as an antioxidant and 10 µL of a stock solution of pentadecanoic acid (10 mg/mL in toluene) as an internal standard. The samples were incubated at room temperature for 4 hours.

After incubation, 2 mL of 1% sulfuric acid in methanol (v/v) was added as a methylation catalyst. The samples were then incubated overnight in a water bath set at 50°C. Following incubation, FAMES were isolated by partitioning, which involved adding 5 mL of hexane and 5 mL of 5% sodium chloride (NaCl) solution (b/v) to the samples. The supernatant was carefully separated using a pipette and transferred to a clean vial. The remaining solid residue was washed with an additional 5 mL of hexane, and the supernatants were pooled together. The combined supernatants were dried at 50°C using a rotary evaporator for 5–10 minutes. The resulting residue was dissolved in 1 mL of hexane and transferred into 2 mL vials for GC-MS analysis (Aytar and Kömpe 2024). The identification and quantification of FAMES were performed using a Gas Chromatography-Mass Spectrometry (GC-MS) system (GC2010, Shimadzu, Japan). FAMES were separated on a Zebron™ ZB-FAME column with a high cyano phase capillary column (30 m x 0.25 mm internal diameter, 0.2 µm film thickness, with a 5 m Guardian) (Pamplona et al. 1998).

The data were analyzed semiquantitatively based on peak area percentages obtained from the GC-MS (Aytar and Kömpe 2024), which were used to calculate the total fatty acid content. The highest fatty acid content data was selected from two samples that were representative in this study. The data on fatty acid content with the unit % area is converted to % mol using the following formula:

$$\% \text{ mol} = \% \text{ area of each fatty acid} \times 100 / \% \text{ area total fatty acids}$$

Fatty acid analysis using the formula of Pamplona et al. (1998):

- Ratios of Unsaturated Fatty Acids (UFA = MUFA+PUFA) to Saturated Fatty Acids (SFA), or UFA/SFA
- Ratios of Monounsaturated Fatty Acids (MUFA) to Polyunsaturated Fatty Acids (PUFA), or MUFA/PUFA
- The double bond index was calculated as $\text{DBI} = \sum \text{mol\% of unsaturated fatty acids} \times \text{number of double bonds of each unsaturated fatty acid}$,
- and the peroxidizability index (PI) = $[(\% \text{ Monoenoic} \times 0.025) + (\% \text{ Dienoic} \times 1) + (\% \text{ Trienoic} \times 2) + \dots]$.

Biothermal profiling by Differential Scanning Calorimetry (DSC) (dup: abstract ?)

The biothermal status of lipids in orchid seeds under varying temperature and storage conditions was determined using Differential Scanning Calorimetry (DSC) analysis. The fresh orchid seed samples used for this analysis were the same as those tested for FAME, prepared by removing fibers and reducing moisture content. The seeds were stored in a desiccant for 3–5 days, lowering the relative humidity (RH) to 50%, as measured using an RH meter. Approximately 5 mg of each sample was weighed and placed into a small aluminum DSC pan (standard aluminum pan), which was then sealed for analysis.

The DSC 8000 system, equipped with a cooling mechanism utilizing liquid nitrogen, was employed. The method used was an optimized version of the protocol by Diantina et al. (2023). The temperature programming for the DSC analysis was as follows: (a) Cooling the sample from 25°C to -125°C at a rate

of -10°C/min, (b) Holding the temperature steady at -125°C for 3 minutes, (c) Heating the sample from – 125°C to 50°C in increments of 10°C/min.

This process allowed for detailed monitoring of the thermal transitions in the seed lipids, providing insights into their stability and behaviour under storage conditions.

Results

Seed viability and longevity during storage

Before biochemical (FAME) and biophysical (DSC) analyses, in vitro germination tests were conducted to assess the initial viability of the seed lots and to monitor changes in germination during storage. The germination percentages of the four orchid species stored at -20°C for 12 months are presented in Table 1. Fresh seeds of all four species exhibited germination percentages above 50%.

Table 1
Germination Test Results of Orchid Seeds After 12-Month Storage at -20°C.

Species	Percentage of germination (%)					P ₅₀ (Month)
	Fresh (0 Month)	3 Months	6 Months	9 Months	12 Months	
<i>D. aphyllum</i>	70.17 ± 7.08b	23.96 ± 2.60a	14.98 ± 2.38a	18.51 ± 2.85a	10.52 ± 4.06a	0.62
<i>P. amabilis</i>	92.42 ± 2.77c	72.74 ± 4.26c	77.74 ± 4.68c	69.16 ± 2.51c	90.06 ± 3.40d	115.97
<i>P. cornu cervi</i>	86.23 ± 3.21c	78.82 ± 1.98c	77.02 ± 5.34c	49.71 ± 5.26b	64.64 ± 5.87c	14.83
<i>V. tricolor</i>	56.74 ± 3.78a	43.78 ± 6.44b	31.77 ± 8.63b	58.04 ± 5.99b	34.84 ± 7.12b	0.96

Note: Germination values ending with different letters mean significantly different at the 0.05 level.

P₅₀ calculation to find out the storage period when the germination ability of the seeds is only 50%

Marked interspecific differences in germination were observed during storage at -20°C (Table 1). *D. aphyllum* exhibited the largest decline in germination, decreasing from 70.17% in fresh seeds to 10.52% after 12 months. The predicted P50 value was 0.62 months. In contrast, *P. amabilis* maintained germination above 69% throughout the storage period and reached 90.06% after 12 months. This species showed the highest predicted P50 value of 115.97 months. *P. cornu-cervi* exhibited intermediate storage performance, with a predicted P50 of 14.83 months, whereas *V. tricolor* had a predicted P50 of 0.96 months and relatively low initial germination.

The predicted P50 values therefore differed substantially among the four species, with *P. amabilis* showing the highest predicted longevity and *D. aphyllum* and *V. tricolor* showing the shortest predicted longevity. The predicted storage longevity of *P. amabilis* was approximately 10 years under the conditions represented by the viability model.

Fatty Acid Methyl Ester (FAME) profiling by GC-MS

FAME analysis identified 19 fatty acid methyl esters across the four orchid species, comprising saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) (Table 2; Appendix 1).

Table 2
FAME Results of Orchid Seeds before Storage at -20°C

No.	FAME	mol (%)				FA Group
		<i>D. aphyllum</i>	<i>P. amabilis</i>	<i>P. cornu cervi</i>	<i>V. tricolor</i>	
1	Lauric acid methyl ester (C13:0)	6.13	9.61	9.47	10.88	SAFA
2	Tetradecanoic acid, methyl ester (C15:0)	0.31	0.20	0.00	0.43	SAFA
3	Methyl n-pentadecanoate (C16:0)	0.61	0.28	0.22	1.08	SAFA
4	Palmitic acid methyl ester (C17:0)	20.53	24.45	17.87	18.64	SAFA
5	Palmitelaidic acid methyl ester (C16:1)	0.00	0.00	0.45	0.00	MUFA
6	Methyl palmitoleate (C16:1n-7)	0.48	0.99	1.41	0.35	MUFA
7	Methyl behenate (C23:0)	0.00	0.00	0.80	0.00	SAFA
8	Methyl heptadecanoate (C18:0)	0.39	0.21	0.00	0.45	SAFA
9	Methyl erucate (C22:1)	0.00	0.00	8.66	0.00	MUFA
10	Methyl lineoleate (C18:2)	0.10	0.14	0.07	0.00	PUFA
11	Octadecanoic acid, methyl ester (C18:0)	7.36	4.24	5.94	5.13	SAFA
12	Methyl elaidate (C18:1)	0.00	9.55	12.19	14.66	MUFA
13	Methyl oleate (C18:1)	17.19	7.42	6.50	4.40	MUFA
14	Methyl lineoleate C18:2)	44.60	40.85	32.50	39.60	PUFA
15	Methyl linolenate (C18:3)	1.26	1.52	3.18	1.82	PUFA
16	Methyl arachidate (C21:0)	0.00	0.00	0.62	0.77	SAFA
17	cis-11-Eicosenoic acid methyl ester (C20:1 n-9)	0.77	0.23	0.00	0.87	MUFA
18	formyl 7E-hexadecenoate (C16:1)	0.00	0.00	0.00	0.15	MUFA
19	Cis-11,14-eicosadienoic acid methyl ester (C20:2)	0.26	0.32	0.12	0.77	PUFA
	Total	100	100	100	100	
	UFA/SFA	1.830	1.565	1.863	1.674	

No.	FAME	mol (%)				FA Group
		<i>D. aphyllum</i>	<i>P. amabilis</i>	<i>P. cornu cervi</i>	<i>V. tricolor</i>	
	MUFA/PUFA	0.399	0.425	0.814	0.484	
	DBI	112.15	105.37	104.12	106.61	
	PI	47.94	44.81	39.78	44.51	

Methyl linoleate (C18:2) was the most abundant FAME in all four species, accounting for 32.50–44.60 mol% of the total fatty acids. Methyl palmitate (C16:0) was the predominant SFA, representing 17.87–24.45 mol%, followed by methyl laurate (C12:0), which accounted for 6.13–10.88 mol%. Among the MUFAs, methyl elaidate (C18:1 trans) was the predominant component in *P. amabilis*, *P. cornu-cervi*, and *V. tricolor*, with values ranging from 9.55 to 14.66 mol%. Methyl elaidate was not detected in *D. aphyllum*, in which methyl oleate (C18:1 cis) represented the predominant MUFA at 17.19 mol%, compared with 4.40–9.18 mol% in the other three species.

Several minor fatty acids were consistently detected in all four species, including methyl pentadecanoate (C15:0), methyl linolenate (C18:3), methyl palmitoleate (C16:1n-7), and cis-11,14-eicosadienoic acid methyl ester (C20:2), each contributing less than 4 mol% of the total fatty acids. Methyl behenate (C22:0) and methyl erucate (C22:1) were detected only in *P. cornu-cervi*, whereas formyl 7E-hexadecenoate (C16:1) was detected only in *V. tricolor* (Table 2; Appendix 1).

The calculated lipid indices showed differences in fatty acid unsaturation among species (Table 2). *P. cornu-cervi* had the highest UFA/SFA ratio (1.863), whereas *P. amabilis* had the lowest ratio (1.565). *D. aphyllum* exhibited the lowest MUFA/PUFA ratio (0.399) and the highest DBI (112.15) and PI (47.94). *P. cornu-cervi* had the lowest PI (39.78).

Biothermal profiling by Differential Scanning Calorimetry (DSC)

DSC analysis was conducted for *P. amabilis*, *P. cornu-cervi*, and *V. tricolor*. The available seed mass of *D. aphyllum* was below the required minimum of 5 mg and was therefore insufficient for DSC analysis. The thermal parameters obtained during heating are presented in Table 3, and representative thermograms are shown in Fig. 2.

No detectable exothermic transitions were observed during cooling from 25°C to -125°C in any of the three species analysed. During subsequent heating from -125°C to 50°C, three endothermic transitions were detected in all three species.

The first endothermic transition occurred within a narrow temperature range of -77.95 to -77.84°C. The second transition occurred between -37.47 and -33.47°C, whereas the third occurred between -18.53 and -16.56°C (Table 3). The second transition exhibited the highest peak height among the three

transitions. The peak height during this transition was 0.6084 W g⁻¹ in *P. cornu-cervi*, 0.4569 W g⁻¹ in *P. amabilis*, and 0.4151 W g⁻¹ in *V. tricolor*.

The second transition also showed the highest enthalpy values, ranging from 3.8198 to 5.7438 J g⁻¹. *P. cornu-cervi* exhibited the highest enthalpy at 5.7438 J g⁻¹, followed by *P. amabilis* at 4.1490 J g⁻¹ and *V. tricolor* at 3.8198 J g⁻¹ (Table 3).

Table 3
The Detail DSC Analysis Results of Four Orchid Species

Crystallization: Cool from 25.00°C to -125.00°C at 10.00°C/min				
Species				
Peak Temperature (°C)	<i>P. amabilis</i>	Undetectable		
Tg	<i>P. cornu cervi</i>	-		
	<i>V. tricolor</i>	-		
Melting: Heat from – 125.00°C to 50.00°C at 10.00°C/min				
	Species	Peak 1	Peak 2	Peak 3
Peak Temperature (°C)	<i>P. amabilis</i>	-77.95	-37.47	-16.56
Tm	<i>P. cornu cervi</i>	-77.84	-36.11	-18.53
	<i>V. tricolor</i>	-77.9	-33.47	-16.97
Peak height (W/g)	<i>P. amabilis</i>	0.1814	0.4569	0.4877
hm (mW)	<i>P. cornu cervi</i>	0.2139	0.6084	0.3619
	<i>V. tricolor</i>	0.1704	0.4151	0.1992
Enthalpy (J/g)	<i>P. amabilis</i>	0.5575	4.1490	3.8262
ΔHm	<i>P. cornu cervi</i>	1.4625	5.7438	2.2302
	<i>V. tricolor</i>	1.4098	3.8198	1.234

Discussion

Species-specific differences in orchid seed storability

The current research showed significant variations in seed durability among the four orchid species despite being stored at the same temperature of -20°C. *P. amabilis* maintained high germination rates throughout the 12-month storage period, in contrast to *D. aphyllum*, which exhibited the most rapid decline in germination. *P. cornu-cervi* displayed intermediate storage performance, whereas *V. tricolor's*

relatively low initial germination led to a short predicted P50. Orchid seed longevity varies significantly between species, and this variation is typically linked to differences in seed physiology, biochemical composition, and ability to withstand desiccation and low-temperature storage conditions (Seaton and Pritchard 2003; Merritt et al. 2014a).

The predicted lifespan of *P. amabilis* in this study was consistent with the approximately nine-year storage period reported by Puspitaningtyas and Handini in 2021 for seeds stored at -20°C. The slightly longer P50 estimated here may be due to variations in the initial physiological quality of the seed lots and the fact that germination rates remained relatively high, ranging from approximately 70 to 90%, throughout the 12-month observation period. The observed decline in germination had not yet reached a significant phase, so extrapolating the viability curve indicated a relatively long predicted storage period. Studies based on the Ellis–Roberts seed viability model (Ellis and Roberts 1980; Walters et al. 2005) have also acknowledged the impact of initial seed quality on longevity estimates.

Differences in longevity among species offer a key basis for investigating whether biochemical and biophysical seed characteristics are linked to their storage capabilities. The contrasting behaviour of *D. aphyllum* and *P. amabilis* offers a chance to investigate whether variations in lipid composition and thermal properties are associated with differences in seed longevity.

Fatty acid composition and potential association with seed longevity

FAME profiles revealed that all four orchid species were distinguished by a high proportion of unsaturated fatty acids, with methyl linoleate being the most prevalent FAME. Previous research on orchid seeds has shown similar results. Researchers such as Diantina et al. (2022) found significant amounts of unsaturated fatty acids in five orchid species and discovered that fatty acid profiles altered over time in conjunction with decreasing seed viability. Colville et al. (2015) also showed interspecific differences in the fatty acid composition of *Dactylorhiza fuchsii* and *Grammatophyllum speciosum* through direct transesterification GC-MS.

The predominance of linoleic acid found in this study is also consistent with findings in other seed systems, such as tomatillo (*Physalis philadelphica*), where linoleic acid was identified as a key storage fatty acid (Pichardo-González et al. 2014). The high abundance of linoleic acid across all four orchid species suggests that unsaturated lipids are a significant component of their seed lipid profiles. The overall fatty acid profiles of the species are similar, but the relative proportions of MUFAs, PUFAs, and SFAs differ, indicating that the degree and pattern of unsaturation may be more useful for understanding species-specific storage behaviour than the presence of individual major fatty acids alone.

The interpretation is particularly relevant to *D. aphyllum*, which exhibited the lowest MUFA/PUFA ratio and the highest DBI and PI values. DBI characterises the overall density of double bonds within the fatty acid pool, as stated by Wang et al. (2020), whereas PI provides an estimation of the relative susceptibility of lipids to peroxidative attack based on their degree of unsaturation, according to Jové et al. (2020).

Greater DBI and PI values indicate greater theoretical unsaturation and peroxidizability, respectively. Observations of high DBI and PI in *D. aphyllum* indicate that its lipid pool is theoretically more susceptible to oxidative damage compared to others.

The link between fatty acid unsaturation and oxidative susceptibility has a biological basis due to multiple double bonds offering potential sites for free-radical-mediated oxidation. Pamplona et al. (1998) showed a link between fatty acid unsaturation and lipid peroxidizability, and DBI and PI have since been employed as indicators of lipid unsaturation and peroxidation potential. The high DBI and PI of *D. aphyllum* in this study coincided with its rapid loss of germination and the shortest predicted longevity ($P_{50} = 0.62$ months). The highly unsaturated lipid profile of *D. aphyllum* may be linked to its poorer storage performance, as suggested by this correspondence. This interpretation should be approached with caution as lipid oxidation products were not directly measured in the present study. The data indicate a correlation between lipid unsaturation properties and seed durability, rather than showing that lipid peroxidation directly causes a reduction in viability.

In contrast, *P. cornu-cervi* showed the lowest PI (39.78) and a significantly greater predicted lifespan than *D. aphyllum*. Because the peroxidizability index provides a theoretical estimate of the relative susceptibility of a fatty-acid profile to peroxidative damage, the lower PI of *P. cornu-cervi* suggests a comparatively lower theoretical susceptibility to lipid peroxidation than that of *D. aphyllum* (Pamplona et al. 1998). This difference is consistent with, although it cannot by itself explain, the relatively greater storage longevity observed in *P. cornu-cervi*. The disparities among species further suggest that seed durability cannot be inferred solely from the prevalence of a single fatty acid. Rather, the balance among lipid classes and the overall degree of fatty-acid unsaturation may provide a more informative biochemical context for understanding interspecific differences in storage performance.

These findings imply that the fatty acid composition should be taken into account in relation to both oxidative susceptibility and the physical properties of lipids at low temperatures. Researchers led by Diantina et al. (2023) showed that seeds from several Indonesian and New Zealand orchid species can be cryopreserved, thereby providing an additional method for conserving orchid germplasm. The degree of fatty acid unsaturation can also affect lipid phase-transition behaviour, with more highly unsaturated lipids typically showing lower transition temperatures than more saturated lipids. The high PUFA content identified by FAME analysis provides a biochemical basis for examining whether the observed lipid composition is accompanied by characteristic thermal transitions as a consequence. The central rationale for combining FAME profiling with DSC is that the two methods characterize complementary aspects of the seed lipid system: FAME profiling provides information on fatty-acid composition, whereas DSC characterizes the temperature-dependent thermal behaviour of the lipid matrix. Their integration may therefore provide a more comprehensive indication of species-specific seed storability than either biochemical or biophysical characterization alone (Francisqueti et al. 2024).

Thermal behaviour of orchid seed lipids

DSC profiles showed three endothermic transitions in *P. amabilis*, *P. cornu-cervi*, and *V. tricolor*, with no measurable exothermic crystallization transition observed during cooling to -125°C . The lack of a detectable crystallisation peak should be cautiously interpreted as a characteristic of the experimental conditions rather than proof that crystallisation cannot occur in these seeds. The low moisture content of the desiccated seeds may have decreased the likelihood of detectable ice formation and other crystallisation events, consistent with the desiccation tolerance of orthodox orchid seeds discussed by Merritt et al. (2014b).

Three endothermic transitions took place at roughly -78°C , between -37 and -33°C , and between -19 and -17°C . In all three species, the second transition had the highest enthalpy value, with *P. cornu-cervi* displaying the greatest value of 5.7438 J g^{-1} . The largest heat absorption related to the observed thermal transitions took place within this temperature range. The relatively high enthalpy of *P. cornu-cervi* is noteworthy, as it also displayed the highest UFA/SFA ratio, but its relationship with seed longevity cannot be directly inferred from enthalpy alone.

The thermal behaviour observed here is consistent with the idea that seed lipids can undergo multiple phase transitions across a wide temperature range. Researchers van der Walt and Nadarajan (2023) found that lipid crystallization occurs in several Myrtaceae species over a range of approximately -18 to -52°C , suggesting that conventional seed-bank temperatures around -20°C can overlap with lipid phase-transition regions in some seeds. Repeated or prolonged changes in lipid physical state may affect membrane and cellular organisation, which could be relevant to long-term seed stability. Merritt et al. (2014b) also highlighted the significance of lowering seed moisture prior to cryopreserving Australian orchid seeds, noting that successful cryopreservation correlated with moisture levels below $0.17\text{--}0.20 \text{ g H}_2\text{O g}^{-1}$ dry weight.

The current DSC results provide evidence that orchid seed lipids undergo measurable thermal transitions at temperatures below those conventionally used for storage. The observed transitions should not be taken as direct melting points of individual fatty acids. Seed lipids occur within complex biological matrices and comprise different fatty acids and lipid forms, including storage triacylglycerols, whose composition and molecular organization can contribute to the observed thermal behaviour. Accordingly, DSC provides a thermal fingerprint of the collective behaviour of the seed lipid system rather than necessarily allowing each thermal transition to be assigned to a single fatty acid (Francisqueti et al. 2024).

Integrating fatty acid composition and thermal behaviour to interpret seed storability

The combined FAME and DSC results offer complementary information on the biochemical and biophysical properties of orchid seed lipids. FAME analysis revealed that the four species were rich in linoleic acid and varied in their levels of fatty acid unsaturation, whereas DSC indicated distinct thermal transitions in the temperature range below approximately -15°C . The research suggests that seed

storability cannot be attributed solely to the presence of a specific fatty acid or the overall level of unsaturation.

D. aphyllum exhibited the highest DBI and PI, and displayed the fastest decline in germination, indicating an association between its highly unsaturated lipid profile and poor storage performance. In contrast, *P. cornu-cervi* had the highest UFA/SFA ratio but a lower PI and a longevity that was intermediate. *P. amabilis* exhibited the lowest UFA/SFA ratio and maintained high germination throughout the 12-month storage period. The differences among species indicate that the relationship between lipid composition and seed longevity is unlikely to be explained solely by the overall degree of fatty-acid unsaturation. Other factors, including lipid class composition, the physical organization and thermal behaviour of lipids, seed moisture status, and the capacity of endogenous antioxidant systems to limit oxidative damage, may also contribute to variation in seed storage behaviour (Vertucci and Roos 1990; Nadarajan et al. 2023; Francisqueti et al. 2024).

The DSC results add an additional dimension to this interpretation. The second major endothermic transition took place between roughly -37 and -33°C , and the third occurred between approximately -19 and -17°C . At least part of the thermal behaviour detected in the present study occurs relatively close to the conventional -20°C storage temperature. Van der Walt and Nadarajan (2023) found that lipid phase transitions in some seeds can coincide with conventional seed-bank temperatures. The current data do not show that the -20°C storage condition directly led to the observed viability losses via lipid phase transitions. Instead, they suggest that the storage temperature falls within a temperature range where seed lipid systems display observable thermal properties.

The integration of FAME profiling and DSC therefore complements germination-based assessment by showing that differences in seed longevity among orchid species are accompanied by differences in fatty-acid composition and thermal behaviour (Francisqueti et al. 2024). The findings suggest that seed storability is associated with the combined biochemical and biophysical characteristics of the lipid system rather than being explained by a single lipid component (Diantina et al. 2022; Francisqueti et al. 2024). FAME profiling provides information on the degree and pattern of fatty-acid unsaturation, whereas DSC provides information on the temperature-dependent thermal behaviour of the seed lipid system. These approaches can therefore serve as complementary indicators for evaluating species-specific seed storability and may contribute to a more comprehensive characterization of the factors associated with differences in orchid seed longevity (Francisqueti et al. 2024).

The integrated interpretation also has relevance to orchid conservation efforts. Researchers led by Diantina et al. (2023) showed that cryopreservation can be used on multiple orchid species, and the current study found that orchid seed lipids undergo thermal transitions at temperatures significantly below 0°C . Conventional -20°C storage may therefore be associated with relatively rapid loss of viability in certain species, making cryogenic storage a potentially useful strategy for them. The current study did not directly compare long-term viability under liquid-nitrogen storage with storage at -20°C . The present DSC results should therefore be regarded as a starting point for determining potentially suitable

cryogenic temperature ranges rather than as definitive proof that -196°C is the optimal storage temperature for the four species studied.

Implications for orchid seed conservation

The substantial interspecific variation in seed storage performance observed in the present study highlights the need for species-specific approaches to orchid seed conservation (Diantina et al. 2023; Francisqueti et al. 2024). The long lifespan of *P. amabilis* at -20°C implies that conventional seed-bank storage could be suitable for at least some orchid species, whereas the rapid decline observed in *D. aphyllum* suggests that -20°C may be less suitable for maintaining long-term viability in other species. The relatively short predicted longevity of *V. tricolor* should also be considered in conjunction with its lower initial germination, as low starting viability can impact P50 estimation.

The integration of biochemical and biophysical profiles may provide a useful framework for identifying orchid species that may require alternative storage strategies when conventional -20°C storage results in poor seed longevity (Francisqueti et al. 2024). Species exhibiting rapid viability decline, coupled with lipid profiles indicative of high theoretical peroxidizability, may merit further examination of alternative storage temperatures and cryogenic methods. Establishing causal mechanisms and determining optimal conservation conditions would require validation through direct storage experiments at cryogenic temperatures, as well as measurements of lipid oxidation, membrane integrity, and post-storage germination.

The present study supports the view that variation in orchid seed longevity may be associated with differences in the biochemical composition and thermal behaviour of seed lipids (Diantina et al., 2022). Combining germination testing, FAME profiling, and DSC analysis offers a comprehensive method for assessing orchid seed viability and for guiding the development of improved storage protocols for long-term preservation.

Conclusion

This study shows considerable variation in the storage ability of orchid seeds when kept at -20°C in a conventional seed bank. *Dendrobium aphyllum* showed the most rapid decline in germination and the shortest predicted lifespan, whereas *Phalaenopsis amabilis* maintained high germination levels throughout the 12-month storage period. Differences in seed lipid characteristics were accompanied by these contrasting storage behaviours. *D. aphyllum* showed the lowest MUFA/PUFA ratio and the highest DBI and PI, suggesting a highly unsaturated lipid profile that is more susceptible to lipid peroxidation.

Further profiling by FAME revealed linoleic acid as the predominant fatty acid in all four orchid species, and DSC analysis showed distinct endothermic lipid transitions occurring between approximately -78 and -17°C . The occurrence of thermal transitions within and around the conventional -20°C storage range implies that lipid phase behaviour may be relevant to the storage response of orchid seeds. The current findings do not indicate that lipid oxidation or thermal transitions directly result in viability loss.

This suggests that species-specific seed longevity is linked to the biochemical composition and thermal properties of seed lipids.

The integration of germination assessment, FAME profiling, and DSC therefore offers complementary biochemical and biophysical indicators for characterising orchid seed storability and may assist in the optimisation of species-specific conservation conditions. The findings also support further investigation of cryogenic storage as an alternative for orchid species that show poor longevity under conventional – 20°C storage. Future studies should incorporate broader taxonomic sampling, direct measurements of lipid oxidation and membrane stability during storage, and controlled long-term experiments under cryogenic conditions to validate the predictive value of these lipid characteristics and establish optimal preservation protocols.

Declarations

Compliance with ethical standards

No experiments involving human participants or animals were conducted by the authors for this study.

Competing interest

The authors have no relevant financial or nonfinancial interest to disclose.

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Author Contributions

Surya Diantina, Windri Handayani, and Yasman Yasman contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Elizabeth Handini. The formulation of treatments was assisted by Popi Aprilianti. The work was supervised by Surya Diantina, Windri Handayania, and Yasman Yasman, who also contributed to writing the manuscript. The figures and first draft of the manuscript were prepared by Elizabeth Handini, and all authors commented on previous versions. All authors read and approved the final manuscript.

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Data availability

All data supporting the findings of this study are available within the paper.

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Figures



Figure 1

Flowers and Seeds of Orchid Species Used in This Study: *Dendrobium aphyllum* (Roxb.) C.E.C.Fisch. *Phalaenopsis amabilis* (L.) Blume, *Phalaenopsis cornu cervi* (Breda) Blume & Rchb.f, and *Vanda tricolor* Lindl.

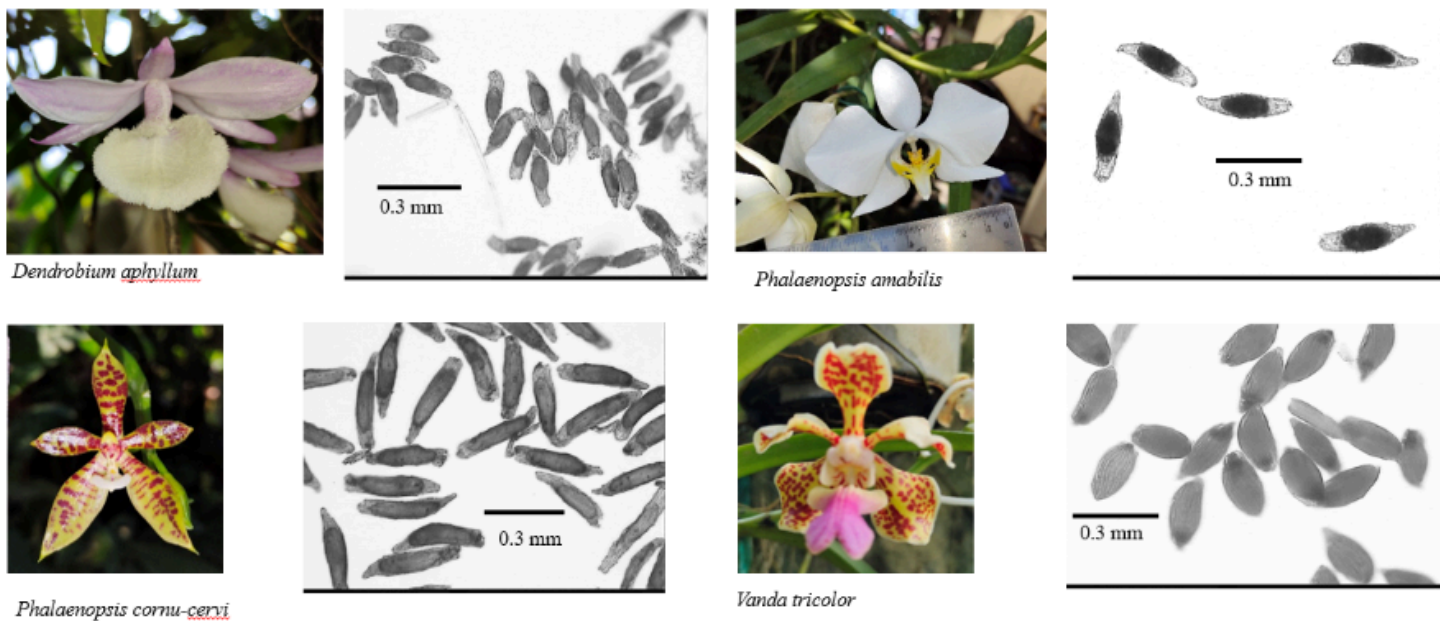


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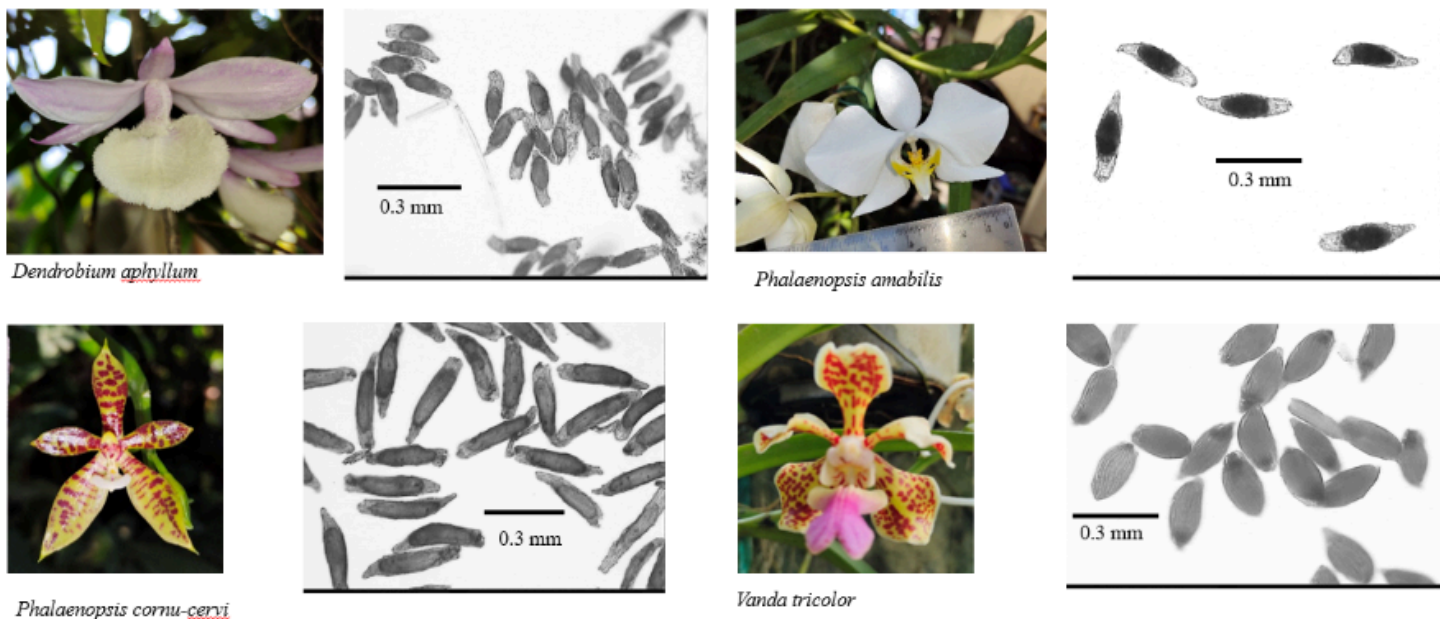
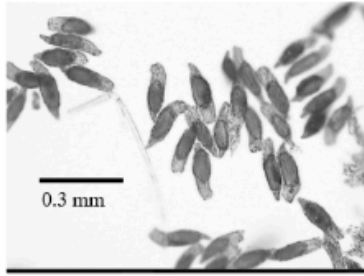


Figure 1

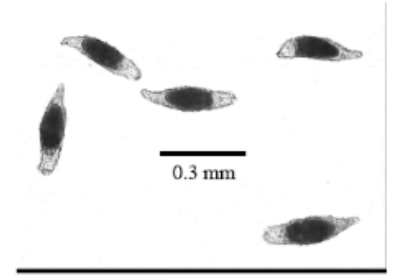
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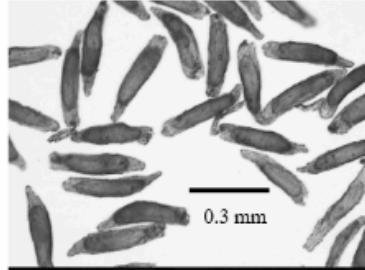
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Phalaenopsis amabilis



Phalaenopsis cornu-cervi



Vanda tricolor

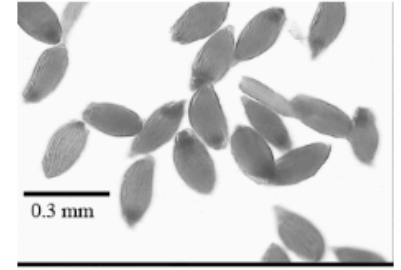


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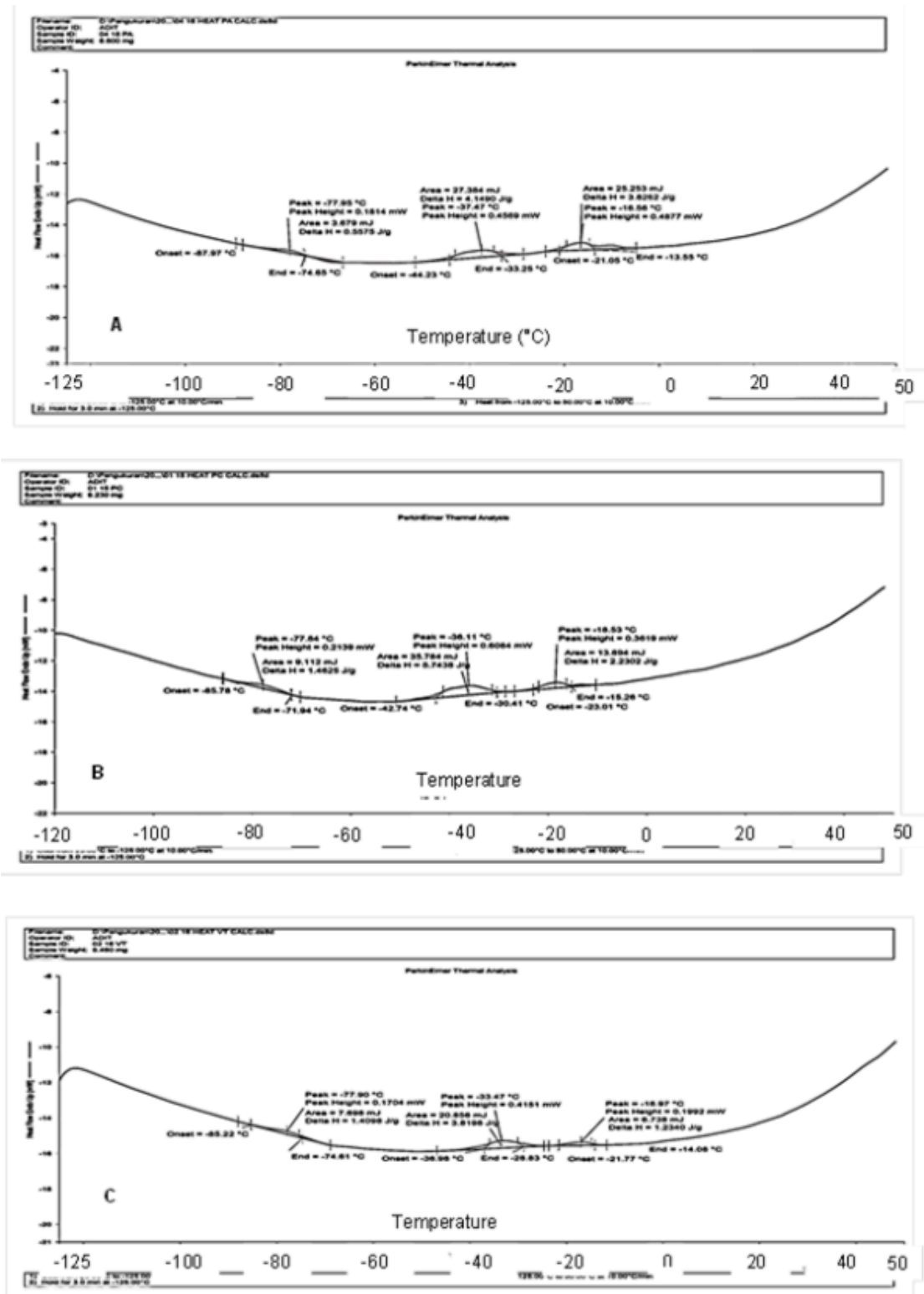


Figure 2

Comparison of DSC Thermograms from the Heating Process (-125°C to 50°C at a Rate of 10°C/min) Showing: **A.** *Phalaenopsis amabilis*, **B.** *Phalaenopsis cornu cervi*, and **C.** *Vanda tricolor*

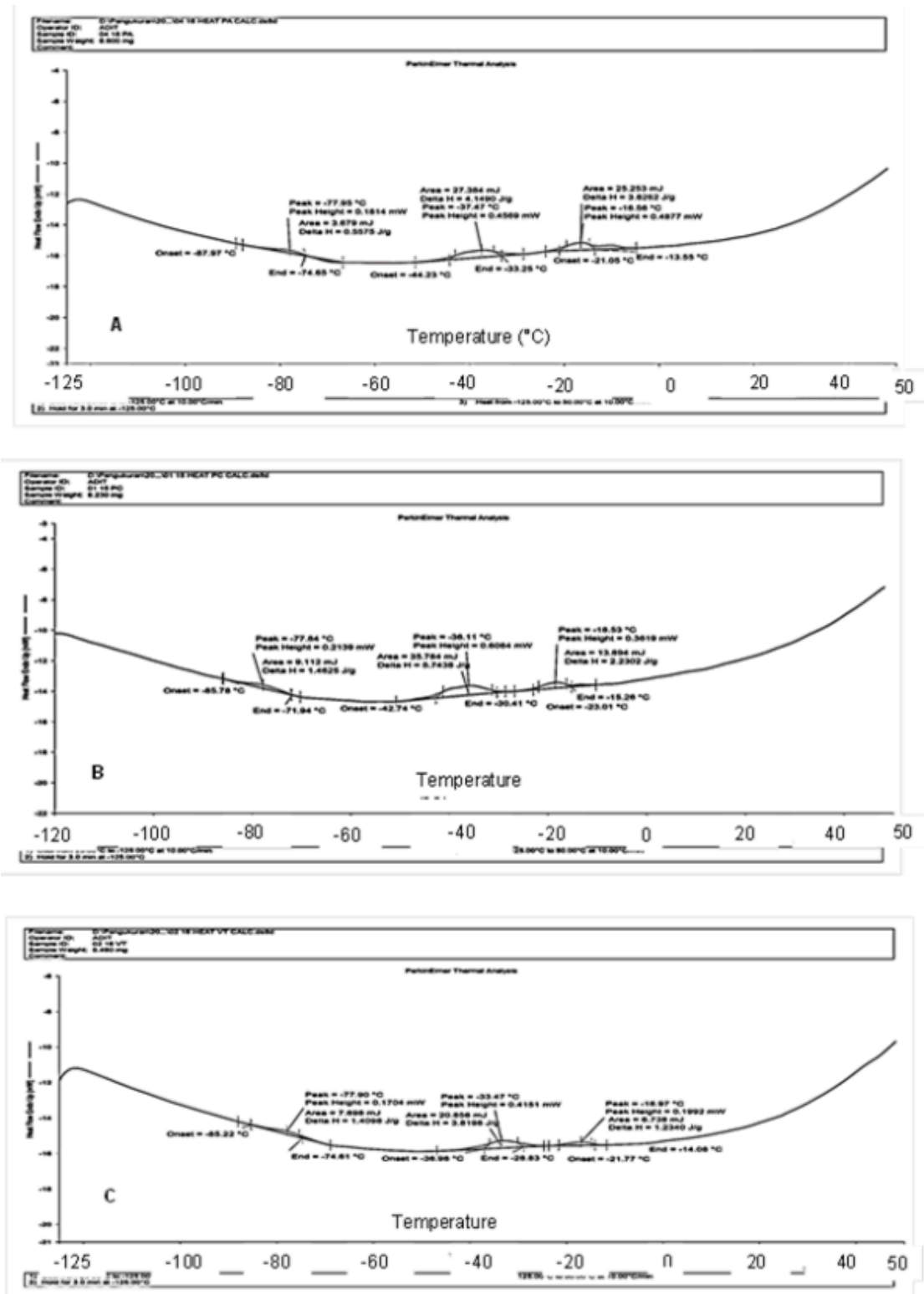


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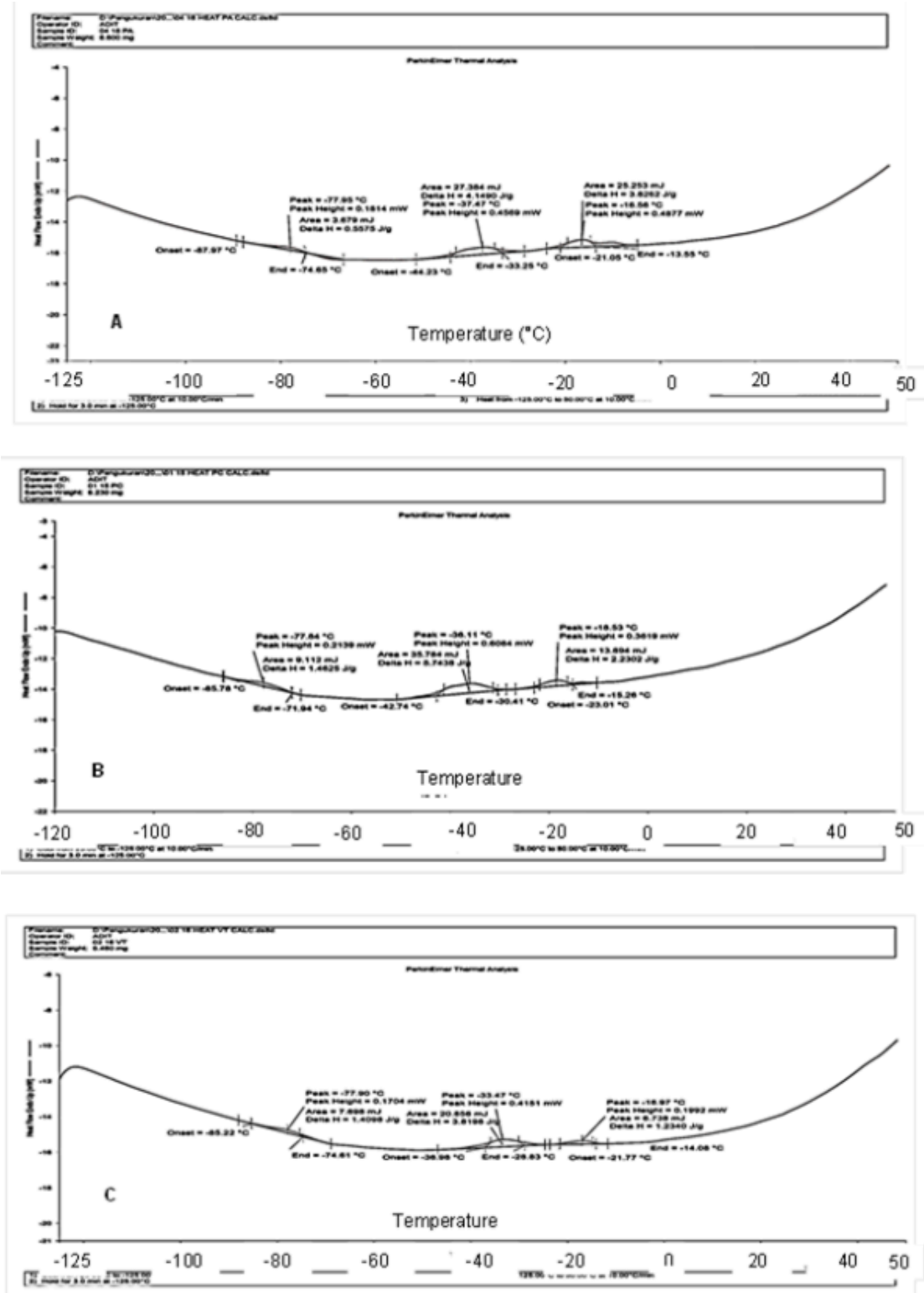


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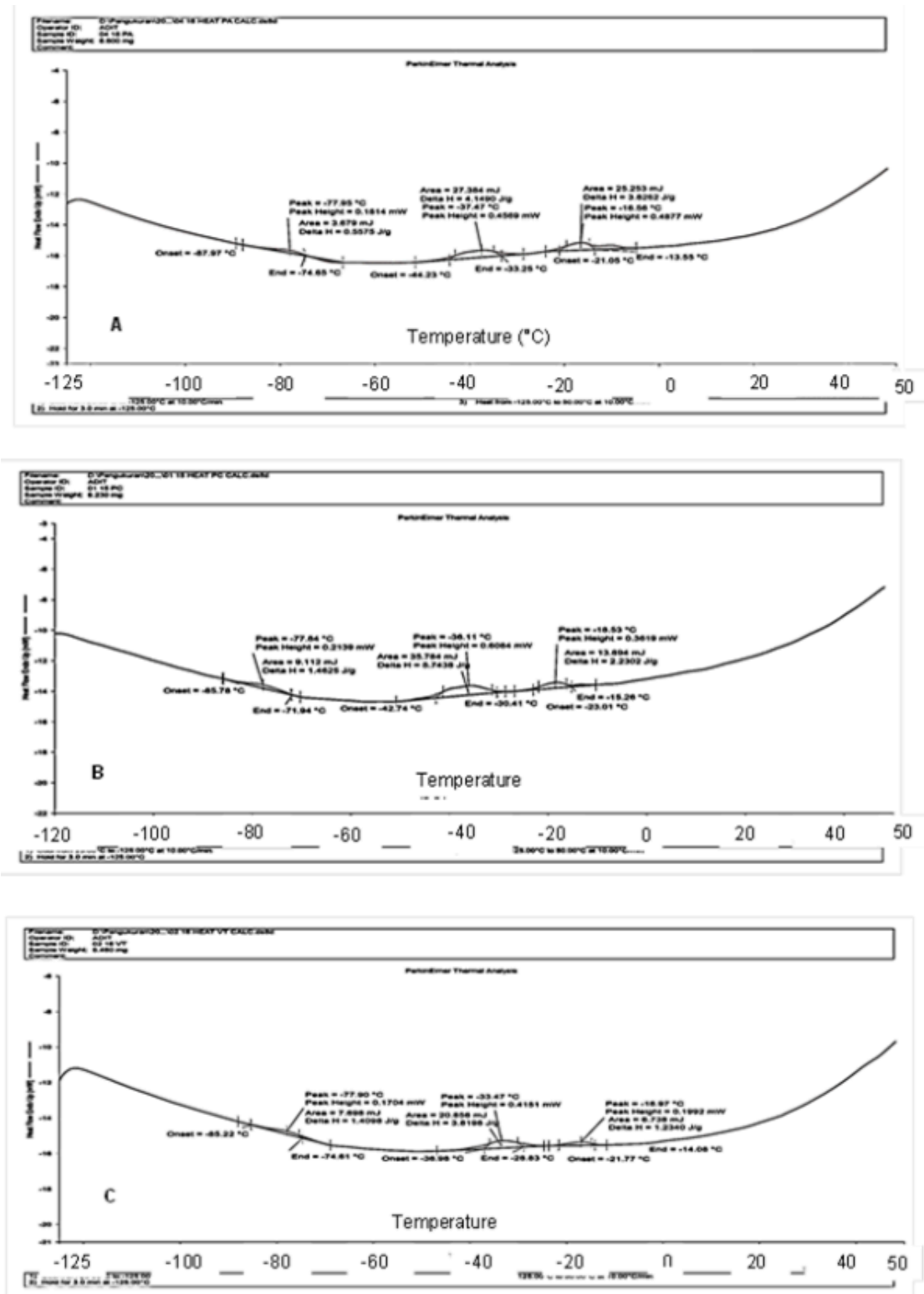


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