Received: 17 June 2023 | Revised: 30 October 2023 | Accepted: 9 November 2023

DOI: 10.1002/leg3.212

ORIGINAL ARTICLE

Legume Science Open Access WILEY

# Effect of temperature during flowering, pod set, and seed development on yield components and accumulation of protein, starch, and low molecular weight carbohydrates in two faba bean (*Vicia faba* L.) cultivars

Anne Marthe Lundby[1,2] | Wendy Waalen[1] | Anne Kjersti Uhlen[2] | Svein Halvor Knutsen[3] | Anne-Berit Wold[2]

[1]Division of Food Production and Society, Norwegian Institute for Bioeconomy Research (NIBIO), Ås, Norway

[2]Faculty of Biosciences, Norwegian University of Life Sciences (NMBU), Ås, Norway

[3]Department of Food and Health, Norwegian Institute for Food, Fisheries and Aquaculture Research (NOFIMA), Ås, Norway

**Correspondence**
Anne Marthe Lundby, Division of Food Production and Society, Norwegian Institute for Bioeconomy Research (NIBIO), PB 115, NO-1431, Ås, Norway.
Email: anne.marthe.lundby@nibio.no

**Funding information**
Norwegian Research Council, Grant/Award Numbers: 267858, 319049

## Abstract

The aim was to explore the impact of temperature during seed development on yield performance and seed quality in faba bean when grown at cool temperatures representative for high latitude regions. Two varieties, an early and a medium late maturing, were grown in climate chambers with three temperature regimes (day/night temperatures of 14°C/12°C, 19°C/12°C, and 24°C/12°C) from onset of flowering to maturation. Yield components were recorded, and the accumulation of protein, starch, and low molecular weight carbohydrates including the raffinose family oligosaccharides was followed during the accumulation phase until physiological maturity. The lower temperature regimes strongly delayed pod and seed development compared with 24°C/12°C. Temperature affected the number of pods per plant for the upper node group. Plants grown at 19°C had the highest total dry seed weight compared with plants grown at 14°C and 24°C. Temperature per se did not influence the content of starch, protein, and low molecular weight carbohydrates, while their accumulation followed the moisture content in the seed, and thus the seed development stage. The content of raffinose family oligosaccharides increased sharply when the seed moisture dropped below 70% and leveled off at about 40% and 50% moisture for verbascose and stachyose, respectively, coinciding with physiological maturity. The results provide more knowledge about the seed maturation and accumulation in faba bean under low temperatures, important for cultivation under high latitude regions.

**KEYWORDS**
faba bean, maturation, seed development, storage compounds, temperature

## 1 | INTRODUCTION

Faba bean (*Vicia faba* L.) belonging to the Fabaceae family, widely used for both feed and food (Crépon et al., 2010), is one of the most grown cool-season legumes, covering a wide latitude range up to 70°N (Stoddard et al., 2022). Globally, the production of faba bean in 2021 was 5.9 million tons (Faostat, 2023), China being the largest producer followed by Ethiopia, Australia, and United Kingdom (Warsame et al., 2018). Protein and starch are the main energy components in faba bean seeds, and protein content ranges from 24% to 35% of the seed dry matter (Hamid & Albert, 2020). Starch is the most abundant carbohydrate in faba bean seeds, contributing to about 45% of the total seed weight (Hoover & Sosulski, 1991; Morad et al., 1980; Punia et al., 2019).

Low molecular weight carbohydrates (LMWC) are important contributors to seed quality and include digestible glucose, sucrose, and fructose (GFS) and undigestible raffinose, stachyose, and verbascose referred to as the raffinose family of oligosaccharides (RFO) (Landry et al., 2016; Saldanha do Carmo et al., 2022). The antinutritional properties of RFOs may limit the use of faba bean for food because they may cause discomfort and digestible disorder due to the production of flatulence gases (Elango et al., 2022). On the other hand, RFOs may have positive impact on gut microflora (Anggraeni, 2022). The RFOs play an important role in the plant's tolerance to biotic and abiotic stress (Elango et al., 2022) and are involved in regulation of seed germination and desiccation tolerance (Salvi et al., 2022).

Faba bean seed development can be divided into two phases: prestorage phase when potential seed size is determined and a storage phase when storage products are accumulated (Patrick & Stoddard, 2010). In the prestorage phase, glucose and fructose are the dominating sugars, while this shifts to sucrose in the storage phase (Patrick & Stoddard, 2010). Sucrose is supplied to the embryo and declines toward the end of grain filling simultaneously with an increase in starch (Heim et al., 1993; Smith & Denyer, 1992). In mature faba bean seeds, there are negligible amounts of fructose and glucose (Landry et al., 2016; Lattanzio et al., 1986). The RFOs accumulate late in legume seed maturation (Sonali et al., 2015). Verbascose is found to be the main RFOs in faba bean, followed by stachyose and raffinose (Landry et al., 2016; Lattanzio et al., 1986).

Storage compounds accumulate and displace water in the storage phase. Harrington et al. (1997) found that dry matter accumulation declined abruptly when the moisture content dropped below 60% in faba bean, and it ceased at 47.5% indicating physiological maturity.

Faba bean is sensitive to heat and cold stress during reproductive and grain filling phases. Daily temperature above 25°C is considered as a threshold level for heat stress in cool-season legume crops (Gogoi et al., 2018). Dekhuijzen and Verkerke (1986) demonstrated that temperature increases during pod and seed development resulted in faster development that shorten the period for seed growth and accumulation of storage compounds, resulting in lower final seed weight. Challenges with high temperatures during the reproduction phase have previously been reported (McDonald & Paulsen, 1997), but so far, few studies have investigated effects of lower temperatures during grain filling and seed maturation in faba bean.

There is a growing interest in cultivation of faba bean as a protein crop at northern latitudes to meet demands of local plant proteins. In these areas, faba bean is spring-sown (Duc, 1997) and is often limited by a short growing season and decreasing temperatures during seed development and maturation. Few investigations have studied temperature responses in faba bean under such climatic conditions. In the present study, faba bean was grown in controlled climate at a low temperature regime mimicking areas toward a northern border of cultivation. The aim was to elucidate the impact of temperature during seed development and maturation on yield components and the interplay between protein, starch, and LMWC in early and medium late maturing varieties of faba beans.

## 2 | MATERIAL AND METHODS

### 2.1 | Plant materials

The experiment included two varieties of faba bean (*V. faba* L.): cv Vertigo (NPZ), a medium-sized seed and late maturing cultivar (140 ± 10 days) under Norwegian growing condition, and cv Sampo (Boreal), a small seed and earlier maturing cultivar (120 ± 10 days). Seeds were sown in 3 L plastic pots with soil mixture (86 vol.% sphagnum peat, 10 vol.% sand, and 4 vol.% granulated clay) (Gartnerjord, LOG, Oslo) in greenhouses at the Norwegian University of Life Sciences (59°40′N, 10°45′E). The experiment was conducted from late February to mid-July. Four seeds of the same cultivar were sown per pot and thinned to one seedling after germination, in total 162 pots for each cultivar. The pots were kept at 20 ± 1°C during daytime (16 h) and 15 ± 1°C at night (8 h) until germination. After germination, the day temperature was set to 18°C. The plants were watered as needed, alternating between tap water and a full nutrient solution (1:1 Yara Kristalon Indigo™ and YaraLiva Calsinit™), with electric conductivity of 1.5 mS/cm.

### 2.2 | Controlled experiment

At the onset of flowering, plants were transferred to controlled climate chambers, with natural daylight. The plants were grown at daytime temperatures of 14°C, 19°C, and 24°C (±1°C) for 16 h and night temperature of 12°C (±1°C) for 8 h. A total of six chambers were used, allowing for two replicates of each temperature regime. During the defined daytime, set to be well above the critical photoperiod for flower induction, additional light was added whenever the photosynthetic photon flux fell below 200 μmol quanta/$m^2$/s. Figure S6 shows the daylight through the experiment.

Plants were harvested at three timepoints, based on growing degree days (GDD) from the onset of flowering (Table 1). Mean daily temperatures were used ($T_{base} = 0$°C) to calculate degree days. For each variety, nine pots of single plants were placed in trolleys (harvest unit). Pods from nine plants were pooled and divided into three node groups (node 1–5 = lower, 6–10 = mid, and 11–15 = upper), counting from the first pod bearing node. Number of pods and seeds for

**TABLE 1** Dates for the three harvest times and temperatures, for each of the variety Sampo and Vertigo.

| | Harvest 1 | | | Harvest 2 | | | Harvest 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Variety: Sampo | | | | |
| Temp (°C) | 14 | 19 | 24 | 14 | 19 | 24 | 14 | 19 | 24 |
| Date | 6/11 | 5/29 | 5/20 | 6/21 | 6/6 | 5/27 | 7/5 | 6/17 | 6/5 |
| Days after flowering | 65 | 52 | 43 | 75 | 60 | 50 | 89 | 71 | 59 |
| Degree days after flowering | 866.7 | 866.7 | 860.0 | 1000.0 | 1000.0 | 1000.0 | 1186.7 | 1183.3 | 1180.0 |
| Degree days after sowing | 1573.0 | 1573.0 | 1566.3 | 1706.3 | 1706.3 | 1706.3 | 1893.0 | 1889.7 | 1886.3 |
| | | | | | Variety: Vertigo | | | | |
| Temp (°C) | 14 | 19 | 24 | 14 | 19 | 24 | 14 | 19 | 24 |
| Date | 6/18 | 6/5 | 5/27 | 6/28 | 6/13 | 6/3 | 7/15 | 6/27 | 6/14 |
| Days after flowering | 65 | 52 | 43 | 75 | 60 | 50 | 92 | 74 | 61 |
| Degree days after flowering | 866.7 | 866.7 | 860.0 | 1000.0 | 1000.0 | 1000.0 | 1226.7 | 1233.3 | 1220.0 |
| Degree days after sowing | 1692.0 | 1692.0 | 1685.3 | 1825.3 | 1825.3 | 1825.3 | 2052.0 | 2058.7 | 2045.3 |

*Note*: Harvest times is based on the degree days after start of flowering. The table also show the number of days after flowering and the degree days after sowing.

each node group were registered. Seed samples were dried at 60°C for 48 h (tested and found to be suitable for drying faba bean seed) to determine moisture content. The remaining seeds were frozen in liquid nitrogen, crushed, and stored at −50°C.

## 2.3 | Freeze drying and milling

The seeds were freeze dried for 4 days (Gamma 1-16 LSC plus, Martin Christ Freeze Dryers GmbH, Germany), milled to 0.5 mm particle size using a Retsch ZM 100 rotor mill (Retsch GmbH, Germany), and stored at −20°C. Before analysis, the samples were dried at 60°C for ~16 h, to stabilize dry matter.

## 2.4 | Analysis of protein and starch

Total nitrogen content was quantified using the Dumas method (ISO 16634, 2008). Combustion was performed using a Vario El Cube (Elementar Analysensysteme GmbH, Germany) and total nitrogen converted to protein by multiplying with 6.25. Starch was quantified using the Total Starch Assay Procedure K-TSTA (Megazyme, Ireland), after removal of free glucose.

## 2.5 | Analysis of LMWC

LMWC consisting of GFS, raffinose, stachyose, and verbascose (RFO) were analyzed using high-performance anion-exchange chromatography with pulsed amperometric detection, following a method adapted from Helgerud et al. (2016) and described in Saldanha do Carmo et al. (2022). Standards for determination of retention time were D-glucose, sucrose, fructose, raffinose, stachyose, and verbascose. For extraction of LMWC, ~50 mg of milled sample was dissolved in 10 mL of 50% ethanol in 15 mL Falcon tube and shook at 50°C with melibiose as an internal standard. Extracts were centrifuged, diluted with water, and filtered through a 0.22 μM filter, injected into the PA1 column with PAD detections, and quantified via individual calibrations for each LMWC constituent. The content was calculated as grams per 100 g of dry weight for the individual carbohydrates.

## 2.6 | Data analysis

Statistical analyses were performed using MiniTab® Statistical Software program package (Version 20.2. Minitab. Inc., State College, PA, USA). Analysis of variance (ANOVA) was performed using the mixed effects model. The effects of temperature (fixed effect, three levels), harvest (fixed effect, three levels), variety (fixed effect, two levels), and chamber (nested within temperature, random effect, six levels) were analyzed for each node group individually, and Tukey's multiple comparison test with significance level 0.05 was used.

Regression models were developed for each variety for the accumulation of starch, sucrose, verbascose, stachyose, and protein (grams per 100 g) in relation to moisture percentage in seed. A split-line model was used for starch, sucrose, verbascose, and stachyose given by $E(y) = \alpha + \beta \cdot x$ when $x \leq \tau$ and $E(y) = \alpha + (\beta - \delta) \cdot \tau + \delta \cdot x$ when $x > \tau$. E(y) is the expectation of the response variable y, x is the regressor, and α, β, δ, and τ are parameters to be estimated, where τ is the breakpoint value; β and δ are the slopes of the lines below and above τ, respectively. To estimate and test the difference in breakpoints (τ), for the two varieties in the split line-models, we used PROC NLMIXED in SAS (SAS 9.4, SAS Institute Inc., Cary. NC. USA). For accumulation of protein in relation to moisture percentage, a linear model given by $E(y) = \alpha + \beta \cdot x$ was used.

## 3 | RESULTS

### 3.1 | Seed yield components

ANOVAs of yield components for individual node groups are presented in Table 2. Full results including fitted means can be found in Tables S1–S3.

There were no significant effects of temperature or variety on the number of pods for the two lowest node groups (Figure 1). For the lowest node group, number of pods ranged from 1.4 to 10.1, whereas for the middle node group, number of pods ranged from 2.5 to 14.

For the upper node group, however, significant effects of temperature and variety were found. The number of pods was significantly higher for plants grown at 19°C compared with 24°C, with Sampo having a significantly higher number of pods compared with Vertigo. At the last harvest of the upper node group, Vertigo had approximately 10 pods at 19°C compared with three pods at 24°C, which was significantly lower, whereas Sampo had approximately 15 pods at 19°C compared with nine pods at 24°C which was not significant.

There was no significant effect of temperature, harvest time, nor variety on the number of seeds per pod for any of the three node groups, with a total mean value of 2.8 seeds per pod (data not shown).

The number of seeds per plant followed the same pattern as pods per plant (data not shown). For the two lowest node groups, there was no significant effect of temperature nor variety on the number of seeds per plant. The number of seeds increased throughout plant development and was highest at the last harvest. For the upper node group, significant effects of temperature and variety on number of seed per plant were found. Sampo had a significantly higher number of seeds per plant compared with Vertigo. The number of seeds per plant was significantly higher for plants grown at 19°C compared with 24°C.

Dry weight per seed increased during plant development, while moisture content decreased. As expected, seeds from the lowest node groups had the highest weight at the first harvest, because these were the first initiated (Figure 2). At the latest harvest at 24°C, all node groups within variety had reached similar seed weights, indicating that the accumulation of storage compounds was completed.

In general, the moisture content in Sampo for the lowest node group declined sharply from the second to third harvest at 19°C and from the first to the second harvest at 24°C, indicating that desiccation had started and accumulation of storage compounds in the seed was terminating (Figure 2b). For Vertigo, the late variety, a sharp decline in moisture content was observed from the second to the third harvest at 24°C.

At last harvest, the seed moisture content in the lower node group for Sampo varied from 11.4% for the seed grown at 24°C to 55.9% for the seed grown at 14°C. The moisture content for Vertigo at the last harvest varied from 23.2% to 60.5%. The difference in moisture for Vertigo and Sampo is due to the difference in earliness.

**TABLE 2** Results (*p*-values) from analysis of variance, for each node group (1–5, 6–10, and 11–15) individually for the parameters number of pods per plant, number of seeds per plant, moisture content (%), dry weight per seed (mg), and total dry weight of seeds per plant (g).

| | *p*-value | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pods/plant | | | Seeds/plant | | | Moisture content (%) | | | Dry weight (mg/seed) | | | Tot. dry weight of seeds (g/plant) | | |
| Node | 1–5 | 6–10 | 11–15 | 1–5 | 6–10 | 11–15 | 1–5 | 6–10 | 11–15 | 1–5 | 6–10 | 11–15 | 1–5 | 6–10 | 11–15 |
| Temp (°C) (T) | .055 | .418 | .002 | .113 | .436 | .011 | <.001 | .01 | <.001 | <.001 | .011 | .016 | .051 | .159 | <.001 |
| Harvest (H) | .006 | <.001 | <.001 | .028 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |
| Variety (V) | .184 | .201 | <.001 | .546 | .075 | <.001 | <.001 | .235 | .032 | <.001 | <.001 | <.001 | <.001 | <.001 | .219 |
| T × H | .08 | .009 | .001 | .372 | .02 | .008 | <.001 | <.001 | <.001 | <.001 | .011 | .083 | .051 | <.001 | <.001 |
| T × V | .003 | .001 | .001 | .058 | .002 | .014 | .001 | .067 | .48 | 0 | .001 | .001 | .575 | .113 | .006 |
| H × V | .097 | .12 | .02 | .173 | .017 | .019 | .003 | .099 | .591 | <.001 | <.001 | <.001 | .039 | .155 | .227 |
| T × H × V | .513 | .464 | .028 | .416 | .816 | .322 | .003 | .404 | .291 | .131 | .526 | .984 | .768 | .275 | .152 |

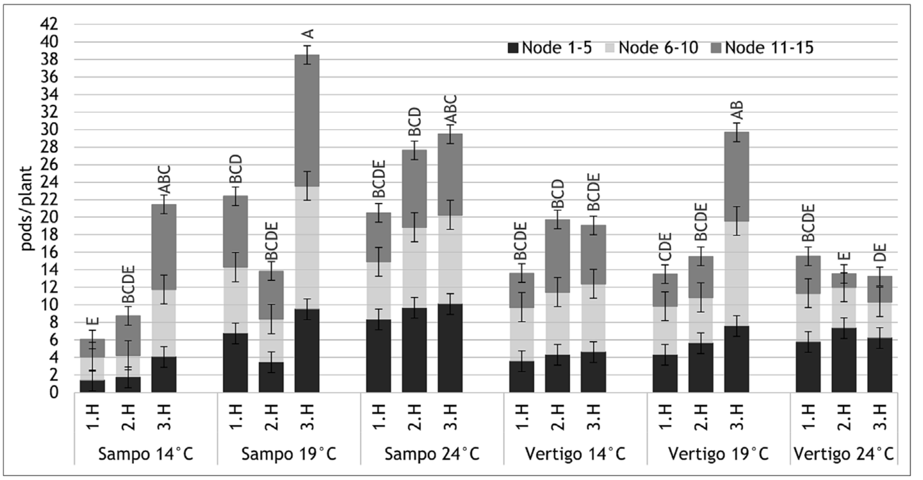


**FIGURE 1** Number of pods per plant for the two varieties grown at the three temperatures and for the three harvest time points, recorded for three node groups individually. 1.H = 1. Harvest (~860 growing degree days [GDD]), 2. H = 2. Harvest (1000 GDD), and 3.H = 3. Harvest (Sampo ~1180 GDD and Vertigo ~1220 GDD). Data are mean ± standard error (SE). Significant differences for the upper node group, and results from Tukey test are given above the bars ($p < .05$ level).

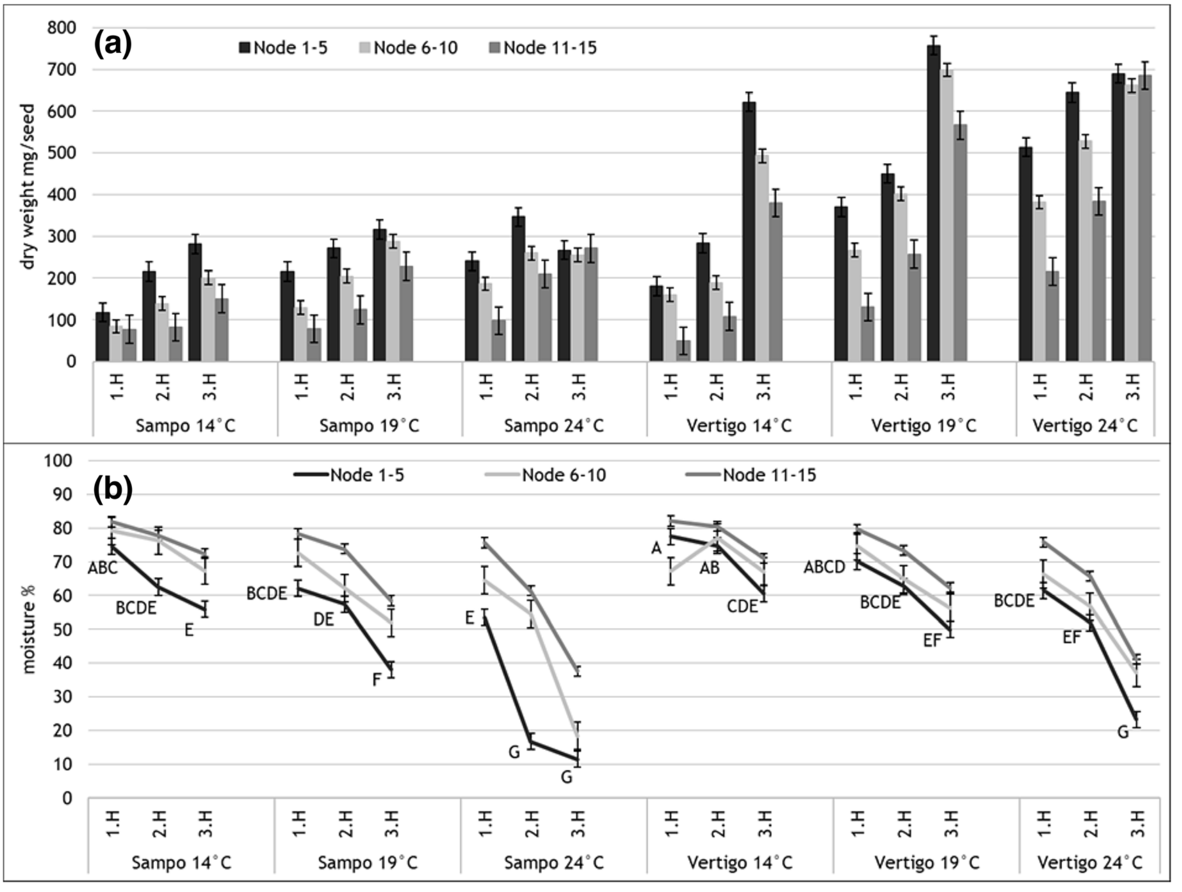


**FIGURE 2** (a) Dry weight (milligrams per seed) for each variety, temperature, and harvest, for each node group individually and (b) the corresponding moisture content in percentage in seeds for variety, temperature, and harvest, for each node group. 1.H = 1. Harvest (~860 growing degree days [GDD]), 2.H = 2. Harvest (1000 GDD), and 3.H = 3. Harvest (Sampo ~1180 GDD and Vertigo ~1220 GDD). Data are mean ± SE. Significant differences for the moisture content at the lowest node group, and result from Tukey test is shown ($p < .05$ level).

Although each variety was harvested based on degree days, seed from plants grown at 14°C and 19°C had not reach the same maturity as those grown at 24°C, which means that the plants grown at 19°C and 14°C are harvested at an earlier stage of growth.

At the last harvest, a higher total dry seed weight was observed at 19°C compared with 14°C and 24°C, for both varieties (Figure 3). This was mainly due to a higher number of seeds for the two upper node groups. For the lowest node group, increasing temperatures resulted in a higher dry seed yield, with the highest total yield at 24°C. This response was not observed for the middle node group. For the upper node group, plants grown at 19°C had significantly higher total dry seed yield.

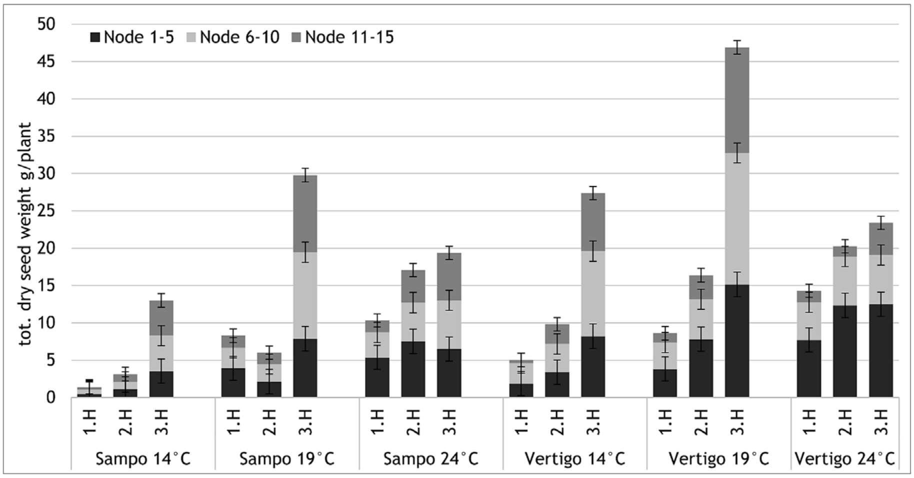


**FIGURE 3** Total dry weight of seed in grams per plant for each variety, temperature, and harvest, for each node group individually. 1.H = 1. Harvest (~860 growing degree days [GDD]), 2. H = 2. Harvest (1000 GDD), and 3.H = 3. Harvest (Sampo ~1180 GDD and Vertigo ~1220 GDD). Data are mean ± SE.

### 3.2 | Protein and carbohydrate accumulation

The accumulation of storage compounds such as protein, starch, and LMWC were followed for the lowest node group (Table 3). Full results from ANOVA including fitted means can be found in Table S4.

Seed development was followed during the storage phase to seed desiccation, covering the range in moisture content from about 80% to below 20%. The seed dry weight increased as the accumulation of protein and starch proceeded (data not shown). Because there is a genetic difference in seed size between the two varieties, we compared the accumulated content in grams per 100 g.

The protein content (data not shown) tended to be stable throughout the period of seed storage, and no significant effect of temperature was observed. Sampo had a significantly higher protein content compared with Vertigo (32.5 vs. 28.7 g/100 g).

Starch content increased, whereas sucrose content decreased (Figure 4) as the period of storage phase proceeded. For Sampo, the accumulation of starch ceased at the second harvest at 19°C, while for Vertigo, this was observed for the second harvest at 24°C. There was no significant difference in sucrose content for the two varieties, but they have different accumulation patterns, due to the differing maturity stages.

Among the GFS, sucrose made up 98–99% of the monosaccharides and disaccharides, and only small traces of glucose and fructose were found (data not shown). The sucrose content (grams per 100 g) decreased with increasing temperature and with the later harvest times. Vertigo had a higher sucrose content compared with Sampo. For Sampo, the decrease in sucrose content leveled off at 24°C, while for Vertigo, the sucrose content continued to decrease, due to differences in maturation for the two varieties.

Accumulation of RFOs showed opposite pattern compared with accumulation of sucrose, as they accumulated later during seed development. Among the RFOs, verbascose was the predominant oligosaccharide, followed by stachyose. Only low contents of raffinose were found. The content of RFOs (grams per 100 g) for each variety, temperature, and harvest time for the three oligosaccharides is presented in Figure 5. At the lowest temperature, there was a slight increase in raffinose throughout the harvesting period, while an opposite trend was observed for the highest temperature. Accumulation of stachyose followed the same pattern as raffinose, but the concentration was higher. Verbascose, on the other hand, accumulated slightly later, and no decrease was observed when approaching desiccation.

### 3.3 | Accumulation of storage compounds in relation to moisture content

Harvest times were based on degree days; however, plants grown at 14°C and 19°C did not obtain the same developmental stage compared with those grown at 24°C. Therefore, the content of main storage compounds is presented and discussed related to seed moisture content (Figures 6a–d and 7 and Table S5). This is based on the assumption that the moisture content is a good indicator of seed development stage in seed crops (Sripathy & Groot, 2023). Due to genetic difference in seed size for the two varieties, the content was calculated as grams per 100 g.

The content of starch increased rapidly during seed development, and a break point was observed at a moisture content of 62.5% and 57.7% for Sampo and Vertigo, respectively, which is significantly different (Figure 6a). The associated starch content at these break points is 38.9 and 46.0 g/100 g for Sampo and Vertigo, respectively. In the early storage phase, the seeds contained 14.6–18.6 g/100 g sucrose. Sucrose content then rapidly decreased before leveling out at 2.1 g/100 g for Sampo and 3.9 g/100 for Vertigo (Figure 6b). The split-line model gave a break point for Sampo at 51.0% moisture and 54.2% for Vertigo; however, the difference was not significant.

Verbascose was the predominant oligosaccharide of RFOs for both varieties. During early accumulation phase, only a small content of verbascose was found (Figure 6c), which increased during the accumulation phase. The break point for Sampo and Vertigo was found to be at 41.9% and 40.7% moisture content, respectively (no significant difference). The associated verbascose content at the break points was 3.0 and 2.3 g/100 g for Sampo and Vertigo, respectively. After the break point, the content of verbascose in Sampo was relatively

**TABLE 3** Results (*p*-values) from analysis of variance for the lowest node group (node 1–5), for compounds in grams per 100 g for protein, starch, sucrose, raffinose, stachyose, and verbascose.

| | *p*-value | | | | | |
|---|---|---|---|---|---|---|
| | Protein (g/100) | Starch (g/100 g) | Sucrose (g/100 g) | Raffinose (g/100 g) | Stachyose (g/100 g) | Verbascose (g/100 g) |
| Temp (°C) (T) | .426 | <.001 | <.001 | .262 | .014 | .001 |
| Harvest (H) | .113 | <.001 | <.001 | .462 | .004 | <.001 |
| Variety (V) | <.001 | .102 | <.001 | .862 | .001 | <.001 |
| T × H | .107 | <.001 | <.001 | <.001 | <.001 | <.001 |
| T × V | 1 | .032 | .014 | .001 | .001 | .002 |
| H × V | .57 | <.001 | .007 | .005 | .018 | .346 |
| T × H × V | .448 | .021 | .474 | .002 | .008 | .001 |

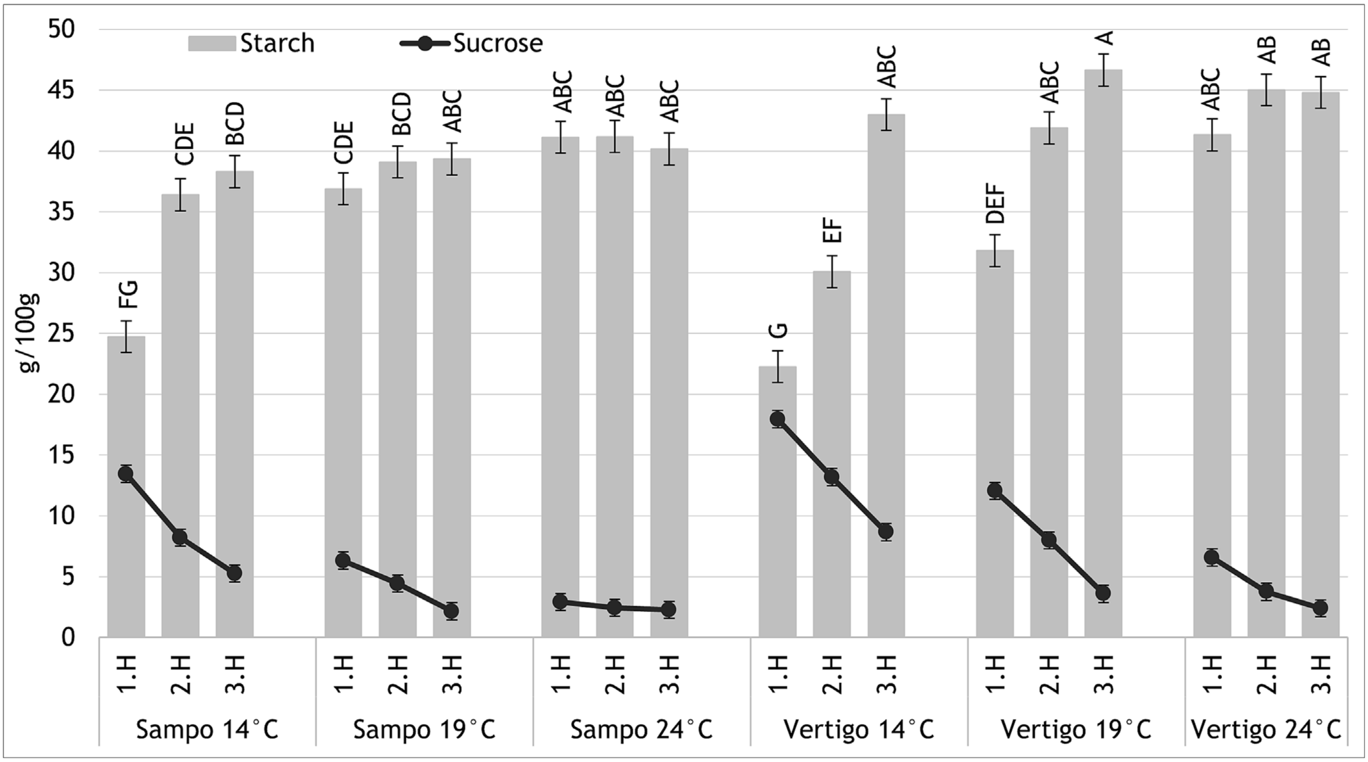


**FIGURE 4** Starch and sucrose content (grams per 100 g) for each variety, temperature, and harvest. 1.H = 1. Harvest (~860 growing degree days [GDD]), 2.H = 2. Harvest (1000 GDD), and 3.H = 3. Harvest (Sampo ~1180 GDD and Vertigo ~1220 GDD). Data are mean ± SE. Significant differences for the starch content, and results from Tukey test are given above the bars ($p < .05$ level).

stable, whereas for Vertigo, the accumulation increased slightly, most likely due to the later seed desiccation.

Stachyose was found in low concentrations at the early accumulation phase; however, the accumulation increased, and break points were reached at 59.5% and 54.2% moisture contents, for Sampo and Vertigo, respectively (Figure 6d). This corresponds to a stachyose content of about 1.0 g/100 g for both varieties. The break point was not significantly different for the two varieties. After the break point, stachyose content decrease slightly for both varieties, but at a higher rate for Sampo.

Sampo had a higher protein content than Vertigo. At the early phase of accumulation, seeds had a protein content of ~33 and ~28 g/100 g for Sampo and Vertigo, respectively (Figure 7). At the end of accumulation, the protein content was ~33 and ~30 g/100 g for the two varieties.

## 4 | DISCUSSION

Cultivation of faba bean is expanding to higher latitudes due to interest in alternatives for soya bean in the feed industry. There also a growing popularity for plant protein in the human diet. Faba bean can be grown successfully in the Nordic countries in areas around 60°N. In Norway, faba bean cultivation has the potential to expand from the

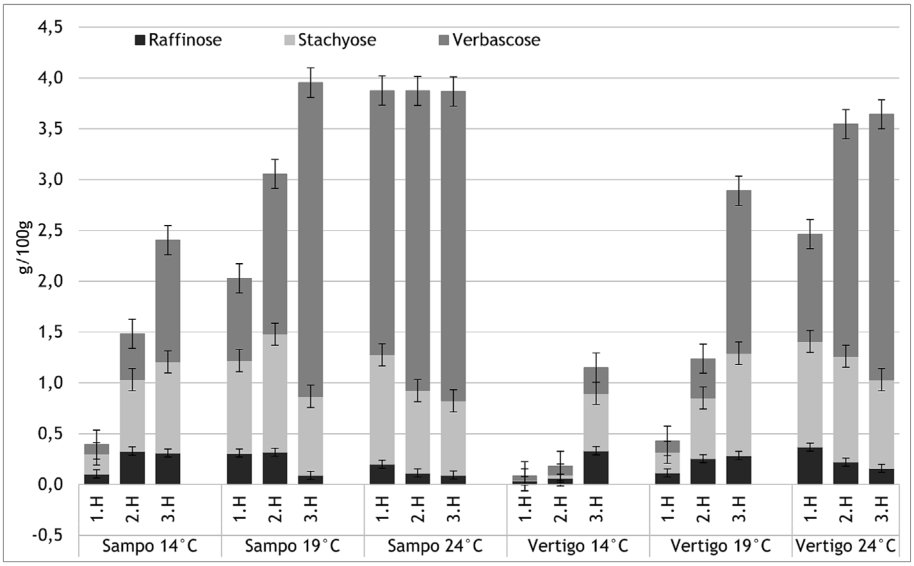


**FIGURE 5** Content of raffinose family oligosaccharides in grams per 100 g for each variety, temperature, and harvesting time, divided into raffinose, stachyose, and verbascose. 1.H = 1. Harvest (~860 growing degree days [GDD]), 2.H = 2. Harvest (1000 GDD), 3. H = 3. Harvest (Sampo ~1180 GDD and Vertigo ~1220 GDD). Data are mean ± SE. Result from Tukey test is shown in Table S4.

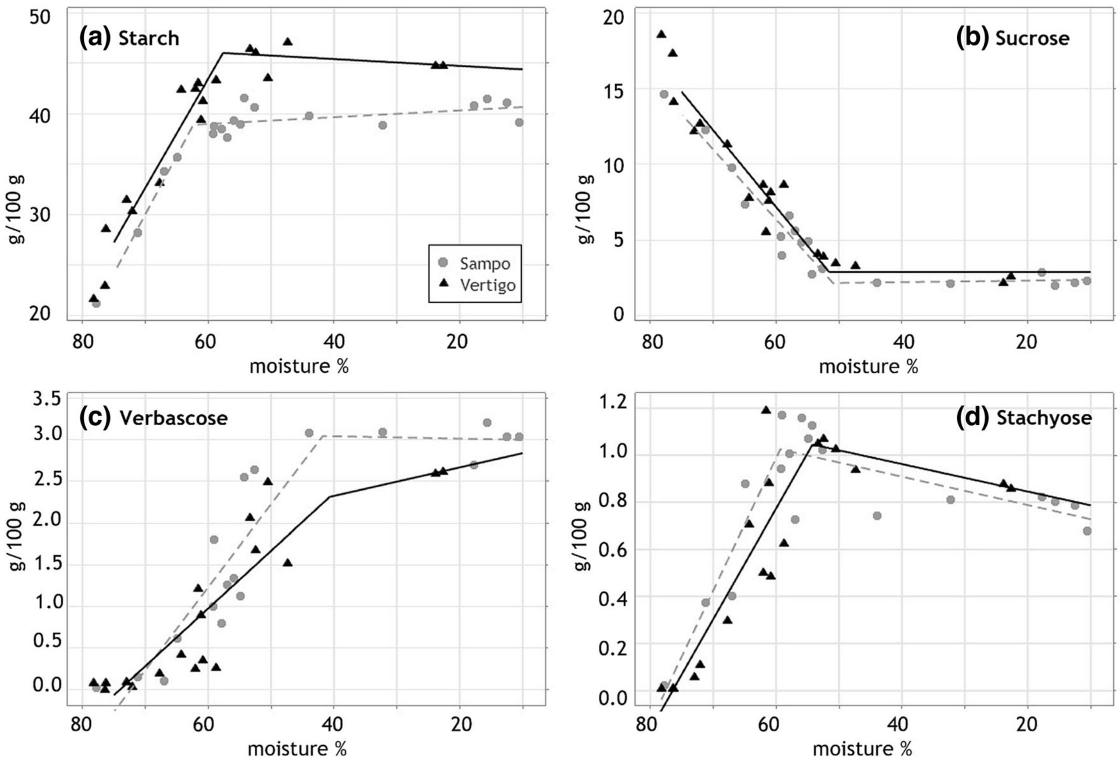


**FIGURE 6** Accumulation of (a) starch, (b) sucrose, (c) verbascose, and (d) stachyose (grams per 100 g) in relation to moisture percentage in seed, divided into the two varieties Sampo and Vertigo.

southeastern regions surrounding the Oslofjord to regions further north surrounding lake Mjøsa (Innlandet).

In these areas, growth can be limited by a short growing season and challenged by decreasing temperatures during maturation, potentially leading to delayed harvest. The region surrounding lake Mjøsa can be characterized by a growth season of theoretical ~175 days (Apelsvoll) and mean temperature in July, August, and September of 16.1°C, 14.7°C, and 10.5°C compared with the areas surrounding the Oslofjord having ~190 days (Aas) and a mean temperature in July, August, and September of 16.7°C, 15.7°C, and 11.5°C (NIBIO, 2023; Stabbetorp et al., 2023). In the present study, day temperature of 14°C was compared with 19°C and 24°C, which, in the Oslofjord region, is considered as normal and high temperatures, respectively. The predicted rise in temperature due to climate changes can lead to a longer growing season in the higher latitudes but also the risk of heat stress during some physiological phases. Therefore, breeding for

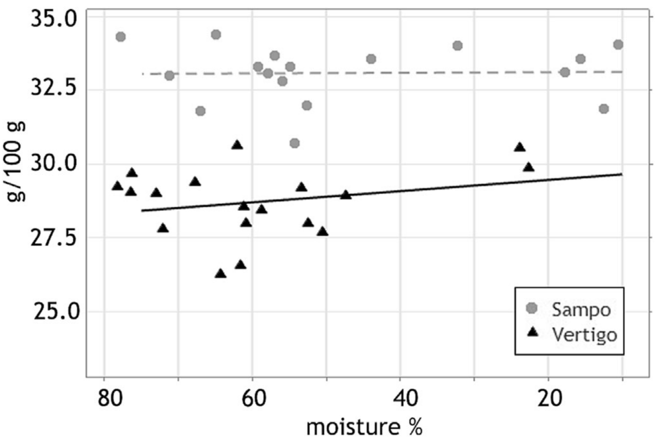


**FIGURE 7** Accumulation of protein (grams per 100 g) in relation to moisture percentage in seed, divided in the two varieties Sampo and Vertigo.

early varieties well adapted for the Nordic climate is important, which also are tolerant to periods causing heat stress.

Faba bean has an indeterminate growth habit giving continued vegetative growth after flower initiation which can result in tall plants with many internodes if grown under favorable conditions (Hughes et al., 2020; Knott, 1990). Subjected to optimal conditions in the greenhouse, plants produced new phytomers for a much longer period compared with field conditions. The indeterminate inflorescence growth extends the time to ripening, so that late developed pods are still unripe while the earliest are mature (Stoddard, 1993). For this reason, pods from the lower, medium, and upper sections of the plants were harvested and pooled into different node groups, to follow the various stages of seed development and maturity.

The main effect of temperature was the strongly delayed pod and seed development with decreasing temperatures. For the early maturing variety Sampo, the last harvest was conducted 89, 71, and 59 days after flowering (~1180 GDD) for the temperature regime 14°C, 19°C, and 24°C, respectively (Table 1). Harvest times were based on GDD calculations using $T_{base} = 0$, as is used by others (Bodner et al., 2018; Boote et al., 2002; Patrick & Stoddard, 2010; Stützel, 1995) However, plants grown at 14°C and 19°C did not reach the same maturity stage compared with those grown at 24°C. Lizarazo et al. (2015) calculated the GDD in faba bean grown under Nordic conditions using 5°C as base temperature, which is used for several crops. Confalone et al. (2011) found that the base temperature varied across phenological phases, with a base temperature from flowering to maturity likely >0°C. To our knowledge, consensus about base temperatures to be used for faba bean is lacking and, particularly, for the period of seed development and maturation.

For Sampo, days after flowering to reach the stage of ~55% moisture were estimated to be 89, 60, and 43 days, for the temperature regimes of 14°C, 19°C, and 24°C, respectively. Using $T_{base} = 0$, this corresponds to 1187, 1000, and 860 GDD, while a $T_{base} = 5$ gives corresponding values of 742, 700, and 645 GDD. Regardless of base temperature, this experiment gave important knowledge on how the maturation process is delayed under low temperatures, in line with results from Lizarazo et al. (2015) reporting a great delay in maturation in cool/wet seasons in Finland.

Temperature affected the number of pods per plant, but only for the upper node group. The plants were transferred to controlled climate chambers with different day temperatures at the onset of flowering at the lower nodes. The initiation and progress toward flowering for the upper nodes would hence to a larger extend be influenced by the different day temperatures, compared with the lowest nodes. At the final harvest, the highest number of pods per plant were found in the upper node groups for plants grown at 19°C.

Number of seeds per pod was not found to be influenced by temperature, harvest time, nor variety, for any of the node groups. Number of seeds per pod appears to be a trait that is consistent regardless of environments, and previous reports have demonstrated that number of seeds per pod within cultivars was consistent over a range of plant densities and sowing dates (Thomsen & Taylor, 1977).

Seed weight needs to be interpreted related to progressing seed development and stage of maturation, of which the seed moisture content is a good indicator. The increase in seed dry weight together with a decrease in seed moisture reflects the dynamic of temperature regime and variety. At the last harvest at 24°C, the seed moisture content had dropped to 41% or lower for all node groups, indicating that seeds had proceeded to the desiccation phase. Seed size is reported to vary depending on the location of the pod (Etemadi et al., 2019), where seeds from the lowest node group are first initiated, and therefore gain most weight. Interestingly, seeds from last harvest at 24°C appeared to obtain the same size for all three node groups. This was found for both varieties, and the seed size was as expected for these varieties (Waalen et al., 2022).

An increase in temperature will give a faster development of pods and seeds, while shortening the duration of growth phases. Seed from plants grown at 19°C was found to have the highest total dry seed yield, primarily due to a high total seed weight for the upper node groups, while seeds from plants grown at 14°C would probably continue to gain weight if the harvest had been postponed. This is in line with the results of Dekhuijzen and Verkerke (1986), showing decreasing size with increasing temperatures in the range of 16°C, 21°C, and 26°C.

Accumulation of the storage compounds protein, starch, and LMWC were analyzed for the lowest node group and covered the seed storage phase with decreasing moisture contents from about 80% to below 20%. The results demonstrated the dynamics of increased starch and decreased sucrose content (given as grams per 100 g dry matter) as maturation proceeded and moisture content decreased. Fructose and glucose contents were found to be negligible in mature seeds, which correspond with previous results (Lattanzio et al., 1986). The sucrose content stabilized at 2.1 g/100 g for Sampo and 3.9 g/100 for Vertigo. Previously, sucrose content in mature faba beans seeds has been found to range from 0.02% to 5.23% of the dry weight, and larger seeded varieties generally have a higher sucrose content than smaller seeded (Landry et al., 2016).

The starch content in the break points was 38.9 and 46.0 g/100 g for Sampo and Vertigo, respectively, in line with the literature (Haase & Shi, 1991).

The break points for verbascose were found to be at 3.0 and 2.3 g/100 g for Sampo and Vertigo, respectively; however, for Vertigo, the accumulation tended to increase slightly, due to later seed desiccation. The break point for stachyose was corresponding to a content of about 1.0 g/100 g for both varieties. Landry et al. reported the content of RFO in mature faba bean seed, with verbascose (2.4%) being the predominant, followed by stachyose (1.9%), whereas others found that verbascose was the major RFO, followed by stachyose and raffinose (Lattanzio et al., 1986).

This study demonstrated that accumulation of RFOs increased as the moisture content in the seeds decreased, indicating that RFO accumulation is an important part of seed maturation. This implies that RFO accumulation is affected by maturation stage, and not the temperature directly. In line with others, the RFOs were found to accumulate late during seed development and are present in only small amounts during the early storage phase.

## 5 | CONCLUSION

This study investigated pod and seed development and the dynamics of storage compound accumulation in seeds for three temperature regimes. The lower temperature regimes strongly delayed pod and seed development compared with 24°C/12°C.

The temperature affected the number of pods per plant for the upper node group. No effects of temperature were detected on the number of seeds per pod, while seed weights varied with the seed development stage, indirectly effected by temperature. Plants grown at 19°C had the highest dry seed yield, primarily due to the highest number of pods at the upper nodes.

Temperature per se did not influence the content of starch, protein, and LMWC, while their accumulation followed the moisture content in the seed, and thus the seed development stage. This is illustrated by the regression models, showing contents of the main storage compounds (grams per 100 g) in relation to moisture content. The results give more knowledge about seed maturation and accumulation in faba bean under low temperatures, important for cultivation under high latitude regions.

### ACKNOWLEDGMENTS

This study was financially supported by the Norwegian Research Council, Project "FoodProFuture" Nr 267858 and "GreenPlantFood" Nr 319049. Additionally, the authors would like to thank Torfinn Torp (NIBIO) for help with the statistical analysis and Karin Svinnset (NMBU), Hanne Homb (NIBIO), Ulrike Böcker, and Hanne Zobel (NOFIMA) for technical support with experiment and analyses.

### CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

### DATA AVAILABILITY STATEMENT

Data that support the findings of this study are available from the corresponding author upon reasonable request.

### ORCID

*Anne Marthe Lundby* https://orcid.org/0009-0006-0734-9477

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**How to cite this article:** Lundby, A. M., Waalen, W., Uhlen, A. K., Knutsen, S. H., & Wold, A.-B. (2023). Effect of temperature during flowering, pod set, and seed development on yield components and accumulation of protein, starch, and low molecular weight carbohydrates in two faba bean (*Vicia faba* L.) cultivars. *Legume Science*, e212. https://doi.org/10.1002/leg3.212