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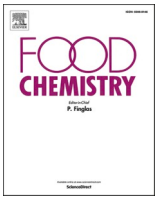

Impact of temperature and humidity conditions as abiotic stressors on the phytochemical fingerprint of oat (*Avena sativa* L.) sprouts

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ABSTRACT

This study aimed to evaluate the effect of temperature (20, 25, and 30 °C) and relative humidity (RH, 50, 55, and 60 %) as abiotic stressors during oat (*Avena sativa* L.) germination using a 2-level factorial design with central point. UPLC-QToF-MS^E identified eighty polyphenols, nine avenanthramides, twelve lignans, and five phytosterols Notably, 100 % germination was achieved at 25 °C/60 % RH from day 3, yielding the longest radicle size. The highest content of most phenolic acids, avenanthramides, and lignans occurred at 30 °C/65 % RH, where 100 % germination was attained by day 5, but with a shorter radicle size. The best flavonoid and phytosterol profle was obtained at 20 °C/55 % RH, achieving only a 67 % germination rate. Therefore, while these conditions enhance the bioactive compound profile, the associate decrease in germination metrics suggests potential distress effects. Consideration of both photochemical outcomes and germination yield is crucial for comprehensive assessments in future applications.

1. Introduction

Consumers are increasingly interested in acquiring food products with beneficial health properties, such as oat (*Avena sativa* L.). This cereal has attracted attention from researchers over the years resulting in increased interest from worldwide food industries to use this cereal as an ingredient in several food products, including breakfast cereals, bread, infant foods, and beverages.

Several studies have demonstrated the biological effects of oats, such as their antioxidant and hypolipidemic properties. In addition, this cereal is known to provide essential nutrients and health benefits associated mainly with its high dietary fiber content. Oat is also a rich source of a wide range of phenolic compounds avenanthramides, a group of *N*-cinnamoylanthrannilic acid derivatives exclusively found in this cereal (Gramza-Michałowska et al., 2018).

Recently, cereal sprouts have been the subject of different research studies due to their improved bioactive compounds and health benefits compared to grains, including antioxidant, hypolipidemic, hypoglycemic, anti-inflammatory, cardioprotective activities (Benincasa et al., 2019). During germination, protein content and quality improve, mineral availability increases, the glycemic index decreases, and antinutrients are reduced. This results in a nutritionally enhanced cereal (Singh et al., 2015). Moreover, the concentration of several bioactive compounds, including polyphenols, can be augmented during germination. For example, oat contains a high concentration of quinones, benzoic and cinnamic acids, flavonols, flavones, flavanones, anthocyanidins, aminophenols and their precursors, which have been increased by 3- to 4-fold during germination. Furthermore, avenanthramides concentration significantly increases during this process (Schendel, 2019).

Seed germination and seedling emergence are the first critical steps for plant establishment. These processes depend highly on environmental conditions, such as temperature, water, light, and oxygen, which can affect their macro and micronutrient concentration**.** Particularly temperature and humidity are crucial factors that regulate germination, which both can act as abiotic stressors by inducing a local or systemic response during sprout development (Świeca et al., 2014), increasing the production of reactive oxygen species (ROS) that activate enzymatic and non-enzymatic detoxification systems such as the overproduction of phenolic compounds and other endogenous antioxidants that play an

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essential role in the regulation of plant metabolism (Benincasa et al., 2019).

Previous studies have explored the impact of these environmental factors on oat germination. For instance, El-Mouhamady et al. (2014) identified 25 °C as the optimal temperature for the activity of enzymes related to germination, highlighting the significance of temperature regulation in this process. Ding et al. (2019) and Jiménez-Pulido et al. (2022) observed time-dependent increases in total phenolic content during oat germination, effect that was also observed in avenanthramide levels by Feng et al. (2022) indicating the dynamic nature of phytochemical changes. Altogether, these previous studies demonstrate that germination combined with environmental stress could be a cheap and efficient process to improve the bioactive composition of oats. Unlike previous research that has focused on specific aspects such as growth, nutritional changes, polyphenols, and avenanthramides as isolated variables, this study stands out for its holistic approach, simultaneously measuring a wide range of bioactive compounds, including lignans and phytosterols, which have not been previously reported during oat germination. Therefore, the aim of this study was to evaluate the impact of temperature and relative humidity as abiotic stressors on oat sprouts' growth and phytochemical fingerprint.

2. Materials and methods

2.1. Vegetable materials

Oat (*Avena sativa* L.) grains were donated by Campo Experimental Bajío-Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (CEBAJ-INIFAP, Celaya, Guanajuato, México). First, oat grains (100 g) were soaked in 600 mL of 1.5 % sodium hypochlorite for 30 min at 30 °C. Then, oat grains were drained, washed, and soaked in distilled water at a 1:6 w/v ratio for 12 h. Afterward, as previously reported, hydrated grains were allocated in trays covered with a wet filter paper in a germination chamber for five days in darkness (Damazo-Lima et al., 2020). Afterward, oat sprouts were dried at 50 °C for 12 h and ground to a particle size < 0.5 mm. Finally, samples were stored at 4 °C protected from light until analysis.

The germination conditions evaluated in this study included a temperature range from 20 to 30 °C and a relative humidity (RH) range from 55 to 65 %, which were selected based on a preliminary study aimed at identifying a range of abiotic stressors for oat germination to avoid an important delay or inhibition in germination due to low temperature and/or relative humidity, and to avoid fungal growth due to high relative humidity. To assess the impact of these conditions as abiotic stressors on oat germination, we adopted a 2-level factorial design with a central point. The experiment was laid out in a randomized complete block design, consisting of three blocks, with each block having three replicates. Additionally, we included a non-germinated sample (oat grain) as a control for comparison.

2.2. Germination characteristics

Germination parameters were determined daily for five days. Germination percentage was determined as the number of seedlings fully emerged. Radicle length (mm) was measured with a vernier caliper.

2.3. Nutritional and non-nutritional composition

The nutrient composition was analyzed in oat grains and sprouts at different temperatures and RH conditions on the fifth day of germination, which was carried out as indicated in the official methods of the Association of Official Agricultural Chemists (AOAC). Protein (method 920.87), crude fat (method 920.85), crude fiber (method 962.09), ash (method 923.03), and moisture (method 925.10) (Horwitz, 2010). The content of carbohydrates was estimated by difference. As reported by Latta and Eskin (1980), phytic acid was quantified in all samples.

2.4. Phytochemicals extraction

The phytochemical composition was analyzed in oat grains and sprouts at different temperatures and RH conditions on the fifth day of germination. Samples (25 mg) were extracted by ultrasound with 500 µL of a 50:50 (v/v) methanol:water solution acidified with HCl to pH2 for 60 s. Then, samples were centrifuged at 25,000*g* for 5 min at 4 °C, and supernatants were recovered. The residue was re-extracted with 500 µL of a 70:30 v/v acetone:water solution and centrifuged. Both supernatants were mixed, and the solvent was evaporated to dryness in a vacuum concentrator (SpeedVac, ThermoFisher Scientific, MA, USA). The samples were redissolved in 500 µL of methanol (Mendoza-Sánchez et al., 2019). This extract was used to identify polar phytochemicals such as polyphenols, avenanthramides, and lignans.

Samples (50 mg) were extracted by ultrasound with 500 µL of *n*-hexane for 60 s. Then, samples were centrifuged at 25,000*g* for 5 min at 4 °C, and supernatants were recovered. The residue was re-extracted with 500 µL of *n*-hexane. Both supernatants were mixed, and the solvent was evaporated to dryness in a vacuum concentrator (Mendoza-Sánchez et al., 2019). Samples were redissolved in 500 µL of isopropanol. This extract was used for the identification of non-polar phytochemicals such as phytosterols.

2.5. Phytochemical profile

An ultra-performance liquid chromatography (UPLC) coupled to a photodiode array detector (PDA) and a quadrupole time-of-flight mass spectrometer (QToF MS^E) with an atmospheric pressure electrospray ionization (ESI) probe (Vion, Waters Co., MA, USA) were used for the identification of the polyphenols, avenanthramides, lignans, and phytosterols composition. The concentrated extracts previously described in section 2.3 were resuspended in 1 mL of mobile phase and filtered (PVDF syringe filters, 0.2 µm).

For the polyphenol and avenanthramide profile, samples (2 µL) were injected into a C18 BEH Acquity column (100 × 2.1 mm, 1.7 µm, Waters Co., MA, USA) at 35 °C. The mobile phases used for this analysis were (A) water with 0.1 % formic acid and (B) acetonitrile with 0.1 % formic acid at 0.5 mL/min. The gradient settings were as follows: the initial conditions were 95/5 (A/B), which was maintained for 2 min, then modified to 5/95 (A/B) in a lineal gradient for 20 min, and was maintained for 3 min. Finally, the column was re-equilibrated at 95/5 (A/B) in 2 min and was maintained for 3 min, with a total time of 30 min.

For the phytosterol and lignan profile, samples (2 µL) were injected into a C18 BEH Acquity column (100 × 2.1 mm, 1.7 µm, Waters Co., MA, USA) at 35 °C. The chromatographic separation was achieved with water (A) and methanol (B) at 0.3 mL/min under the following gradient conditions: the initial conditions were 90/10 (A/B), which were modified to 100/0 in a lineal gradient for 5.5 min, and was maintained for 2 min. Then, the column was re-equilibrated at 90/10 in 2 min and was maintained for 1 min, with a total time of 10 min (Reynoso-Camacho et al., 2021).

The mass spectrometry conditions were as follows: the source temperature was set at 120 °C, the desolvation gas (N_2) was set at 450 °C and 800 L/h, and the cone gas flow at 50 L/h. Data were acquired at MS^E with the sensitivity analyzer mode using a mass scan range of 50–1800 *m/z*. The collision energy was set at 5 eV for low energy and 15–45 eV for the high energy ramp. Leucine-enkephalin (50 pg/mL, 556.2766 *m/z*) was used for lock mass correction, which was infused in a 5 min interval for 2 s at 10 µL/min (Rodríguez-González et al., 2018). Data were acquired using the UNIFI software (Waters Co., MA, USA).

The phytochemical analysis performed can be characterized as a semi-targeted approach. Molecular formulas of the target components were sought during data processing, guided by predefined specifications including expected adducts, mass error < 10 ppm, isotopic similarity >

98 %, and characteristic fragments from literature and mass spectra databases. Total ion chromatograms (TIC) at low and high collision energy are included in Fig. 1S.

In this study, the quantification of various phytochemical compounds was conducted using the external standard method with commercial standards or, when specific standards were not available, similar compounds were used. Gallic acid was employed as the standard for the quantification of gallic acid and its derivatives, protocatechuic acid for hydroxybenzoic acids and their derivatives, caffeic acid for derivatives of caffeic acid, chlorogenic acid for all caffeoylquinic acids, p-coumaric acid for coumaric acid and its derivatives, ferulic acid for ferulic acid and its derivatives, epicatechin for epicatechin and its glucoside derivative, catechin for catechin and its glucoside derivative, quercetin for quercetin and its derivatives, kaempferol for kaempferol and its derivatives, luteolin for luteolin and its derivatives, apigenin for apigenin and its derivatives, daidzin for daidzin and its derivatives, genistin for genistin derivatives, avenanthramide A for all avenanthramides, and β-sitosterol for all phytosterols except β-campesterol, which had its own standard. Additionally, syringic acid, vanillic acid, benzoic acid, cinnamic acid, synaptic acid, ellagic acid, gallocatechin, and epigallocatechin gallate were used for the quantification of their respective compounds.

2.6. Statistical analysis

Data are shown as mean ± standard deviation of three replicates. Kolmogorov-Smirnov's and Levene's tests were used for identifying data distribution and homoscedasticity. Statistical significance was determined by ANOVA followed by Tukey's test ($p < 0.05$). All univariate statistical analyses were carried out with JMP software (v10). Multivariate statistical analysis was carried out through Partial Least Square-Discriminant Analysis (PLS-DA) and Variable Importance in the Projection (VIP) score vs. coefficient plots were obtained. Multivariate analyses were carried out in Metaboanalyst v5.0 software.

3. Results

3.1. Effect of temperature and humidity on germination percentage and radicle size of oat sprouts

The growth parameters of oats sprouted at different temperatures and relative humidity are shown in Table 1. Germination at 60 and 65 % RH improved the germination percentage to 97–100 %, whereas treatments with a lower RH did not reach a high germination percentage on day 5. Interestingly, an accelerated germination rate was observed at 25 °C and 60 % RH since the highest germination percentage (99.7 %) was reached on the third day. Moreover, this germination condition led to the most significant radicle size of oat sprouts from the fourth day, 1.5–1.7-fold higher than the maximum radicle size obtained with the other germination conditions on day 5.

Table 1
Effect of temperature and relative humidity conditions on the percentage of germination and radicle size of oat (*Avena sativa* L.) sprouts.

Germination day	Oat sprouts				
	20 °C/55 % RH	20 °C/65 % RH	25 °C/60 % RH	30 °C/55 % RH	30 °C/65 % RH
Germination percentage (%)					
1	4 ± 1.5d	5 ± 0.6e	4 ± 1.2c	2 ± 0.6c	2 ± 0.6d
2	17 ± 2.9c	47 ± 2.6d	40 ± 13.1b	19 ± 14.4b	26 ± 4.6c
3	44 ± 0.6b	55 ± 0.0c	100 ± 0.6a	36 ± 2.1ab	83 ± 8.1b
4	55 ± 4.2a	83 ± 2.5b	100 ± 0.0a	65 ± 2.5a	97 ± 2.9ab
5	67 ± 8.0a	97 ± 1.5a	100 ± 0.0a	69 ± 1.7a	100 ± 0.0a
Radicle size (cm)					
1	0.14 ± 0.04c	0.13 ± 0.01d	0.15 ± 0.01c	0.10 ± 0.00c	0.11 ± 0.02c
2	0.23 ± 0.85bc	1.13 ± 0.41c	0.22 ± 0.06c	0.51 ± 0.24c	0.62 ± 0.47bc
3	1.54 ± 0.38b	1.17 ± 0.50b	3.63 ± 0.65b	1.69 ± 0.29b	1.88 ± 0.25b
4	3.08 ± 0.26a	2.47 ± 0.88ab	6.49 ± 0.32a	3.01 ± 0.31a	2.93 ± 0.76ab
5	3.75 ± 0.12a	4.17 ± 0.91a	6.57 ± 0.24a	4.01 ± 0.26a	4.49 ± 0.41a

Data are expressed as mean values ± standard deviation of three replicates. Different letters indicate significant ($p < 0.05$) differences among germination days within each condition by Tukey's test. RH: relative humidity.

3.2. Effect of temperature and humidity on the nutrient composition of oat sprouts

The nutrient and non-nutrient composition of oats sprouted at different temperatures and relative humidity conditions is shown in Table 2. All germination conditions significantly ($p < 0.05$) increased protein (1.1–1.6-fold) content and decreased total, soluble, and insoluble dietary fiber (2.2–2.9, 3.2–9.3, and 1.8–2.4-fold, respectively) as well as phytic acid (5.5–11.0-fold). Similarly, ash content was decreased with most germination conditions (1.3–3.2-fold), except when sprouting was carried out at 25 °C and 60 % RH. On the other hand, no clear trend was observed in fat content since oats sprouted at 20 °C/55 % RH and 25 °C/60 % RH showed an increased fat content (1.3-fold), whereas oats

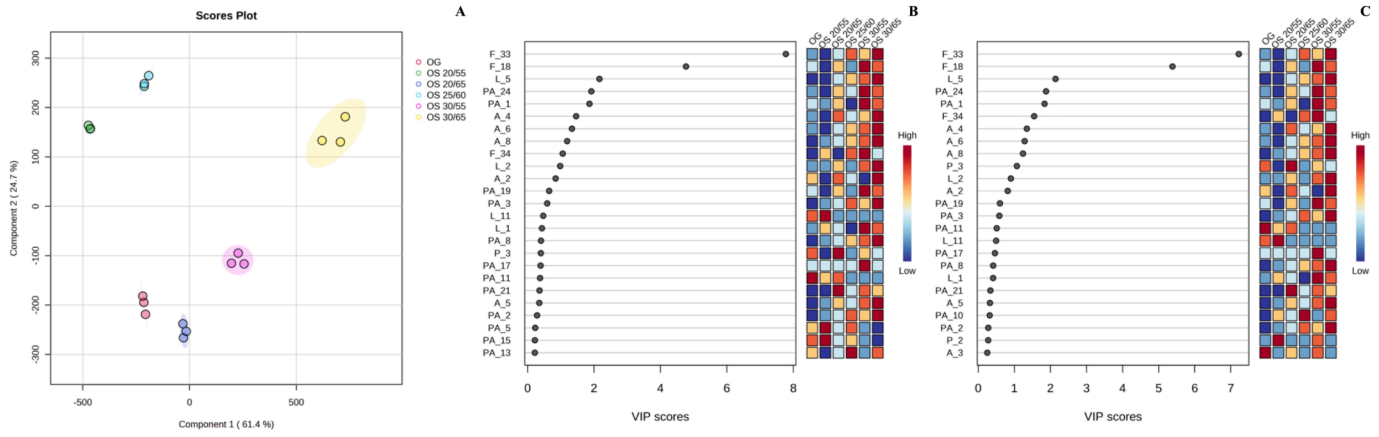

Fig. 1. PLS-DA plot (A) of the phytochemical characterization of oat (*Avena sativa* L.) sprouts grown under different germination conditions, and VIP plots of component 1 (B) and component 2 (C). PLS-DA: partial least square-discriminant analysis; VIP: variable important in the projection; OG: oat grain; OS: oat sprout.

Table 2
Effect of temperature and relative humidity conditions on the nutrient composition and phytic acid of oat (*Avena sativa* L.) sprouts.

Parameter		Oat sprouts				
	Oat grain	20 °C/ 55 % RH	20 °C/ 65 % RH	25 °C/ 60 % RH	30 °C/ 55 % RH	30 °C/ 65 % RH
Protein (%)	8.83 ± 0.23d	14.25 ± 0.20a	12.62 ± 0.17b	10.05 ± 0.04c	14.29 ± 0.38a	14.07 ± 0.05a
Carbohydrates (%)	73.37 ± 1.10b	73.81 ± 1.10b	77.46 ± 0.90a	71.08 ± 0.7c	74.31 ± 0.90b	75.11 ± 1.5ab
Fat (%)	4.41 ± 0.20b	5.59 ± 0.14a	4.87 ± 0.01b	5.55 ± 0.01a	4.43 ± 0.04ab	2.22 ± 0.16c
Ash (%)	3.99 ± 0.30b	2.35 ± 0.11d	1.25 ± 0.06e	3.62 ± 0.10b	4.77 ± 0.23a	3.00 ± 0.27c
Moisture (%)	9.40 ± 0.70a	4.00 ± 0.30c	3.80 ± 0.30c	9.70 ± 0.20a	2.20 ± 0.20d	5.60 ± 0.00b
Dietary fiber (%)						
Total	73.53 ± 2.51a	33.12 ± 4.19b	25.26 ± 2.24b	33.15 ± 4.65b	29.34 ± 2.20b	30.95 ± 2.68b
Soluble	18.49 ± 0.74a	5.83 ± 0.68b	2.44 ± 0.49c	2.28 ± 0.52c	1.99 ± 0.40c	2.02 ± 0.09c
Insoluble	55.04 ± 1.89a	27.29 ± 3.69b	22.82 ± 1.94c	30.87 ± 4.23b	27.35 ± 1.87b	28.92 ± 2.60b
Phytic acid (g)	0.44 ± 0.00a	0.08 ± 0.02b	0.07 ± 0.03b	0.04 ± 0.00c	0.08 ± 0.02b	0.06 ± 0.02bc

Data are expressed as mean values ± standard deviation of three replicates. Different letters indicate significant ($p < 0.05$) differences among germination conditions by Tukey's test. RH: relative humidity.

sprouted at 30 °C/65 % RH showed a decreased fat content as compared to oat grain (2.0-fold).

3.3. Effect of temperature and humidity on the phytochemical composition of oat sprouts

This study identified 80 polyphenols in oat grains and sprouts grown at different temperatures and humidity conditions. These compounds were classified as follows: sixteen hydroxycinnamic acids, eighteen hydroxybenzoic acids, seven flavanols, twenty-four flavonols, ten flavanones, and five isoflavones (Table 3).

Interestingly, twenty-four polyphenols were synthesized *de novo* during oat germination since they were not detected in oat grains, from which only three polyphenols were synthesized under all germination conditions. Conversely, six polyphenols were found at a greater concentration in oat grains as compared to all oat sprouts: dihydroxybenzoic acid hexoside (PA_7), syringic acid (PA_11), gallic acid ethyl ester (PA_14), sinapic acid (PA_22), kaempferide (F_23), and acetyldaidzin (F_46), which were found depleted by 1.2–3.2-fold in oat sprouts. Moreover, one minor polyphenol was only identified in oat grains: quercetin rhamnoside (F_11).

Overall, the most significant content of most polyphenols was found when the oat was sprouted at 30 °C/55 % RH, kaempferol rhamnosyl hexoside rhamnoside (F_18) as the primary compound, followed by gallic acid (PA_1), feruloylquinic acid (PA_24), luteolin apiosyl malonyl hexoside (F_34), which were found increased by 1.4–4.9-, 2.3–10.2-, 2.3–45.2-, and 3.5–55.7-fold as compared to those of other germination conditions. Moreover, caffeic acid hexoside (PA_17) was only identified at this germination condition. On the other hand, oats sprouted at 30 °C/65 % RH synthesized a high content of apigenin apiosyl hexoside (F_33) and kaempferyl rhamnosyl hexoside rhamnoside (F_18), which were 1.3–64.7-fold higher than those of other oat sprouts.

A total of nine avenanthramides were identified in oat grains and sprouts (Table 4). Interestingly, eight avenanthramides were identified in oat grains, of which six were significantly ($p < 0.05$) increased when germination was carried out at 30 °C/65 % RH (3.5–16.9-fold). The major avenanthramides found in oats sprouted at this condition were avenanthramide B (A_4), G (A_2), and L (A_6). In addition, Avenanthramide D was produced during germination since this compound was not identified in oat grains but was found in all oat sprouts.

Regarding lignans, seven compounds were identified in oat grains, of which five were significantly increased by some germination conditions (L_1, L_3, L_7, L_9, and L_11). In contrast, two lignans, secoisolariciresinol-sesquilignan (L_8) and matairesinol (L_12) were maintained or decreased during oat germination (1.4- and 1.2-fold down, respectively). Conversely, five lignans were synthesized during oat germination: medioresinol (L_2), hydroxymatairesinol (L_4), acetoxypinoresinol (L_5), syringaresinol (L_6), and isolariciresinol (L_10). Interestingly, oats sprouted at 30 °C and 65 % RH condition showed the highest amount of lignans, closely followed by oats sprouted at 30 °C and 55 % RH (Table 4).

Finally, five phytosterols were identified in oat grain and oats sprouts (Table 4). The major phytosterol identified in the oat grain was stigmasterol hexoside, which was maintained when the oat was sprouted at 20 °C and 65 % RH. However, it was significantly decreased under the other germination conditions (1.1–7.9-fold). Conversely, campesterol hexoside was synthesized *de novo* when the oat was sprouted at 20 and 30 °C and 55 % RH.

3.4. Chemometric analysis

Multivariate analyses were carried out to better understand the effect of germination temperature and relative humidity as abiotic stressors that caused the modification of the oat phytochemical profile. The PLS-DA model plot of the phytochemical (polyphenol, avenanthramide, lignan, and phytosterol) profile of oat grain and sprouts is shown in Fig. 1. The model explained an overall variance of 86.9 %, showing clear discrimination between the experimental groups. According to the cross-validation, the performance of the PLS-DA model showed an R2 value of 0.83 and a Q2 value of 0.74 with two components, obtaining a $p < 0.01$ with the separation distance random permutation test (100 cycles), indicating adequate predictability and goodness of fit.

Component 1 (explained variance of 61.4 %) separated oat grains (OG) and oats sprouted at 20 °C and 55 % RH and at 25 °C and 60 % RH from oats sprouted at 30 °C and 55 and 65 % RH, whereas the component 2 (explained variance of 24.7 %) separated oat grains and oats sprouted at 20 °C and 65 % RH and at 30 °C and 55 % RH from oats sprouted at 20 °C and 55 % RH, at 25 °C and 60 % RH, and 30 °C and 65 % RH (Fig. 1A). The main phytochemicals responsible for the separation in both components were apigenin apiosyl hexoside (F_33), which was one of the significant components of oats sprouted at 30 °C and 65 % RH, followed by kaempferol rhamnosyl hexoside rhamnoside (F_18), the major phytochemical of oats sprouted at 30 °C at 55 and 65 % RH (Fig. 1B and 1C).

The coefficient score plot of each experimental group is shown in Fig. 2, where the main phytochemicals that characterized each group are distinguished. Interestingly, oat grains (Fig. 2A) and oats sprouted at 30 °C and 55 % RH (Fig. 2E) were distinguished by their lack of luteolin apiosyl malonyl hexoside (F_34) and their relatively low content of gallic acid (PA_1). In contrast, oats sprouted at 20 °C and 65 % RH were characterized by a high content of these polyphenols. On the other hand, oats sprouted at 30 °C and 65 % RH (Fig. 2F) were distinguished by their high content of kaempferol rhamnosyl hexoside rhamnoside (F_18) and apigenin apiosyl hexoside (F_33), which were found at low concentration in oats sprouted at 20 °C and 55 % RH (Fig. 2B).

Table 3
Polyphenol profile assessed by UPLC-QTOF MS^E of oat (*Avena sativa* L.) sprouts grown under different germination conditions.

Code	Tentantive identification	Rt (min)	Molecular formula	Expected mass (Da)	Observed mass (Da)	Mass error (ppm)	Concentration (μg/g)					
							Oat grain	Sprout 20 °C/ 55 % RH	Sprout 20 °C/ 65 % RH	Sprout 25 °C/ 60 % RH	Sprout 30 °C/ 55 % RH	Sprout 30 °C/ 65 % RH
Hydroxybenzoic acids												
PA_1	Gallic acid*	1.03	C7H6O5	170.0215	170.0216	0.61	48.2 ± 1.1c	42.1 ± 3.2c	51.9 ± 3.6c	29.2 ± 1.8d	298.1 ± 1.2a	130.8 ± 7.7b
PA_2	Hydroxybenzoic acid	1.15	C7H6O3	138.0317	138.0320	1.98	3.7 ± 0.6c	5.4 ± 1.3c	14.4 ± 1.7b	16.5 ± 0.1b	14.6 ± 1.2b	33.4 ± 0.7a
PA_3	Hydroxybenzoic acid hexoside	1.15	C13H16O8	300.0845	300.0838	−2.38	8.0 ± 0.4d	17.8 ± 1.8c	31.8 ± 1.6b	37.5 ± 1.6b	32.3 ± 3.7b	71.7 ± 2.7a
PA_4	Protocatechuic acid*	1.15	C7H6O4	154.0266	154.0259	−4.43	ND	5.0 ± 0.2d	8.5 ± 0.1c	9.2 ± 1.5bc	11.1 ± 0.4b	18.1 ± 1.3a
PA_5	Gallic acid hexoside	1.26	C13H16O10	332.0743	332.0714	−8.82	24.2 ± 0.4b	35.4 ± 4.3a	19.1 ± 0.3c	35.4 ± 0.7a	12.3 ± 1.1d	6.0 ± 0.1e
PA_6	Gallic acid gallate	1.39	C14H10O9	322.0325	322.0344	5.90	48.1 ± 2.1c	81.7 ± 4.8b	ND	ND	121.9 ± 2.2a	ND
PA_7	Dihydroxybenzoic acid hexoside	1.54	C13H16O9	316.0794	316.0801	1.96	62.2 ± 0.7a	30.3 ± 2.7c	22.7 ± 2.3d	47.2 ± 3.2b	23.8 ± 1.7d	50.1 ± 1.3b
PA_8	Gallic acid hexoside	1.82	C13H16O10	332.0743	332.0734	−2.71	20.0 ± 1.0c	24.3 ± 2.2c	39.1 ± 2.0b	40.0 ± 1.1b	48.2 ± 2.5a	55.8 ± 1.5a
PA_9	Dihydroxybenzoic aicd	1.87	C7H6O4	154.0266	154.0263	−2.05	5.3 ± 1.1c	5.7 ± 0.8c	6.1 ± 0.1bc	16.9 ± 0.4a	7.7 ± 1.1b	16.9 ± 0.5a
PA_10	Hydroxybenzoic acid*	3.17	C7H6O3	138.0317	138.0315	−1.33	4.6 ± 1.1d	12.8 ± 3.1b	7.2 ± 0.2c	45.9 ± 1.2a	5.1 ± 0.6d	13.7 ± 0.7b
PA_11	Syringic acid*	3.21	C9H10O5	198.0528	198.0512	−8.02	39.8 ± 2.6a	12.4 ± 0.5c	19.7 ± 1.0b	ND	ND	ND
PA_12	Vanillic acid*	4.05	C8H8O4	168.0423	168.0416	−4.14	5.1 ± 0.7b	6.9 ± 1.0a	5.5 ± 0.3b	5.2 ± 0.5b	5.5 ± 0.2b	6.0 ± 0.5a
PA_13	Benzoic acid*	4.20	C7H6O2	122.0368	122.0370	1.55	29.4 ± 0.3c	14.6 ± 1.2d	24.2 ± 0.1c	58.4 ± 2.1a	20.2 ± 1.5c	46.4 ± 3.0b
PA_14	Gallic acid ethyl ester	4.41	C9H10O5	198.0528	198.0512	−8.44	9.5 ± 1.6a	6.9 ± 0.5b	3.8 ± 0.1e	5.5 ± 0.2c	4.4 ± 0.5d	6.9 ± 0.4b
PA_15	Dihydroxybenzoic acid	4.79	C7H6O4	154.0266	154.0265	−0.58	22.8 ± 3.0b	32.4 ± 1.9a	16.9 ± 0.7c	19.6 ± 0.9b	9.5 ± 0.4d	8.5 ± 0.7d
PA_16	Hydroxybenzoic acid	6.64	C7H6O3	138.0317	138.0320	1.98	12.5 ± 0.9a	11.2 ± 0.3ab	10.7 ± 0.5b	9.6 ± 0.5b	13.2 ± 0.7a	10.4 ± 0.9b
Hydroxycinnamic acids												
PA_17	Caffeic acid hexoside	1.44	C15H18O9	342.0951	342.0919	−9.34	ND	ND	ND	ND	100.1 ± 5.4	ND
PA_18	Caffeoylquinic acid	2.39	C16H18O9	354.0951	354.0935	−4.51	ND	1.8 ± 0.1b	3.3 ± 0.2a	ND	3.5 ± 0.2a	3.3 ± 0.1a
PA_19	Ferulic acid hexoside	3.55	C16H20O9	356.1107	356.1104	−0.80	23.7 ± 1.9d	17.7 ± 0.7e	57.4 ± 0.4c	19.0 ± 0.7e	89.9 ± 1.9a	69.8 ± 2.9b
PA_20	Caffeoylquinic acid*	3.81	C16H18O9	354.0951	354.0923	−7.96	2.4 ± 0.1c	3.5 ± 0.1b	ND	12.0 ± 0.6a	ND	14.0 ± 0.7a
PA_21	Coumaric acid hexoside	3.99	C15H18O8	326.1002	326.1002	−0.05	ND	ND	52.6 ± 1.6a	4.5 ± 0.3c	51.0 ± 2.1a	25.1 ± 1.6b
PA_22	Sinapic acid*	4.01	C11H12O5	224.0685	224.0676	−3.74	12.9 ± 0.9a	5.3 ± 0.3c	9.3 ± 0.7b	6.2 ± 0.6c	9.2 ± 0.2b	10.0 ± 0.4b
PA_23	Caffeoylquinic acid	4.05	C16H18O9	354.0951	354.0949	−0.58	ND	5.1 ± 0.0c	12.9 ± 0.5b	5.8 ± 0.3c	12.0 ± 0.4b	16.3 ± 0.7a
PA_24	Feruloylquinic acid	4.10	C17H20O9	368.1107	368.1110	0.84	16.0 ± 0.9d	5.7 ± 0.9e	67.7 ± 1.2c	17.3 ± 0.8d	257.6 ± 21.1a	113.5 ± 4.5b
PA_25	Caffeic acid*	4.16	C9H8O4	180.0423	180.0416	−3.90	5.9 ± 0.7a	5.8 ± 0.4a	3.6 ± 0.2b	4.5 ± 0.5ab	3.0 ± 0.1b	3.5 ± 0.4b
PA_26	Diferuloylquinic acid	4.58	C27H28O12	544.1581	544.1533	−8.70	ND	33.7 ± 0.6	ND	ND	ND	ND
PA_27	Feruloylquinic acid	5.09	C17H20O9	368.1107	368.1109	0.45	3.2 ± 0.0e	ND	11.0 ± 0.4c	4.1 ± 0.6d	27.5 ± 2.1a	14.3 ± 0.5b
PA_28	*p*-Coumaric acid*	5.13	C9H8O3	164.0473	164.0475	1.16	3.4 ± 0.7e	3.0 ± 0.5e	37.2 ± 3.2a	6.7 ± 0.6d	18.0 ± 1.2b	11.1 ± 0.6c
PA_29	Cinnamic acid*	5.37	C9H8O2	148.0524	148.0524	−0.37	20.5 ± 0.8a	4.4 ± 0.2c	3.0 ± 0.1d	6.9 ± 0.7b	2.8 ± 0.3d	3.3 ± 0.3d
PA_30	Ellagic acid*	5.60	C14H6O8	302.0063	302.0058	−1.65	56.2 ± 0.3b	63.8 ± 5.9a	60.8 ± 1.9ab	59.4 ± 1.2ab	68.9 ± 6.4a	56.5 ± 0.8b
PA_31	Ferulic acid*	5.65	C10H10O4	194.0579	194.0579	0.15	5.2 ± 0.8d	11.8 ± 1.0c	16.8 ± 1.0b	16.0 ± 0.9b	21.5 ± 1.0a	19.6 ± 1.0a
PA_32	(iso)Ferulic acid	6.01	C10H10O4	194.0579	194.0582	1.53	3.8 ± 0.3d	8.6 ± 0.3a	7.3 ± 0.1b	6.0 ± 0.2c	8.1 ± 0.5a	5.6 ± 0.3c
PA_33	Diferuloylquinic acid	6.28	C27H28O12	544.1581	544.1527	−9.82	ND	5.9 ± 0.7b	6.1 ± 0.1b	6.5 ± 0.6b	6.1 ± 0.2b	11.2 ± 0.8a
PA_34	Feruloylquinic acid	6.62	C17H20O9	368.1107	368.1119	3.24	ND	ND	17.5 ± 0.2a	ND	4.0 ± 0.2b	ND

(*continued on next page*)

Table 3 (*continued*)

Code	Tentative identification	Rt (min)	Molecular formula	Expected mass (Da)	Observed mass (Da)	Mass error (ppm)	Concentration (μg/g)					
							Oat grain	Sprout 20 °C/ 55 % RH	Sprout 20 °C/ 65 % RH	Sprout 25 °C/ 60 % RH	Sprout 30 °C/ 55 % RH	Sprout 30 °C/ 65 % RH
Flavanols												
F_1	(+)-Gallocatechin*	1.36	C15H14O7	306.0740	306.0730	−3.08	1.4 ± 0.1d	1.9 ± 0.1c	ND	3.4 ± 0.1a	2.6 ± 0.1b	ND
F_2	(-)-Epigallocatechin gallate*	1.38	C22H18O11	458.0849	458.0875	5.68	ND	ND	2.8 ± 0.1b	ND	3.7 ± 0.1a	2.9 ± 0.0b
F_3	(-)-Epigallocatechin*	4.61	C15H14O7	306.0740	306.0712	−9.02	3.9 ± 0.1b	3.9 ± 0.2b	2.5 ± 0.1c	6.4 ± 0.2a	ND	3.8 ± 0.2b
F_4	(-)-Epicatechin*	5.04	C15H14O6	290.0790	290.0763	−9.60	6.0 ± 0.3ab	6.0 ± 0.3ab	6.6 ± 0.1	6.9 ± 0.4a	6.3 ± 0.2ab	5.5 ± 0.1b
F_5	(+)-Catechin*	5.71	C15H14O6	290.0790	290.0776	−4.92	ND	7.0 ± 0.2b	ND	11.5 ± 0.2a	7.1 ± 0.4b	ND
F_6	(-)-Epicatechin hexoside	5.89	C21H24O11	452.1319	452.1349	6.70	ND	4.2 ± 0.3b	10.0 ± 0.5a	ND	ND	4.5 ± 0.2b
F_7	(+)-Catechin hexoside	6.87	C21H24O11	452.1319	452.1350	6.99	7.4 ± 0.3c	13.1 ± 0.4a	10.1 ± 0.3b	7.9 ± 0.6c	5.1 ± 0.7d	11.5 ± 1.3b
Flavonols												
F_8	Quercetin dihexoside	3.15	C27H30O17	478.0747	478.0786	8.02	3.6 ± 0.1e	7.4 ± 0.2d	8.7 ± 0.3c	ND	32.4 ± 0.9a	17.4 ± 2.6b
F_9	Kaempferol acetyl hexoside	3.53	C23H22O12	490.1111	490.1144	6.72	ND	ND	4.9 ± 0.1	ND	ND	ND
F_10	Myricetin rutinoside	5.13	C27H30O17	626.1483	626.1498	2.41	7.9 ± 0.4a	8.0 ± 0.2a	6.1 ± 0.3c	6.8 ± 0.2b	6.2 ± 0.2c	7.9 ± 0.3a
F_11	Quercetin rhamnoside	5.19	C21H20O11	448.1006	448.0997	−1.93	6.3 ± 0.8	ND	ND	ND	ND	ND
F_12	Quercetin hexoside rhamnosyl hexoside	5.48	C33H40O21	772.2062	772.2015	−6.10	6.6 ± 0.6e	7.2 ± 0.5de	10.6 ± 0.3c	8.5 ± 0.3d	12.4 ± 0.6b	17.3 ± 0.0a
F_13	Kaempferol dihexoside	5.67	C27H30O16	610.1534	610.1517	−2.80	19.0 ± 1.5c	ND	26.2 ± 0.6b	15.8 ± 0.6	ND	33.4 ± 0.8a
F_14	Quercetin rutinoside*	5.67	C27H30O16	610.1534	610.1545	1.88	ND	18.5 ± 1.3b	21.1 ± 0.3a	19.1 ± 0.6b	22.9 ± 0.3a	ND
F_15	Quercetin malonyl hexoside	5.89	C24H22O15	550.0959	550.0973	2.69	ND	25.9 ± 0.9b	32.4 ± 1.1a	24.4 ± 0.7b	35.3 ± 3.7a	ND
F_16	Kaempferol hexoside	5.94	C21H20O11	448.1006	448.1000	−1.27	ND	ND	ND	9.8 ± 0.6b	ND	19.1 ± 0.2a
F_17	Kaempferol trihexoside	6.33	C33H40O21	772.2062	772.2037	−3.24	10.5 ± 1.0b	6.3 ± 0.1c	12.3 ± 0.6a	ND	11.5 ± 1.5ab	14.2 ± 0.6a
F_18	Kaempferol rhamnosyl hexoside rhamnoside	6.33	C34H40O21	740.2164	740.2148	−2.07	681.3 ± 22.5c	208.2 ± 8.3e	877.6 ± 18.0b	284.8 ± 6.2d	1024.9 ± 24.3a	902.6 ± 30.3ab
F_19	Kaempferol acetyl hexoside rhamnoside	6.73	C29H32O16	636.1690	636.1639	−8.13	ND	4.5 ± 0.1	ND	ND	ND	ND
F_20	Kaempferol malonyl hexoside	6.73	C24H22O14	534.1010	534.1025	2.81	14.7 ± 1.3b	16.6 ± 1.1a	14.2 ± 0.8b	15.6 ± 0.9ab	13.6 ± 0.5b	13.3 ± 0.9b
F_21	Quercetin*	7.86	C15H10O7	302.0427	302.0398	−9.51	5.2 ± 1.3ab	4.2 ± 0.1b	6.0 ± 0.2a	ND	4.4 ± 0.2b	4.4 ± 0.2b
F_22	Kaempferol*	8.85	C15H10O6	286.0477	286.0469	−3.03	2.5 ± 0.1b	3.6 ± 0.2a	ND	ND	ND	ND
F_23	Kaempferide	8.97	C16H11O6	299.0556	299.0578	7.39	8.9 ± 0.5a	ND	3.4 ± 0.1b	ND	3.6 ± 0.6b	3.9 ± 0.5b
F_24	(iso)Rhamnetin	9.05	C16H12O7	316.0583	316.0575	−2.65	6.9 ± 1.5ab	7.2 ± 0.7a	6.9 ± 0.1a	5.8 ± 0.2b	5.7 ± 0.2b	ND
F_25	Kaempferol hexoside rhamnoside	9.17	C27H30O15	594.1585	594.1578	−1.06	ND	11.9 ± 0.5	ND	ND	ND	ND
F_26	Quercetin acetyl hexoside rhamnoside	10.08	C29H32O17	652.1639	652.1594	−6.98	4.4 ± 0.9b	3.9 ± 0.0b	ND	ND	6.2 ± 0.2a	ND
F_27	Quercetin hexoside	11.01	C21H20O12	464.0955	464.0956	0.22	6.7 ± 0.4a	7.9 ± 0.6a	5.8 ± 0.5b	6.9 ± 0.4a	6.9 ± 0.7a	7.6 ± 0.7a
F_28	Kaempferol hexoside rhamnosyl hexoside	12.81	C33H40O20	756.2113	756.2123	1.33	ND	8.2 ± 0.2a	ND	ND	ND	6.5 ± 0.3b
F_29	Myricetin rhamnoside	13.28	C21H20O12	464.0955	464.0997	9.14	29.8 ± 1.4b	22.1 ± 0.8c	9.9 ± 0.4e	ND	17.6 ± 1.8d	35.0 ± 1.4a
F_30	Kaempferol xylosyl hexoside	13.41	C26H28O15	580.1428	580.1392	−6.21	7.8 ± 0.0a	8.2 ± 0.8a	7.8 ± 0.2a	7.9 ± 0.6a	ND	7.2 ± 0.6a
F_31	Quercetin hexoside rhamnoside	13.83	C27H30O16	610.1534	610.1560	4.30	ND	ND	ND	ND	6.4 ± 0.3	ND
Flavones												
F_32	Luteolin rutinoside	5.38	C27H30O15	594.1585	594.1578	−1.13	10.6 ± 0.7b	3.1 ± 0.1e	5.5 ± 0.2d	6.1 ± 0.3 cd	7.2 ± 0.2c	25.3 ± 3.0a
F_33	Apigenin apiosyl hexoside	5.48	C26H28O14	564.1479	564.1461	−3.28	50.1 ± 5.7e	13.7 ± 2.2f	110.1 ± 10.9d	252.1 ± 20.4b	182.6 ± 18.8c	886.6 ± 65.2a

(*continued on next page*)

Table 3 (*continued*)

Code	Tentantive identification	Rt (min)	Molecular formula	Expected mass (Da)	Observed mass (Da)	Mass error (ppm)	Concentration (μg/g)					
							Oat grain	Sprout 20 °C/ 55 % RH	Sprout 20 °C/ 65 % RH	Sprout 25 °C/ 60 % RH	Sprout 30 °C/ 55 % RH	Sprout 30 °C/ 65 % RH
F_34	Luteolin apiosyl malonyl hexoside	5.66	C29H30O18	666.1432	666.1464	4.75	ND	16.9 ± 3.2c	ND	59.9 ± 4.4b	211.5 ± 27.3a	3.8 ± 0.2d
F_35	Luteolin hexoside	6.37	C21H20O11	448.1006	448.0997	−1.96	3.1 ± 0.5a	ND	ND	ND	3.3 ± 0.1a	2.2 ± 0.1b
F_36	Luteolin malonyl hexoside	6.73	C24H22O14	534.1010	534.1025	2.81	8.9 ± 1.3a	9.0 ± 1.1a	8.0 ± 0.2b	8.9 ± 0.5a	7.7 ± 0.3b	7.6 ± 0.5b
F_37	Luteolin*	7.80	C15H10O6	286.0477	286.0456	−7.53	ND	ND	ND	ND	ND	4.9 ± 0.2
F_38	Apigenin hexoside	8.30	C21H20O10	432.1056	432.1041	−3.49	52.4 ± 1.3a	47.7 ± 5.4ab	32.4 ± 1.5d	39.3 ± 1.8c	45.7 ± 1.1b	42.2 ± 2.6bc
F_39	Luteolin apiosylhexoside	8.41	C26H28O15	580.1428	580.1392	−6.21	4.5 ± 0.0a	4.7 ± 0.5a	4.4 ± 0.1a	4.4 ± 0.7a	ND	4.4 ± 0.1a
F_40	Apigenin*	8.66	C15H10O5	270.0528	270.0523	−2.10	ND	2.3 ± 0.4a	ND	ND	2.2 ± 0.2a	1.7 ± 0.0b
F_41	Apigenin dihexoside	9.17	C27H30O15	594.1585	594.1578	−1.06	ND	6.9 ± 0.2	ND	ND	ND	ND
Isoflavones												
F_42	Malonyldaidzin	6.77	C24H22O12	502.1111	502.1125	2.79	ND	ND	6.9 ± 0.3b	8.9 ± 0.2a	6.1 ± 0.4b	3.8 ± 0.1c
F_43	Acetylgenistin	6.84	C23H22O11	474.1162	474.1203	8.73	25.5 ± 0.4a	22.4 ± 1.3a	26.5 ± 0.4a	23.9 ± 0.5a	17.5 ± 0.7b	24.3 ± 1.3a
F_44	Daidzin*	7.72	C21H20O9	416.1107	416.1075	−7.81	1.3 ± 0.1b	1.7 ± 0.1a	ND	ND	1.6 ± 0.4a	ND
F_45	Malonylgenistin	8.35	C24H22O13	518.1060	518.1054	−1.24	7.8 ± 0.4a	6.5 ± 0.4b	5.2 ± 0.2c	6.4 ± 0.3b	8.0 ± 0.5a	ND
F_46	Acetyldaidzin	9.12	C23H22O10	458.1213	458.1251	8.39	15.9 ± 0.6a	11.5 ± 0.5bc	11.9 ± 0.3bc	12.6 ± 0.8b	11.2 ± 0.3c	10.0 ± 0.6d

Data are expressed as mean values ± standard deviation of three replicates. Different letters indicate significant ($p < 0.05$) differences among germination conditions by Tukey's test. ND: Not detected. *Identification confirmed by comparison with commercial standard.

4. Discussion

While exploring the combination of temperature and relative humidity conditions on oat germination, it's essential to frame our observations within the context of eustressors and distressors (Godínez-Mendoza et al., 2023). The identified optimal germination condition at 25 °C/60 % RH, yielding 100 % germination with the longest radicle size. Notably, among the tested conditions, no distinct eustressor was observed, which must improve both germination metrics and secondary metabolites synthesis. Germination at 30 °C/65 % RH, resulting in the highest content of phenolic acids, avenanthramides, and lignans but with a shorter radicle size despite 100 % germination, suggest a potential distressor role, indicating a trade-off between secondary metabolites production and growth metrics. Similarly, the germination conditions at 20 °C/55 % RH, showcasing the optimal profile of flavonoids and phytosterols but with a lower germination rate, hints at distressor conditions, where the phytochemical composition may be prioritized at the expense of germination performance. This distinction between eustressors and distressors provides valuable insights into the dynamic interplay of environmental factors and oat germination responses, contributing to a nuanced understanding of how abiotic stressors influence both growth and phytochemical composition.

It is well-known that environmental temperature significantly affects the speed and percentage of seed germination. Each plant species has an optimal germination temperature range that promotes water uptake, thereby facilitating various biochemical reactions and physiological processes essential for germination (de Oliveira et al., 2013). However, it is necessary to distinguish between optimal conditions, where environmental factors are conducive to efficient germination, and abiotic stress conditions, where these factors deviate from the optimal range, potentially hindering germination processes. In our study, we intentionally selected a range of temperature and relative humidity conditions to explore their impact as abiotic stressors on phytochemical composition, evaluating the overall adaptability of oat sprouts by measuring germination growth parameters.

Several authors have determined the optimal germination temperatures for some seeds such as *Senna macranthera* (Collad.), *Ocotea odorifera* (Vellozo), *Tabebuia impetiginosa,* and *T. serratifolia* Vahl Nich, finding that most of them ranged between 20 and 30 °C (de Oliveira et al., 2013). Furthermore, El-Mouhamady et al. (2014) evaluated two different temperatures during oat growth and identified that 25 °C was optimum for the activity of several enzymes related to the germination process. It has been suggested that as the temperature rises, there is increased water energy producing an elevation in the diffusion pressure that promotes the metabolic activity and decreases the internal potential of the seed, accelerating its water absorption rate, which causes an increase in the germination speed. Conversely, germination processes carried out at temperatures higher than the optimal could potentially affect enzymatic activity, reducing the amount of available amino acids essential for the metabolic reactions involved in embryo development and thus restricting seed germination (Maraghni et al., 2010).

On the other hand, when the germination process occurs at temperatures lower than optimal, there is a decreased metabolic activity rate, which affects crucial processes involved in the initial stages of germination. The level of disturbance depends on the plant species, water content, temperature, and exposure time. However, in most cases, it is observed that low temperatures reduce the speed and percentage of germination and increase the average germination time, which is attributed to a reduction proline and fatty acid synthesis rate (Noblet et al., 2017).

On the other hand, several studies have shown the importance of relative humidity on sprout growth rate since high relative humidity levels might improve the production yield during germination. For example, in sesame seeds germinated in a dark chamber maintained at 35 °C and 100 % RH the germination rate was higher than 99 % (Hahm et al., 2009). On the other hand, Limwiwattana et al. (2016) found that increasing relative humidity improved the size of the black gram sprouts, finding greater lengths of sprouts at 80 % RH than those at 60 %

Table 4
Aventhramide, lignin, and phytosterol profiles assessed by UPLC-QTOF MS^E of oat (*Avena sativa* L.) sprouts grown under different germination conditions.

Code	Tentative identification	Rt (min)	Molecular formula	Expected mass (Da)	Observed mass (Da)	Mass error (ppm)	Concentration (μg/g)					
							Oat grain	Sprout 20 °C/ 55 % RH	Sprout 20 °C/ 65 % RH	Sprout 25 °C/ 60 % RH	Sprout 30 °C/ 55 % RH	Sprout 30 °C/ 65 % RH
Avenanthramide												
A_1	Avenanthramide C	6.65	C16H13NO6	315.0743	315.0741	−0.46	5.3 ± 0.0d	3.3 ± 0.2e	6.9 ± 0.0c	ND	8.4 ± 0.6b	18.6 ± 1.1a
A_2	Avenanthramide G	7.33	C16H13NO5	299.0794	299.0789	−1.74	28.2 ± 0.4c	ND	39.2 ± 0.3b	25.5 ± 1.0c	ND	136.0 ± 5.4a
A_3	Avenanthramide 1c	7.34	C16H13NO5	299.0794	299.0789	−1.55	33.5 ± 1.4a	ND	19.6 ± 1.0b	ND	31.3 ± 1.5a	ND
A_4	Avenanthramide B	7.66	C17H15NO6	329.0899	329.0899	−0.24	16.2 ± 1.7d	12.6 ± 1.2e	44.5 ± 0.4b	23.1 ± 0.7c	29.6 ± 1.2c	191.9 ± 4.5a
A_5	Avenanthramide 2 s	8.09	C18H17NO7	359.1005	359.1003	−0.57	2.6 ± 0.4d	2.7 ± 0.2d	5.6 ± 0.8c	4.5 ± 0.3c	7.5 ± 1.4b	43.8 ± 1.7a
A_6	Avenanthramide L	8.49	C18H15NO5	325.0950	325.0957	1.99	6.9 ± 0.1e	6.1 ± 0.4e	35.1 ± 1.8d	48.9 ± 1.5c	71.2 ± 4.2b	131.2 ± 7.4a
A_7	Avenanthramide A*	8.87	C16H13NO5	299.0794	299.0786	−2.44	8.3 ± 0.8ab	7.4 ± 0.4b	2.5 ± 0.1c	1.7 ± 0.0d	1.6 ± 0.1d	9.2 ± 0.4a
A_8	Avenanthramide D	9.71	C16H13NO4	283.0845	283.0867	7.79	ND	7.7 ± 0.9d	63.7 ± 1.2c	71.5 ± 1.2bc	80.7 ± 6.9b	104.2 ± 1.0a
A_9	Avenanthramide E	9.85	C17H15NO5	313.0950	313.0933	−5.45	0.6 ± 0.0c	ND	ND	3.5 ± 0.1a	ND	1.5 ± 0.1b
Lignans												
L_1	Lariciresinol-sesquilignan	6.33	C30H36O10	556.2308	556.2359	9.05	8.1 ± 0.4d	30.7 ± 1.8b	11.8 ± 0.6c	ND	53.4 ± 2.7a	50.3 ± 2.7a
L_2	Medioresinol	6.61	C21H24O7	388.1522	388.1557	9.09	ND	ND	77.1 ± 2.0c	ND	89.5 ± 6.5b	104.8 ± 2.4a
L_3	Isohydroxymatairesinol	7.53	C20H22O7	374.1366	374.1372	1.67	17.3 ± 1.0b	8.7 ± 1.1c	16.7 ± 0.5b	10.0 ± 0.1d	9.7 ± 0.0c	19.2 ± 1.0a
L_4	Hydroxymatairesinol	7.54	C20H22O7	374.1366	374.1343	−6.00	ND	ND	ND	ND	9.6 ± 0.5	ND
L_5	Acetoxypinoresinol	7.75	C22H24O8	416.1471	416.1499	6.81	ND	ND	47.2 ± 1.8d	91.6 ± 3.2c	130.0 ± 7.5b	196.6 ± 8.5a
L_6	Syringaresinol	7.87	C22H26O8	418.1628	418.1610	−4.23	ND	18.4 ± 1.1b	13.5 ± 0.7c	23.2 ± 1.1a	18.5 ± 1.8b	20.7 ± 1.0ab
L_7	Secoisolariciresinol	8.64	C20H26O6	362.1729	362.1701	−7.78	69.2 ± 2.3b	66.8 ± 1.8c	69.6 ± 3.1b	72.5 ± 2.0ab	69.9 ± 2.4b	73.4 ± 3.3a
L_8	Secoisolariciresinol-sesquilignan	8.98	C30H38O10	558.2465	558.2505	7.21	11.5 ± 0.6a	10.8 ± 0.4ab	9.8 ± 0.7b	8.3 ± 0.2b	10.4 ± 1.1ab	9.8 ± 0.5b
L_9	Pinoresinol	9.20	C20H22O6	358.1416	358.1395	−6.02	13.3 ± 0.7b	9.9 ± 0.3c	ND	15.9 ± 0.7a	10.2 ± 0.6c	8.4 ± 0.3d
L_10	Isolariciresinol	9.53	C20H24O6	360.1573	360.1538	−9.68	ND	47.5 ± 0.8a	35.6 ± 1.8b	ND	32.9 ± 1.0b	ND
L_11	Lariciresinol*	9.94	C20H24O6	360.1573	360.1543	−8.28	41.0 ± 1.2b	50.7 ± 2.1a	ND	ND	ND	ND
L_12	Matairesinol*	10.37	C20H22O6	358.1416	358.1428	3.13	17.4 ± 1.4a	16.0 ± 1.4ab	17.6 ± 0.7a	18.2 ± 1.0a	14.2 ± 0.1b	17.2 ± 1.4a
Phytosterols												
P_1	β-Campesterol*	15.25	C28H48O	400.3705	400.3667	−9.64	91.1 ± 3.9c	108.9 ± 4.6b	130.4 ± 0.9a	104.1 ± 5.8b	99.5 ± 5.9b	91.9 ± 2.7c
P_2	Campesterol hexoside	22.53	C34H58O6	562.4233	562.4211	−3.95	ND	70.0 ± 3.6a	ND	ND	57.0 ± 6.1b	ND
P_3	Stigmasterol hexoside	22.58	C35H58O6	574.4233	574.4228	−0.99	162.5 ± 5.8a	20.5 ± 0.7d	175.1 ± 4.0a	64.2 ± 3.0c	155.0 ± 4.9b	153.7 ± 3.6b
P_4	Brassicasterol hexoside	23.86	C34H56O6	560.4077	560.4061	−2.80	97.7 ± 0.6b	113.0 ± 12.9a	96.1 ± 5.9b	110.1 ± 3.6a	104.0 ± 7.2ab	84.4 ± 6.1c
P_5	Sitosterol hexoside	25.04	C35H60O6	576.4390	576.4349	−7.11	13.2 ± 0.7d	17.2 ± 0.5c	ND	31.1 ± 0.8a	23.5 ± 0.7b	ND

Data are expressed as mean values ± standard deviation of three replicates. Data are expressed as arbitrary units (AU). Different letters indicate significant ($p < 0.05$) differences among germination conditions by Tukey's test. ND: Not detected. Identification confirmed by comparison with commercial standard.

and 40 % RH. Accordingly, in this study, we found a 97–100 % of germination percentage when relative humidity was set at 60–65 %, indicating that this humidity is high enough to allow oat sprouting.

Regarding the effect of sprouting on nutrient composition, controversial results have been reported depending on the cereal and germination conditions. In this study, all germination conditions decreased the various fractions of dietary fiber. This result could be associated with the degradation of β-glucans, the primary dietary fiber component of oats, which is associated with an increased activity of endo-β-D-glucanase during sprouting, which depends on the hydration rate of the grains (Lemmens et al., 2019). Similar results were reported by Koehler et al. (2007) in barley sprouted at low temperatures (15–20 °C); nevertheless, these authors reported that dietary fiber remained unchanged at higher sprouting temperatures (25 and 30 °C), contrary to what was observed in this study. These differences could be attributed to the different metabolic rates in each cereal.

On the other hand, the enzymatic activity of endogenous peptidase has been found to increase after one day of cereal germination, which is essential for adequate seedling development. Nevertheless, although sprouting leads to protein hydrolysis, slight modifications can be

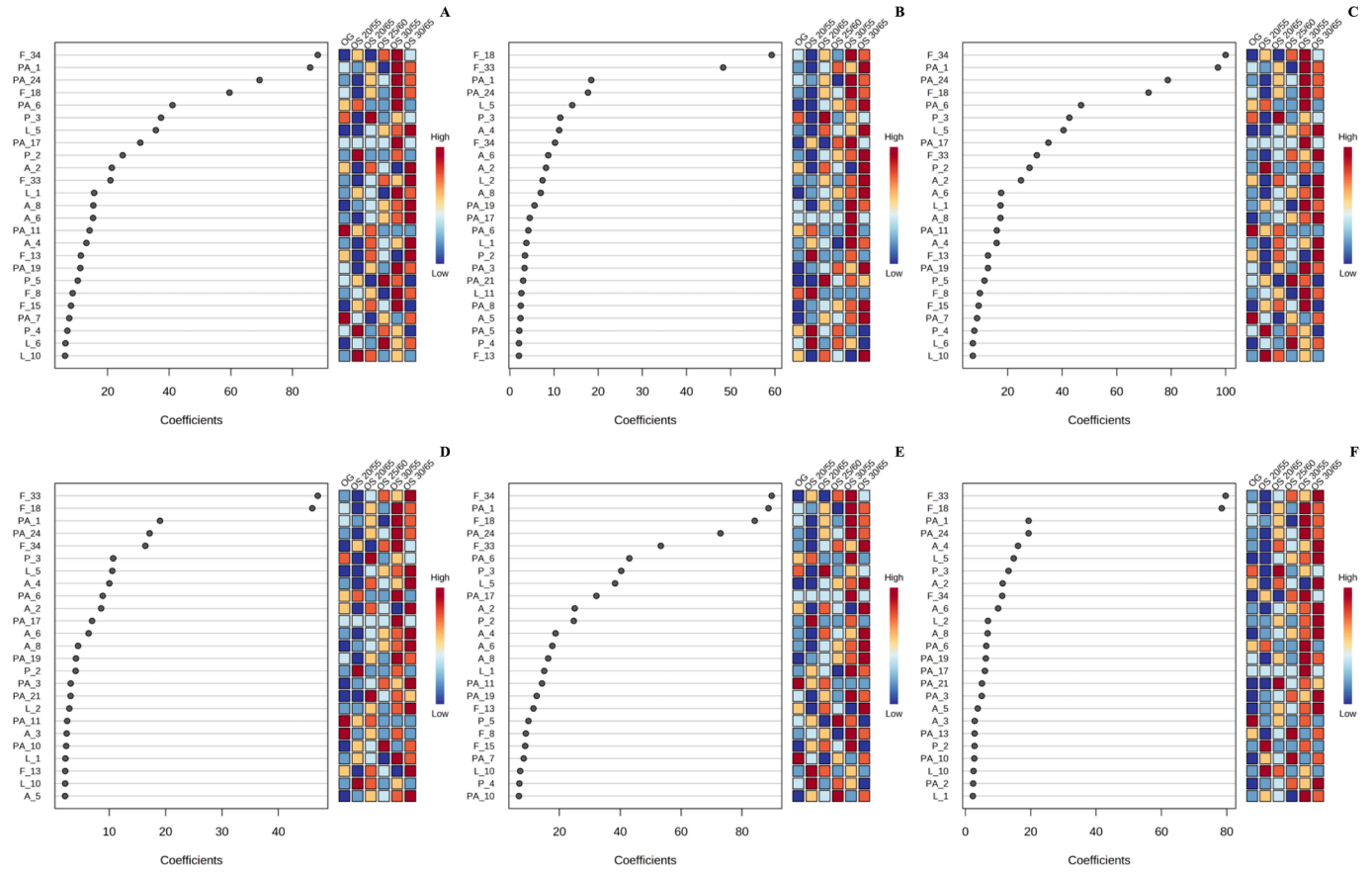

Fig. 2. Coefficient plot extracted from PLS-DA analysis for oat grain (A), oat sprout 20 °C/55 % RH (B), oat sprout 20 °C/65 % RH (C), oat sprout 25 °C/60 % RH (D), oat sprout 30 °C/55 % RH (E), and oat sprout 30 °C/65 % RH (F).

observed in total protein content (Lemmens et al., 2019). In this study, we found that all germination conditions promoted a slight increase in protein content; similar results were observed by Klose and Arendt (2012), which were associated with the degradation and solubilization of storage proteins.

Oat grains are rich in several phytochemicals, including phenolic derivatives, like phenolic acids, flavonoids, avenanthramides, lignans, and phytosterols (Schendel, 2019). In this study, we provide an exhaustive phytochemical characterization of oat sprouted at different conditions combined with chemometrics analyses to understand this phenomenon better. The increased polyphenol content in oat sprouts is linked to the activation of secondary metabolism. This activation occurs due to the generation of oxidative stress during the soaking process (rehydration) of grains, which subsequently leads to the synthesis of antioxidants such as polyphenols. The enzyme phenylalanine ammonia lyase (PAL) plays a key role in this process, as it is one of the key enzymes involved in regultating the phenylpropanoid pathway responsible for polyphenol and lignan synthesis (Benincasa et al., 2019).

In this study, kaempferol rhamnosyl hexoside rhamnoside was the phytochemical found at high concentrations in oat grain and sprouts as compared to the other polyphenols identified in this study. This flavonoid has been identified as one of the contributors of the high antioxidant capacity of papaya and palm fruit (Ma et al., 2019; Soib et al., 2020); however, its specific health beneficial potential has not been reported. Notably, this flavonoid and the flavone apigenin apiosyl hexoside were identified as the main phytochemicals responsible for the discrimination between oat grains and oat sprouted at different conditions in the PLS-DA model. Furthermore, apigenin and its derivatives have been identified in several grains, including oats as well as other food sources such as fruits and teas (Bucar et al., 2021). These compounds have shown potential pharmacological activities, including antioxidant properties. Moreover, preclinical studies have suggested their possible roles in ameliorating inflammation, diabetes, and cancer (Salehi et al., 2019); however, these effects have not been demonstrated in clinical trials.

Studies have yet to be carried out to evaluate the impact of temperature and relative humidity on the polyphenol composition of oat sprouts. For instance, Ding et al. (2019) evaluated the effect of germination time on oat total phenolic content, determining that it was increased up to 3.2 times after 96 h of germination at 24 °C and 95 % RH as compared to non-germinated oat grains. Jiménez-Pulido et al. (2022) sprouted oat grains at 21 °C and 95 % RH for five days, finding a 2.4-fold increase in total phenolic content as compared to oat grains. Moreover, these authors identified four phenolic acids and flavonoids in oat grains; three were lost during sprouting, and only sinapic acid glucoside was increased (1.6-fold). Conversely, these authors observed that sprouting affected the avenanthramide profile since only avenanthramide 2f (B) was identified in oat grains at a very low concentration, which was further increased by 83.7-fold in oat sprouts. In contrast, avenanthramides 2c (C) and 2p (A) were identified only in oat sprouts.

Conversely, Feng et al. (2022) identified these three avenanthramides in oat grains, which were significantly increased after the first day of germination (9.0–20.9-fold) at 20 °C and 80 % RH. Similarly, we identified eight avenanthramides in oat grains; however, some avenanthramides were decreased, and others were augmented depending on the germination conditions. Skoglund et al. (2008) reported that changes in the avenanthramide profile during germination are influced by multiple simultaneous biological processes, including an increased the *de novo* synthesis and activity of the hydroxycinnam hydroxycinnamoyl-CoA:hydroxyanthranilate *N*-hydroxycinnamoyl

transferase (HHT). Additionally, there is an enhanced production or release of phenolic acids such as coumaric, caffeic, and ferulic acids, that are utilized for the synthesis of derivatives, including avenanthramides. Moreover, these authorss demonstrated that both soaking and germination time were the main factors affecting HHT activity, whereas a slight effect was found with germination temperature. Accordingly, avenanthramides were not identified as critical discriminant factors in our PLS-DA model.

Lignans such as lariciresinol, pinoresinol, and hydroxymatairesinol have been reported in oat grains (Bleidere et al., 2022). This group of phenolic compounds have been associated with various potential health benefits, including their antioxidant properties and their possible role in mitigating chronic metabolic diseases. Interestingly, some studies on other grains, like flaxseed sprouts, suggest that lignan biosynthesis-related enzymes, such as dirigent-protein oxidase (DPO) and pinoresinol-lariciresinol reductase (PLR) are activated during germination, possibly contributing to the antioxidant defenses alongside other polyphenols (Wang et al., 2016). Accordingly, we identified five lignans synthesized during oat germination (medioresinol, hydroxymatairesinol, acetoxypinoresinol, syringaresinol, and isolariciresinol), which were further increased under 30 °C and 65 % RH and 30 °C and 55 RH. Although the specific impact of germination on oat lignan composition remains less explored, these findings suggest that germination conditions can influence lignan levels, potentially contributing to antioxidant defenses alongside other polyphenols.

On the other hand, in this study, we identified stigmasterol hexoside as the major phytosterol of the oat grain, whereas beta-sitosterol has been identified as the principal sterol in oat by other authors (Kaukovirta-Norja et al., 2004). Most phytosterols were augmented during germination, including the *de novo* synthesis of campesterol hexoside. Accordingly, it has been reported an increase of up to 1.2-fold of this phytosterol during oat sprouting (Kaukovirta-Norja et al., 2004). Even though phytosterols exert antioxidant capacity, these phytochemicals are not synthesized for this purpose. Phytosterols are critical components for regulating the structure and activity of cell membranes; since they participate in cellular differentiation and proliferation, these compounds are synthesized during the early stages of germination, but this synthesis gradually decreases as the plant matures (Piironen et al., 2000).

5. Conclusions

Temperature and relative humidity influence the phytochemical profile and the growth rate during oat germination; nevertheless, it is essential to recognize that the combination of both environmental parameters collectively determine the outcome since each set of germination conditions yield a unique composition of nutrients, secondary metabolites at a distinct growth rate. Moreover, it is noteworthy that the germination condition that provided the best germination characteristics did not produce the best phytochemical composition. Therefore, germination conditions must be selected depending on the oat sprouts' expected characteristics and nutraceutical potential. By considering germination conditions, end-users can harness the versatility of oat sprouting to meet their specific needs and maximize the potential benefits of this promising cereal crop. For instance, the manufacturers of functional food and beverage could consider using sprouts from conditions that yield high polyphenol content for products targeting antioxidant benefits, whereas nutraceutical manufacturers could focus on sprouting conditions that result in elevated levels of avenanthramides, well-known for their health-promoting properties.

CRediT authorship contribution statement

Marely G. Figueroa-Pérez: Formal analysis, Writing – original draft. **Rosalía Reynoso-Camacho:** Supervision, Writing – review & editing. **Minerva Ramos-Gómez:** Conceptualization, Resources, Supervision, Writing – review & editing. **Magdalena Mendoza-Sánchez:** Formal analysis, Investigation, Writing – review & editing. **Iza F. Pérez-Ramírez:** Formal analysis, Resources, Supervision, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.138173.

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