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ABSTRACT

During germination *sensu-stricto* in pulses, an increase in the content of thiamine (B1) and folate (B9) vitamins is expected, along with a reduction in α-galactoside levels. The aim of our study was to optimize germination to increase the nutritional quality of lentils and cowpeas. An experimental design was carried out at 12 h and 24 h of imbibition to analyze the effects of temperature, light, and water content on thiamine, folate, and α-galactoside content. Germination increased thiamine content by 152% in lentils, while in cowpeas, the increase was only 10%. Folate content in cowpea increased by 33%, while α-galactoside content decreased by 99% in cowpeas and by 48% in lentils. Germination *sensu-stricto* can be safely implemented by any food company worldwide as it is simple and involves less sanitary risk than sprouting. This opens up opportunities for enhancing food nutrient content and new ways of processing pulses.

1. Introduction

Germination *sensu-stricto* is a complex biological process that leads to major metabolic changes and activates pathways for the synthesis of the nutrients required for seedling growth (sprouting). Seed imbibition refers to the process of water absorption by the dry seed. This uptake of water triggers the resumption of metabolic activity in the quiescent dry seed. The uptake of water by a dry mature seed is divided into three phases. Phase I is characterized by rapid initial uptake, followed by phase II, which is a lag phase where water uptake slows down, accompanied by high metabolic modifications in the seed (Galland et al., 2014). Phase III occurs only after germination is completed when the embryonic axe starts elongating, leading to a further increase in water uptake. Germination is considered complete when the embryonic axe elongates, indicating that the seedling is actively growing and developing. The passage mentions that radicle extension, which is the elongation of the primary root, through the structures surrounding the embryo, is the event that terminates germination *sensu-stricto* and marks the commencement of seedling growth (Bewley & Black, 1994; Nonogaki et al., 2007; Rajjou et al., 2012). This event is what we refer to as "sprouting," where the radicle emerges from the seed coat and starts growing into a seedling. Some semantic mistakes have been made in the description of germination *sensu-stricto* and sprouting. During germination *sensu-stricto,* seeds have a heterotrophic metabolism and are still considered as seeds containing large quantities of storage compounds. Depending on the genotype of the seed and environmental conditions, germination can be completed within 24 h, but further thermal processing may be required before consumption. In contrast, sprouting involves autotrophic metabolism and sprouts are classified as ready-to-eat food products. Even if the word 'germination' is used, most studies in the literature actually report the effect of sprouting (Hefni et al., 2015; Shohag et al., 2012). The use of incorrect terms can interfere with the advance of knowledge on the advantages of germination. A short germination period of 24 h and a thermal post-germination process may reduce the microbiological risk of germinated products (Avezum et al., 2022; Bresciani & Marti, 2005). Most studies on germination reported in the literature are carried out between 20 °C and 30 °C, with 12 h to 16 h of light (Coffigniez et al., 2021; Ghavidel & Davoodi, 2011; Joy et al.,

Abbreviations: TDP, Thiamine Diphosphate; TMP, Thiamine Monophosphate; Thia-HCL, Thiamine Hydrochloride; PteGlu, Folic acid; H4folate, Tetrahydrofolate; 5-CH3-H4folate, 5-methyl-tetrahydrofolate; 5-CHO-H4folate, 5-formyl-tetrahydrofolate; 10-CHO-PteGlu, 10-formylfolic acid.

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2022; Sallam et al., 2021). However, all the studies were conducted at water saturation, and no changes in seed water content during germination were reported. Germination and sprouting at 25 ◦C for 24 h to 6 days have been shown to increase the concentrations of water-soluble vitamins and *in-vitro* digestibility of starch and protein in pulses and cereals, while simultaneously reducing the levels of antinutritional factors, such as phytic acid, α-galactoside, trypsin inhibitor, and lectins in the cotyledon (Prodanov et al., 1997; Vidal-Valverde et al., 2002).

Among seeds used for germination, pulses are excellent sources of carbohydrates, proteins, fibers, minerals, and B vitamins (especially thiamine and folate) (Bojn˘ansk´a et al., 2016; Mudryj et al., 2014). Thiamine (B1) is a cofactor for multiple enzymes that catalyze bioenergetic reactions, control amino acid metabolism, and convert some carbohydrates. Thiamine may be present in a free form, or in its phosphorylated derivatives, namely thiamine monophosphate (TMP), diphosphate (TDP), triphosphate (TTP), and adenosine thiamine triphosphate (Tylicki et al., 2018). In pulses, folates are present in different forms, including folic acid (PteGlu), 10-formylfolic acid (10-CHO-PteGlu), 5-formyltetrahydrofolate (5-CHO-H_4folate), 5-methyltetrahydrofolate (5-CH_3-H_4folate), and tetrahydrofolate (H_4folate). Folate co-enzymes mediate two major interrelated metabolic cycles that are responsible for the synthesis of thymidylate, purines, and methionine (Moll & Davis, 2017). The recommended daily intake (RDI) of thiamine and folate for adults, is respectively, 1100–1500 μg/day and 200–400 μg/day (Chunming et al., 2001; Krawinkel et al., 2014; Moll & Davis, 2017). In 100 g, raw lentils contain 51% of the RDI of thiamine and 70% for cowpea; and respectively, 55% and 105% of RDI for folate (Coffigniez et al., 2021; Hall et al., 2017). However along with their health benefits, pulses also contain anti-nutritional factors, such as α-galactoside, in the form of raffinose, stachyose, and/or verbascose (Bessada et al., 2019). Due to the lack of α-galactosidase in human and animal intestinal mucosa, these oligosaccharides escape digestion and are metabolized by colon bacteria, thereby producing gut gas in the form of hydrogen, carbon dioxide, and methane, and resulting in flatulence (Bessada et al., 2019; Naczk et al., 1997).

At laboratory scale, our study is the first specifically designed to simultaneously assess the impact of temperature, light, and water content on the nutritional value of pulses during germination *sensu-stricto*. Few studies have analyzed the effect of temperature on the antioxidant and bioactive compounds in cereals and pulses during germination (Domínguez-Arispuro et al., 2018; Paucar-Menacho et al., 2017; Rico et al., 2020). In this study, we investigated whether optimizing germination *sensu-stricto* enhances vitamins content and reduces α-galactosides content of lentil (*Lens culinaris*) and cowpea (*Vigna unguiculata*). The outcome will allow food companies to better understand the advantages of germination, while improving the nutritional status of pulses.

2. Material and methods

2.1. Material

Two pulses were chosen as models and analyzed: lentil (*Lens culinaris*) and cowpea (*Vigna unguiculata*). Berry green lentil seeds were purchased in 2020 from CIBELE Company, France. White cowpea seeds were purchased directly from the producer in south-east Benin. Cowpea seeds were sowed in November 2021 and harvested in February 2022. Both seeds were stored at —20 ◦C until use.

2.2. Germination

To trigger germination, 80 g of seeds were soaked in 320 mL water (ratio 1:4; w/v) under agitation at 30 ◦C, lentils for 3 h and cowpeas for 5 h. The soaked seeds were removed from the soaking water, immediately drained and placed in a chamber with controlled temperature and relative humidity for germination. The whole process took respectively 12 h and 24 h (including soaking time). For lentil germination, a BIA Climatic chamber with 25 ◦C – 35 ◦C temperature regulation and 90% relative humidity was used. The seeds were placed in a rectangular perforated weighing pan (361 cm^2) equipped with an integrated balance to monitor the seed weight and, consequently, its water content. A spray was automatically activated when the calculated water content dropped below the expected level and stopped after 10 min; spraying was activated roughly once an hour. To verify the water content, 5 g of wet seeds were sampled in five different locations across the rectangular weighing pan, and dried at 105 ◦C for 48 h. Alpheus LED (France) light was used which corresponds to sunlight. The cowpea seeds were germinated in Benin in a BINDER-KMF 240 chamber (~95% relative humidity). In this case, spraying was done manually after 18 h of germination using 60 mL of water, for a targeted water content of 110%, or using 120 mL for 140% water content (db). The spraying was carefully carried out to ensure that the desired water content levels were achieved and maintained under control. The samples were weighed once an hour to monitor the water content. The light used was a normal white LED (Light Emitting Diode). None of the lentil or cowpea seeds, in either of the experimental locations, developed a radicle (i.e. germinated) in the first 12 h. At this stage, the samples were considered as non-germinated, and all the seeds were analyzed. After 24 h of germination, the seeds were sorted into non-germinated (no radicle), germinated (radicle < 5 mm), and sprouted seeds (radicle > 5 mm). At 24 h, only the germinated seeds were analyzed. Lentil seeds were collected and frozen in liquid nitrogen and stored at —80 ◦C. Cowpea seeds were collected and directly stored at —40 ◦C. All the seeds were freeze-dried and then ground in a QIAGEN – TissueLyser II ball mill grinder to obtain a fine flour. The flour was freeze-dried and stored at —20 ◦C until further analyses.

2.3. Experimental design

Response surface methodology (RSM) was used to analyze the effect of germination parameters on the nutritional quality of the pulses and to determine the optimal conditions for enhancing the nutritional quality of germinated lentil and cowpea flour via a desirability analysis. An experimental design with two levels and three factors was used (Supplementary Table S1). The impact of temperature, light, and seed water content on germination rate and nutritional quality of pulses was analyzed. The center point 9 (C) was performed in triplicate. Preliminary trials and data from the literature were used to select the boundaries of the experimental design. The same experiment design was implemented for lentil and cowpea. The desirability function was used to optimize temperature, light, and water to reach the maximum desirable response. The variables considered were germination temperature (T), light (L), and water content (W). The response variables were thiamine (Thia), folate (F), and α-galactoside (G). To optimize the process, the linear equation was applied (1):

$$Y = \beta_0 + \beta_T T + \beta_L L + \beta_w W + \beta_{T,L} TL + \beta_{T,W} TW + \beta_{L,W} LW + \beta_{T,L,W} TLW + \sum \in \quad (1)$$

where Y is the estimated response, β_0 and β_X represent the constant and coefficients of linear and interactive effects, respectively, and ∈ represents the error. Non-significant variables ($p \geq 0.1$) were deleted from the linear equation.

2.4. Germination rate

Given the large number of lentil seeds used for germination, it was not feasible to manually count each individual germinated seed. Instead, 5 g of wet seeds were sampled in five different locations in the rectangle weighing pan (one sample in each corner and one in the center). All germinated and sprouted seeds were counted and the mean and its standard deviation were calculated. Samples were collected at the same

locations in the pan in all the experiments. For cowpea germination, the germination rate was calculated by counting all germinated and sprouted seeds. In both cases, the germination rate was calculated according to equation (2).

$$Germination\ rate\ (\%) = \frac{\Sigma of\ germinated\ seeds + \Sigma\ of\ sprouted\ seeds}{\Sigma\ of\ total\ seed} \times 100 \quad (2)$$

2.5. Thiamine extraction and detection

Thiamine was extracted and quantified as described by Schmidt et al. (2017) with some modifications. The thiamine was extracted from 0.5 g of seed flour in 40 mL of 0.1 M HCL (Hydrochloric Acid) solution for 1 h under agitation. Samples were centrifuged at 8000 rpm for 15 min at 4 °C. The samples were oxidized to fluorescent thiochrome in absence of light. The resulting solutions were filtered through a 0.45 µm and 0.2 µm filter. The thiamine was quantified on an Ultra Performance LC (UPLC™) system equipped with an Acquity™ fluorescence (FLR) detector. The column was operated at a flow rate of 0.40 mL/min. The total cycle was set to 6 min until the following injection. Thiamine diphosphate (TDP), thiamine monophosphate (TMP), and thiamine hydrochloride (Thia-HCL) were quantified using a calibration curve with external standards. The thiamine equivalent (thia-eq) was calculated according to equation (3) and (4).

All samples were prepared in duplicate.

$$Thia-eq = \frac{(nTDP + nTMP + nThiaHCL) \times MM\ Thiamine}{Weight\ of\ samples} \quad (3)$$

nX = number of mols of X (X = TDP, TMP or Thia-HCL) in µmol

$$nX = \frac{[X] \times V}{MMX} \times 10^{6} \quad (4)$$

[X] = Concentration of X in g/mL

V = Extraction volume in mL.

MM X = molar mass of X in g/mol.

2.6. Folate extraction and detection

The folates were extracted and quantified using the methodology described by Coffigniez et al. (2021) and Striegel et al. (2018). Folic acid (PteGlu), Tetrahydrofolate (H4folate), 5-methyl-tetrahydrofolate (5-CH3-H4folate), 5-formyl-tetrahydrofolate (5-CHO-H4folate), and 10-formylfolic acid (10-CHO-PteGlu) were quantified by stable isotope dilution assay with LC-MS/MS detection using isotopologues of the analytes as internal standards. Both extraction and measurements were performed in duplicate.

2.7. Alpha-galactoside extraction and detection

The α-galactoside was extracted using the methodology described by Coffigniez et al., (2018) with slight modifications. α-galactoside was extracted from 80 mg of flour placed in 10 mL of 80% (v/v) ethanol at 80 °C for 1 h. The supernatant was collected and filtered through a 0.45 µm filter. α-galactoside was separated by high-performance anion exchange chromatography using a Dionex ICS-5000 + Ion Chromatography System (Thermo Scientific, France). The sugars were separated at room temperature in a 4 × 30 mm Dionex CarboPac PA210-fast-4 µm pre-column and a 4 × 150 mm Dionex CarboPac PA210-fast-4 µm column (Dionex, Germany). The injection volume was 10 µL. The mobile phase was 12 mM potassium hydroxide solution. The flow rate was 0.8 mL/min. Raffinose, stachyose, and verbascose were quantified using a calibration curve with external standards. Extraction was done in duplicate.

2.8. Statistical analyses

The thiamin, folate, and α-galactoside contents were analyzed using TIBCO Statistica software (StatSoft). For each nutritional compound, an ANOVA of temperature, light, water, and its interaction was performed. For a better understanding of the impact of germination conditions on the nutritional quality, 12 h and 24 h germination periods were statistically analyzed separately. Tukey-HSD test was performed to check for significant differences between samples for each compound.

3. Results and discussion

3.1. Germination rate

The highest germination rate for lentils, 51.4%, was reached after 24 h at 25 °C, including 12 h of lighting and under water saturation (120%) (Supplementary Table S2). In many of the experiments (assay 1, 3, 5, 7, 11), the standard deviation of the germination rate was high (>10%) underlining the heterogeneity of the samples. This heterogeneity may be due to the use of the automatic spraying system, as the effect on the support was not even. However, the heterogeneous germination of the lentil seeds did not directly affect the final nutritional parameters, since only the germinated seeds were analyzed. Another study reported that, at 22 °C for 24 h, the germination rate of the lentils was 80% (Vasilean et al., 2018). This discrepancy may be due to the difference in germination methodology used in the two studies (illumination and HR were not mentioned in the Vasilean et al. study), seed varieties, and seed vigor.

The cowpea germination rate was better (83.3 %) than the lentil germination rate with the same light and water content but at a temperature of 35 °C. Cowpea germination rates at 28 °C for 48 h have been reported to range from 60% to 99% (Ravelombola et al., 2017). Coffigniez et al. (2021) reported that the germination rate of cowpea at 30 °C reached 75% after only 48 h, while under the same germination conditions, but after 24 h, the germination rate was between 40% and 50% (Coffigniez et al., 2021). These results are in agreement with the data we found at the central point (30 °C) for which the average of germination rate was 45.6%.

In our study, in lentil, only temperature had a negative significant impact ($p < 0.05$) on the germination rate. In cowpea, the temperature and water content had a positive significant impact ($p < 0.01$). Thus, lower temperatures favored lentil germination and higher temperatures and water content favored cowpea germination. The effect of temperature showed that the seeds used in our experiments are adapted to their environment. Lentils are cultivated in regions with milder temperatures, e.g. North America, Europe, India, and Australia, while cowpeas are cultivated in tropical regions like West Africa.

3.2. Nutritional analyses

3.2.1. Thiamine content

Germination increased thiamine content and changed the vitamer profile in lentil seeds. Dry lentil seeds contained 154 µg/100 g (db) thiamine-equivalent (thia-eq) (Table 1). The thiamine content in lentil reported in the literature is higher, ranging between 433 µg/100 g and 510 µg/100 g (db) (Ghavidel & Prakash, 2007; Prodanov et al., 1997). The difference is probably due to the use of different varieties, seed conservation, and thiamine extraction methods. In the present study, soaking increased the total thia-eq in lentil by 109%. Between dry lentils and lentils germinated for 24 h (35 °C, 12 h of lighting, and 80% water content) the thia-eq content increased by 169%. However, no significant impact ($p > 0.05$) of the studied factors was found on the total thia-eq in germinating (12 h) and fully germinated (24 h) lentil seeds. Ghavidel & Prakash, (2007) reported different results, i.e. an increase of up to 33%

Table 4
Average total concentration of thiamine equivalent, folate content in µg/100 g and total α-galactoside content in g/100 g on a dry basis for lentil. The percentage increase in thiamine and folate and the percentage reduction in α-galactoside content is calculated between dry seeds and each condition.

Temperature (◦C)	Light (h)	Water Content (% db)	Time (h)	Total thia-eq in lentil [µg/100 g db]	% increase	Total folate content in lentil [µg/100 g db]	% increase	Total α-galactoside content in lentil [g/100 g on db]	% reduction
Dry				154 ± 3.4		147 ± 7.4		5.56 ± 0.25	
Soaked				322 ± 5.1	109%	122 ± 1.2	−17%	4.65 ± 0.56	16%
25	0	80	12	345 ± 16	124%	124 ± 1.8	−16%	4.41 ± 0.007	21%
			24	388 ± 11	152%	157 ± 1.3	6%	2.97 ± 0.17	47%
35	0	80	12	345 ± 9.4	125%	128 ± 7.8	−13%	4.14 ± 0.20	26%
			24	371 ± 3.8	141%	146 ± 7.2	−1%	3.56 ± 0.22	36%
25	12	80	12	343 ± 6.8	123%	123 ± 5.3	−17%	3.92 ± 0.45	30%
			24	392 ± 5.1	155%	148 ± 1.9	1%	3.12 ± 0.024	44%
35	12	80	12	348 ± 13	126%	120 ± 0.87	−18%	3.92 ± 0.0004	30%
			24	418 ± 11	169%	158 ± 6.5	8%	3.88 ± 0.059	30%
25	0	120	12	351 ± 2.3	128%	120 ± 0.34	−18%	3.99 ± 0.011	28%
			24	377 ± 16	145%	147 ± 2.4	0%	3.39 ± 0.37	39%
35	0	120	12	346 ± 13	125%	111 ± 3.4	−24%	3.93 ± 0.16	29%
			24	385 ± 15	150%	155 ± 9.6	5%	3.35 ± 0.037	40%
25	12	120	12	358 ± 13	132%	113 ± 0.35	−24%	4.09 ± 0.59	26%
			24	373 ± 4.7	142%	147 ± 13	0%	3.00 ± 0.12	46%
32	12	120	12	350 ± 2.5	128%	115 ± 6.9	–22%	4.11 ± 0.045	26%
			24	366 ± 7.8	137%	148 ± 1.5	1%	3.18 ± 0.034	43%
30	6	100	12	347 ± 7.9	125%	98 ± 1.3	–33%	4.03 ± 0.75	27%
			24	367 ± 6.3	138%	145 ± 8.5	−2%	3.24 ± 0.26	42%
30	6	100	12	346 ± 5.3	125%	122 ± 1.6	−17%	3.97 ± 0.22	29%
			24	379 ± 14	146%	146 ± 6.8	−1%	3.02 ± 0.33	46%
30	6	100	12	353 ± 12	129%	123 ± 9.4	−17%	4.07 ± 0.19	27%
			24	373 ± 2.5	142%	147 ± 5.0	0%	2.92 ± 0.20	48%

after 36 h of germination in lentil (Ghavidel & Prakash, 2007), and a study by Vidal-Valverde et al. (2002) showed no significant variation in thiamine content when lentils, peas, and beans were germinated at 20 ◦C for between 2 and 4 days. Under the same germination conditions but for 6 days, Prodanov et al. (1997) reported a significant reduction (11% to 14%) in thiamine content in lentils. Thus, a short germination period may increase or at least maintain the thiamine content in lentils while thia-eq may decrease after 6 days of sprouting, as it is used by the plant for seed and seedling metabolism. In both the above-mentioned studies, the seeds were no longer in germination *sensu-stricto* but in the seedling growth stage.

In addition to the increase in thia-eq in lentils after soaking, the vitamer profiles changed during germination (Fig. 1). The changes in the vitamer profile followed the same trend regardless of the germination conditions. Fig. 1 shows the average of each vitamer in all 11 study conditions for 12 h and 24 h. In dry lentils, thia-HCL and TDP accounted for 86% and 11% of the total vitamers, respectively. Soaking and germination induced a higher increase in TDP content than thia-HCL. Soaking increased thia-HCL by 91% and TDP content by 321%. The increase in the percentage of TDP may be the result of the metabolic activation during germination and its production for use by the seedling. TDP is the active form of thiamine and is required for the performance of enzymes linked to carbohydrate metabolism. Temperature and water had a negative significant ($p < 0.01$) effect on thia-HCl content (Supplementary Table S3), whereas temperature had a positive significant ($p < 0.01$) effect on TDP.

Based on an amount of 932 µg/100 g (db), dry cowpea seeds were 6 times richer in total thia-eq than lentil seeds (Table 2). Other authors found lower thiamine content (144 µg/100 g, 640 µg/100 g and 870 µg/100 g) in cowpeas (Akinlosotu & Akinyele, 1991; Ghavidel & Prakash, 2007; Joy et al., 2022). This variation may be linked to the thiamine extraction method used, the variety of seed, and the timing of harvest.

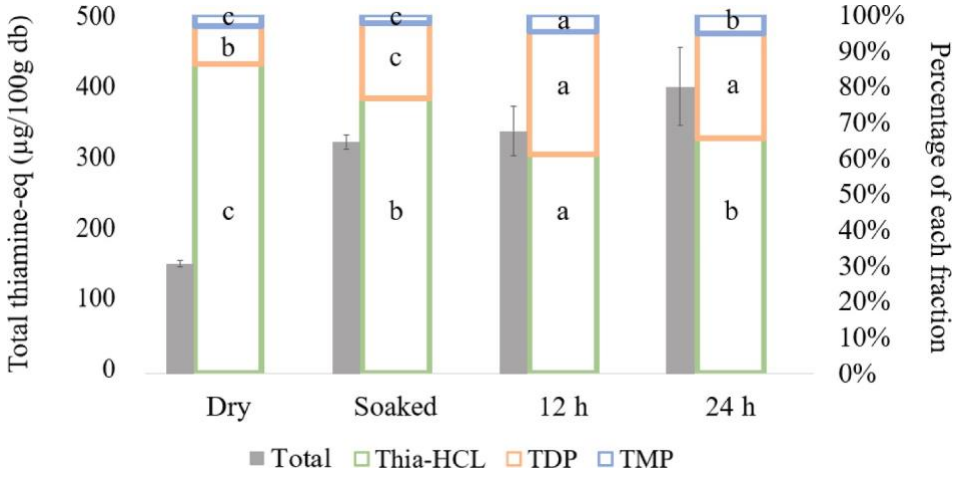

Fig. 1. Total thiamine-eq content (gray) in µg/100 g (db) and the percentage of each thiamine fraction, thiamine hydrochloride (Thia-HCl) (green), thiamine diphosphate (TDP) (orange), and thiamine monophosphate (TMP) (blue), in dry, soaked, germinating (12 h) and germinated (24 h) lentils. Letters indicate statistically (Tukey-HSD test) significant difference ($p < 0.05$) within each compound in comparison with each sample. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Average total concentration of thiamine equivalent, folate content in µg/100 g and total α-galactoside content in g/100 g on a dry basis for cowpea. The percentage increase in thiamine and folate and the percentage reduction in α-galactoside content is calculated between dry seeds and each condition.

Temperature (◦C)	Light (h)	Water Content (% db)	Time (h)	Total thia-eq in cowpea [µg/100 g db]	% increase	Total folate content in cowpea [µg/100 g db]	% increase	Total α-galactoside content in cowpea [g/100 g on db]	% reduction
Dry				932 ± 27		329 ± 14		7.41 ± 0.43	
Soaked				967 ± 7.1	4%	362 ± 13	10%	5.42 ± 0.07	27%
25	0	80	12	980 ± 9.6	5%	334 ± 8.0	2%	6.60 ± 0.85	11%
			24	964 ± 16	3%	345 ± 8.0	5%	5.69 ± 0.21	25%
35	0	80	12	976 ± 28	5%	359 ± 12	9%	4.61 ± 0.09	38%
			24	947 ± 33	2%	381 ± 5.2	16%	0.18 ± 0.01	98%
25	12	80	12	927 ± 35	−1%	328 ± 4.6	0%	6.05 ± 0.34	18%
			24	971 ± 6	4%	355 ± 3.5	8%	2.73 ± 0.04	63%
35	12	80	12	987 ± 20	6%	356 ± 8.4	8%	3.16 ± 0.27	57%
			24	941 ± 16	1%	394 ± 9.6	20%	0.12 ± 0.02	98%
25	0	120	12	980 ± 7.5	5%	335 ± 5.2	2%	6.29 ± 0.29	15%
			24	1023 ± 5.2	10%	383 ± 11	16%	0.69 ± 0.08	91%
35	0	120	12	985 ± 16	6%	368 ± 1.2	12%	2.72 ± 0.08	63%
			24	955 ± 2.9	2%	420 ± 6.3	28%	0.22 ± 0.02	97%
25	12	120	12	995 ± 5.9	7%	351 ± 6.2	7%	0.94 ± 0.22	87%
			24	989 ± 1.4	6%	393 ± 2.8	19%	0.57 ± 0.13	92%
32	12	120	12	975 ± 15	5%	366 ± 0.34	11%	2.57 ± 0.30	65%
			24	979 ± 19	5%	437 ± 16	33%	0.07 ± 0.002	99%
30	6	100	12	985 ± 10	6%	354 ± 6.9	8%	3.18 ± 0.14	57%
			24	973 ± 12	4%	426 ± 17	29%	0.12 ± 0.006	98%
30	6	100	12	999 ± 9.5	7%	361 ± 1.0	10%	3.33 ± 0.31	55%
			24	999 ± 22	7%	426 ± 6.3	29%	0.19 ± 0.03	98%
30	6	100	12	997 ± 32	7%	357 ± 2.5	9%	3.30 ± 0.24	56%
			24	1016 ± 6.1	9%	421 ± 12	28%	0.10 ± 0.01	99%

Soaking and germination had less effect on total thia-eq in cowpeas than in lentils. For instance, soaking only resulted in a 4% increase in total thia-eq in cowpeas and germination only to an increase of between 1% and 10%. Like in lentils, we found no significant impact ($p > 0.05$) of the factors we studied on the total thia-eq in germinating (12 h) and germinated (24 h) cowpea seeds. Ghavidel & Prakash (2007) reported similar results to ours: after 36 h, the thiamin content of germinated cowpea increased by 8% (Ghavidel & Prakash, 2007). As mentioned above, 36 h of germination in fact corresponds to sprouting and the seed is no longer in the germination *sensu-stricto*. However, Akinlosotu & Akinyele, (1991) reported different results: germinating cowpea at room temperature for 24 h reduced thiamine content by 6.9%, and increasing the germination time to 72 h reduced thiamin content to 51% (Akinlosotu & Akinyele, 1991). In the present study, TDP was the main vitamer found in cowpea and represented 89% of total vitamers. Unlike in lentils, in cowpeas, we found no significant difference ($p > 0.05$) in any vitamer percentage in any of the conditions analyzed.

3.2.2. *Folate content*

In dry lentils, the total folate concentration was 147 µg/100 g (db) (Table 1). In the literature, a value of 244 µg/100 g of fresh weight was reported for brown lentil (Sallam et al., 2021). In our study, folate content decreased by 17% after soaking and remained in the same range after partial germination at 12 h. However, after 24 h germination, the total folate content increased back to the original folate content of dry lentils whatever the germination conditions. This shows that in the first hours of germination, lentil seeds used the folate for their metabolism. With a longer germination period, the seeds produce folate to prepare for the next germination stage, which is the seedling. The factors (temperature, light, water content) we studied had no significant impact

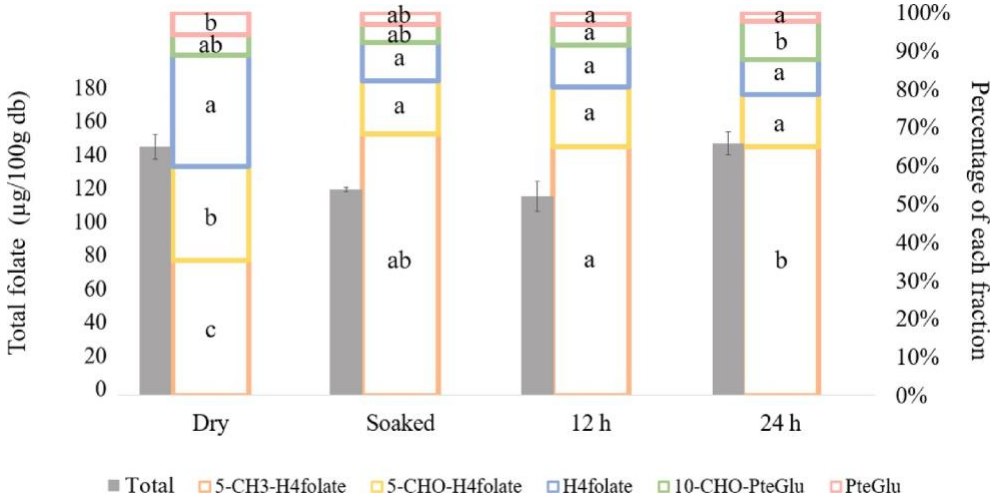

Fig. 2. Total folate content (gray) in µg/100 g (db) and the percentage of each folate fraction, 5-methyl-tetrahydrofolate (5-CH3-H4folate) (orange), 5-formyl-tetrahydrofolate (5-CHO-H4folate) (yellow), tetrahydrofolate (H4folate) (blue), 10-formylfolic acid (10-CHO-PteGlu) (green), and folic acid (PteGlu) (red), in dry, soaked, germinating (12 h) and germinated (24 h) lentils. Letters indicate statistically (Tukey-HSD test) significant difference ($p < 0.05$) within each compound in comparison with each sample. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

($p > 0.05$) on the total folate content in either germinating and germinated lentil seeds.

The vitamin profile of dry lentil seed was diverse: 5-CH3-H4folate was the main vitamer present accounting for 35% of the total content, followed by H4folate (29%), 5-CHO-H4 folate (24%), PteGlu (6%), and 10-CHO-PteGlu (5%) (Fig. 2). However, Sallam et al. (2021) identified 10-CHO-PteGlu as the main folate form in lentil, accounting for 27% of total folate content. The changes in vitamer profiles followed the same trend, irrespective of the germination conditions. Since the factors we studied had the same impact on the total folate content, Fig. 2 shows the average of each vitamer in all 11 study conditions at 12 h and at 24 h. During soaking, the 5-CH3-H4folate content increased by 60% and 5-CHO-H4folate and H4folate content decreased by 54% and 71%, respectively. The changes in vitamer profiles are given by the conversion of 5-CHO-H4folate and H4folate into 5-CH3-H4folate to be used for subsequent seedling metabolism (Coffigniez et al., 2021). In all germination conditions, PteGlu and 10-CHO-PteGlu accounted for a minimum percentage of the total folate content ranging from 2% to 9%. 5-CH3-H4folate was the main vitamer found in lentils, and so the impact of germination on its content was analyzed. Results of ANOVA showed a positive significant ($0.05 < p < 0.1$) impact of temperature on the concentration of 5-CH3-H4folate when the lentils were germinated for 24 h. Sallam et al. (2021) reported that when brown lentils were germinated at 26 °C (during the day) and 21 °C (at night) the maximum folate content (a three-fold increase) was reached on the fifth day. Like in the present study, the vitamer most present in germinated seeds was 5-CH3-H4folate (Sallam et al., 2021).

In contrast to what happens to thiamine metabolism, soaking and germination had a more positive effect in cowpeas than in lentils; germination indeed increased total folate content and changed the vitamer profile in cowpea seeds. Cowpea was twice as rich in folate as lentil, with a concentration of 329 µg/100 g (db) (Table 2). Coffigniez et al. (2021) found total folate contents of 420 µg/100 g in brown cowpea, and a similar concentration (329 µg/100 g, fresh weight) was found by Sallam et al. (2021) in the same variety. In cowpea, soaking increased the total folate content by 10% and germination by up to 33%. Between germinating and germinated seeds, the total folate content increased by 3% to 20%. Temperature was the only factor that had a significant ($p < 0.05$) positive effect on total folate content in germinating cowpea (12 h) (Supplementary Table S4).

In dry cowpea seeds, the 5-CHO-H4folate was the main vitamer present, accounting for 44% of the total content, followed by 10-CHO-PteGlu (23%), PteGlu (15%), H4folate (10%), and 5-CH3-H4folate (8%) (Supplementary Figure S1). Coffigniez et al. (2021) showed a folate profile close to that found in our study: 5-CHO-H4folate was still the main folate found with a percentage of 54%, but 5-CH3-H4folate, PteGu, 10-CHO-PteGlu, and H4folate represented 11%, 16%, 16%, and 3%, respectively (Coffigniez et al., 2021). However, Sallam et al. (2021) found 5-CH3-H4folate (30%) to be the main form of folate in cowpea. These different results indicate that folate vitamers can differ depending on both genetic and environmental conditions.

The changes in vitamer profile followed the same trend independently of the germination conditions. Soaking significantly affected the vitamin profile in cowpeas (Supplementary Figure S1). To trigger germination, seeds produce 5-CH3-H4folate, mostly from 5-CHO-H4folate, to use for their metabolism (Coffigniez et al., 2021). Between dry and soaked cowpea seeds, the 5-CH3-H4folate content increased by 731% whereas 5-CHO-H4folate content decreased by 77%. The total folate concentration and the vitamer profile did not differ significantly ($p > 0.05$) between soaked and germinated seeds. Temperature was the only factor that had a significant ($p < 0.05$) positive impact on the concentration of 5-CH3-H4folate (Supplementary Table S4). The sum of PteGlu, H4folate, and 10-CHO-PteGlu accounted for<20% of total folates in germinated cowpea. Coffigniez et al. (2021) reported that germinated cowpea seeds at 30 °C for 48 h did not significantly increase the total folate content. However, the folate content doubled between 48 h and 96 h of germination (Coffigniez et al., 2021). Germinated cowpeas at 26 °C (during the day) and 21 °C (at night) induced a 2.8 fold increase in total folate content after 5 days (Sallam et al., 2021). Like in this present study, the main vitamer that accumulated during germination was 5-CH3-H4folate, which reached maximum (490.4 µg/100 g) on the fifth day (Coffigniez et al., 2021; Sallam et al., 2021). The difference in results is due to the duration of germination. As already mentioned, 48 h and 5 days of germination correspond to sprouting and the seed is no longer in the germination *sensu-stricto*.

3.2.3. Alpha-galactoside content

Germination considerably reduced total α-galactoside content in both lentil and cowpea seeds. The α-galactoside content of dry lentils was 5.56 g/100 g (db) (Table 1). This value is higher than that reported in the literature, which is between 2.72 g/100 g (db) and 2.96 g/100 g (db) (Abdel-Gawad, 1993; Njoumi et al., 2019; Vidal-Valverde et al., 2002). In the present study, soaking lentils for 3 h at 30 °C reduced α-galactoside content by 16%. Independently of the germination time and condition, there was always a decrease in α-galactoside content in lentils. For instance, the total α-galactoside content decreased by up to 29.5% and 47.5% after 12 h and 24 h of germination on lentil seeds. The ANOVA showed that the interaction of light and water had a significant ($p < 0.05$) negative effect on the total α-galactoside content when lentils were germinating for 12 h; α-galactoside decreased under light and with high water content. α-galactoside content decreased after germination for 24 h, but no significant impact of parameters was found ($p > 0.05$). Vidal-Valverde et al. (2002) reported that germinating lentils at 20 °C reduced the total α-galactoside content by 90% after 2 days, but no effect of light was evidenced during germination.

In the present study, the total α-galactoside content in cowpea seeds was 7.41 g/100 g (db) (Table 2). In the literature, α-galactoside content in cowpea ranged between 2.56 and 4.66 g/100 g (db) (Akisso´e et al., 2021; Coffigniez et al., 2018; Ibrahim et al., 2002; Nnanna & Phillips, 1988). However, Akinlosotu & Akinyele, (1991) found a total α-galactoside content of 9.54 g/100 g, which is in agreement with our findings. Soaking cowpea seeds for 5 h reduced α-galactoside content by 27%. Germination had a more positive effect in cowpeas than in lentils, with an up to 99% reduction in the total α-galactoside content. Between 12 h and 24 h of germination, there was a decrease of up to 97% in α-galactoside. These results show germination is an effective way to reduce α-galactoside content in pulses, with evidence that the longer the process, the greater the reduction. Ibrahim et al. (2002) showed that germinated cowpea seeds at room temperature for 24 h reduced total stachyose and raffinose contents by 100%, which is in agreement with our results. However, other authors obtained different results: for instance, a 25% reduction in total α-galactoside content after 24 h of germination at room temperature (Akinlosotu & Akinyele, 1991). However, when cowpea seeds were germinated for 48 h, the authors reported similar results to ours (-92%), (Akinlosotu & Akinyele, 1991). The difference in the results is due to the different germination methodology and temperature conditions used. The results of ANOVA showed that temperature, light, water, and their interaction had a significative negative impact ($p < 0.05$) on the total α-galactoside content in germinating cowpea (12 h). These results indicate that increasing the values of the factors studied reduced the total α-galactoside content. The temperature had a significant ($p < 0.05$) negative impact on the total α-galactoside content in cowpea germinated for 24 h. We can thus conclude that the conditions used in the present study favor the reduction of α-galactoside in cowpea, and higher temperatures should be preferred.

The α-galactoside profiles of dry, soaked, germinating (12 h) and germinated (24 h) lentil and cowpea seeds were homogenous (Fig. 3). Stachyose was the main α-galactoside identified accounting for>80% of the total concentration. The α-galactoside profile followed the same trend, independently of the germination conditions. Fig. 3 shows the

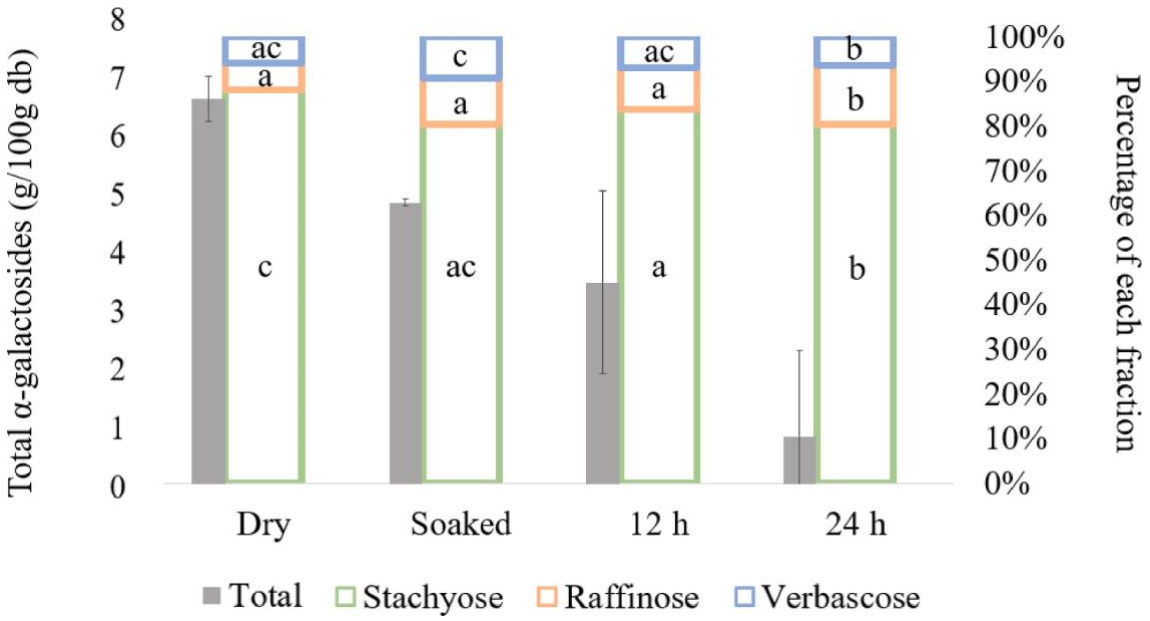

Fig. 3. Total α-galactoside content (gray) in g/100 g (db) and the percentage of each fraction, stachyose (green), raffinose (orange), and verbascose (blue), in dry, soaked, germinating (12 h) and germinated (24 h) cowpea seeds. Letters indicate statistically (Tukey-HSD test) significant difference ($p < 0.05$) within each compound in comparison with each sample. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

average of each vitamer at 12 h and 24 h of germination in all 11 conditions studied. In the literature, stachyose was also the main α-galactoside found in lentil and cowpea seeds (Akissoe´ et al., 2021; Coffigniez et al., 2018; Ibrahim et al., 2002; Njoumi et al., 2019; Vidal-Valverde et al., 2002).

3.3. Optimal germination process

Using the RSM models, it was possible to optimize the parameters during germination in lentil and cowpea seeds. For this purpose, the principal vitamer and α-galactoside was used for each seed variety. By maximizing thiamine (Thia-HCL) and reducing stachyose content at 24 h of germination, the optimal germination condition for lentil was shown to be 25 ◦C, without light, and with 80% water content. In these conditions, the thiamine, folate, and α-galactoside contents were 388 µg/100 g (db), 157 µg/100 g (db), and 2.97 g/100 g (db), respectively (Table 3). Thiamine and folate contents increased by 152% and 6%, respectively, while total α-galactoside content decreased by 47%. The recommended daily intakes (RDI) of thiamine and folate are 1100–1500 µg/day and 200–400 µg/day for adult, respectively (Chunming et al., 2001; Krawinkel et al., 2014; Moll & Davis, 2017). In the above-mentioned conditions, consuming 100 g of germinated lentils will cover 26% and 39% of the RDI of thiamine and folate, respectively. In addition, the low water content may help reduce microbiological risk during germination. By maximizing the 5-CH3-H4folate and reducing the total α-galactoside content at 24 h of germination, the optimal germination condition for cowpeas was shown to be at 35 ◦C, with 12 h of light, and 140% water content. In these conditions, the thiamine, folate, and α-galactoside contents were respectively, 979 µg/100 g (db), 437 µg/100 g (db), and 0.07 g/100 g (db) (Table 3). Between dry and germinated seeds, the thiamine and folate content increased by 5% and 33%, respectively, and the total α-galactoside decreased by 99%. Under these germination conditions, eating 100 g of germinated cowpea seeds will cover 65% and 109% of the RDI of thiamine and folate, respectively.

In our study, we optimized the germination *sensu-stricto* to increase the nutritional quality of lentil and cowpea. Germination *sensu-stricto* has an advantage because it requires less time (24 h), than sprouting (2 to 6 days). We suggest that reducing germination time increases vitamin content and reduces microbiological risks. For instance, sprouts are highly perishable and harbor bacterial pathogens such as *Salmonella* and *Escherichia coli* (Miyahira & Antunes, 2021). It is known that cell populations of these bacteria increase with an increase in the length of the germination period (Liu et al., 2018). To confirm this hypothesis and the safety of germination, microbiological analyzes should now be undertaken. Seeds that undergo germination *sensu-stricto* must undergo thermal treatment before being edible. We suggest that cooking also helps reduce the microbiological risk of germinated seeds, in comparison to sprouting. Moreover, adding germination as a pre-treatment reduces the negative impact of thermal treatment. For instance, folate content is 52% higher in germinated-canned fava beans than conventional canned

Table 3
Final thiamine-equivalent, total folate, and total α-galactoside contents in lentil and cowpea after soaking and optimized germination.

	Lentil				***Cowpea***			
	Temperature, light, water content, duration	Thiamine-eq µg/100 g	Folate µg/ 100 g	α-galactoside g/ 100 g	Temperature, light, water content, duration	Thiamine-eq µg/100 g	Folate µg/ 100 g	α-galactoside g/ 100 g
Soaking	30 ◦C, 0 h, 3 h	322 (+109%)	121 (—17%)	4.65 (—16%)	30 ◦C, 0 h, 5 h	967 (+4%)	362 (+10%)	5.42 (—27%)
Germination	25 ◦C, 0 h, 80%, 24 h	388 (+152%)	157 (+6%)	2.97 (—47%)	35 ◦C, 12 h, 140%, 24 h	979 (+5%)	437 (+33%)	0.07 (—99%)
RDI		1100 – 1500 µg/100 g/day	200–400 µg/ 100 g/day			1100 – 1500 µg/100 g/day	200–400 µg/ 100 g/day	

RDI = Recommended daily intake.

(Hefni et al., 2015). The results of our study could be used to model the nutritional behavior of lentil and cowpea seeds during food processing. The impact of other kinds of food processing, such as soaking/cooking/autoclaving, should also be modeled. A first model to predict folate and α-galactoside activity during soaking-sprouting-cooking in cowpea seeds was recently published (Coffigniez & Briffaz, 2023), with sprouting at 30 °C for 72–92 h, followed by cooking (95 °C or 100 °C) for<30 min. This type of model will advance our understanding and check the effects and benefits of germination *sensu-stricto* as a pre-treatment before processing of pulses into food.

4. Conclusion

Our study was the first to simultaneously analyze the impact of temperature, light, and water on the nutritional quality of lentil and cowpea. Temperature and water showed a more significant impact on the nutritional quality when compared to light. This finding could provide valuable insights for researches to consider including this parameter as a critical factor in their germination analyses. The use of RSM models allowed for the optimization of the technological parameters. For lentils, low temperature, no light, and low water content should be used. On the other hand, cowpea germination should be at higher temperature, light, and water content. Optimized germination increased thiamine content by 152% in lentils and by 5% in cowpeas. In addition to increasing the total thiamine content, germination changed its vitamer profile in lentil by increasing TDP through a metabolic shift. Germination increased folate content by up to 6% and 33% between dry and germinated lentil and cowpea seeds, respectively. The 5-CH3-H4folate content increased in both seeds during germination. This vitamer is the main form of folate found in germinated lentil and cowpea and is produced for seed metabolism. The α-galactoside content decreased by up to 47% in germinated lentil seeds and by 99% in germinated cowpea seeds. In both seeds, stachyose was the main form of α-galactoside found at all the germination times and conditions. To further advance our understanding of germination, we now need to analyze the metabolomics changes that take place during germination to identify the different metabolites used by the seed to produce nutrients. This will provide more accurate information about the different chemical pathways used during germination. This information can be used to improve the nutritional quality of pulses, even by industrial food companies as a novel strategy to process pulses.

CRediT authorship contribution statement

Luiza Avezum: Writing – original draft, Methodology, Formal analysis. **Yann E. Madode:** Methodology, Writing – review & editing. **Christian Mestres:** Writing – review & editing, Conceptualization. **Nawel Achir:** Writing – review & editing, Formal analysis. **Charlotte Delpech:** Methodology, Resources. **Morgane Chapron:** Methodology, Resources. **Olivier Gibert:** Conceptualization, Writing – review & editing. **Loïc Rajjou:** Writing – review & editing, Conceptualization, Funding acquisition, Supervision. **Eric Rondet:** Writing – review & editing, Supervision, Conceptualization, Funding acquisition.

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

No data was used for the research described in the article.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.138027.

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