Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

The variety, growing location, and year of harvest influence chickpea content in fat-soluble micronutrients and antinutritional compounds, with no foreseeable impact on the bioavailability of fat-soluble micronutrients

Angélique Berthomé
Aix-Marseille University, INRAE, INSERM, C2VN
Stéphane Georgé
CTCPA – Technical Center Food
Quentin Lambert
Terres Inovia
Yan Laberdesque
Terres Inovia
Emmanuelle Reboul
Emmanuelle.Reboul@inrae.fr

Aix-Marseille University, INRAE, INSERM, C2VN

Article

Keywords: Fat-soluble vitamins, Carotenoids, Antinutrients, Bioaccessibility, Chickpea

Posted Date: July 9th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10081049/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Chickpea content in antinutritional compounds can negatively affect fat-soluble micronutrient (FSM) bioaccessibility, whether the FSM come from the chickpeas themselves or from the meal containing the chickpeas. In this study, FSM and antinutrient contents of two chickpea varieties, cultivated in two French regions over two consecutive years, were characterised, prior and following a cooking process. Effects on FSM bioaccessibility were then assessed using an *in vitro* digestion model. Our data show that agronomic factors (variety, growing location and year of harvest) impacted both FSM and antinutrients contents, leading to distinct chickpea profiles. The cooking process further modified these profiles and led to differences in test meal initial γ-tocopherol, zeaxanthin and α-tocopherol amounts. FSM bioacessibility was neither explained by agronomic factors nor by chickpea content in antinutrients, which could be attributed to insufficient distinction in antinutrient content between the chickpea batches. Although selecting specific conditions for a chosen chickpea variety can help modulating its saponin and tannin contents, this will likely be insufficient to significantly improve FSM bioaccessibility.

Introduction

In the context of global climate change and the urgent shift towards sustainable diets, the cultivation and consumption of protein-rich pulse seeds as an alternative to meat are critical. Chickpea (C*icer arietinum* L.), as other seed pulses, is able to fix atmospheric nitrogen through symbiosis with *Rhizobium* species, preventing excessive nitrogen fertilizer use (Nour et al., 1994; Udvardi and Poole, 2013). This crop is also well adapted to dry climate such as those of Mediterranean areas, due to its drought and water stress tolerance (Karalija et al., 2022). It also provides about 7 g of proteins per 100-g portion of cooked chickpeas (Margier et al., 2018).

All higher plants synthesise secondary metabolites to handle biotic and abiotic stress (Kumar et al., 2020; Chaturvedi et al., 2024). These metabolites can also be found in their seeds (Smolikova and Medvedev, 2015). Among the small molecules produced, tocopherols (vitamin E) and carotenoids display beneficial properties for humans when consumed. The beneficial traits of these fat-soluble micronutrients (FSM) owe to their antioxidant activity (Jukanti et al., 2012), their ability to modulate gene expression (Landes et al., 2003; Landrier et al., 2009; Landrier et al., 2010; Bohn, 2019), and to the provitamin-A activity of some carotenoids (Tang, 2010). Among carotenoids, both carotenes (α- and β-carotene) and their oxygenated derivatives xanthophylls (lutein and zeaxanthin) are found in chickpeas. Tocopherols, synthetised from the same metabolic precursor as carotenoids, can also be found in this seed in α- and γ- forms ,and to a lesser extent in δ- and β- forms (Padhi et al., 2017; Rodriguez-Concepcion et al., 2018). Conversely, no or limited amounts of tocotrienols are found in chickpea and legume seeds in general (Fernández-Marín et al., 2017). Although chickpea is not the richest food item in these molecules, it can contribute to their intake as part of a diversified diet (Jukanti et al., 2012).

However, the possibility to take advantage of the seed beneficial micronutrients is limited by the presence of some other secondary metabolites and storage molecules also present in the seeds, such

as tannins, saponins and phytates. Also called “antinutrients”, these molecules have been described to interfere with mineral and protein digestion (Ghavidel and Prakash, 2007), thus affecting their bioaccessibility (i.e., the fraction of a molecule released from the food matrix after ingestion and accessible for absorption). More recently, negative association between pulse tannins, saponins and phytates and FSM bioaccessibility has also been observed (Margier et al., 2019; Antoine et al., 2021; Antoine et al., 2023). This detrimental effect is mainly due to the ability of these compounds to chelate either directly minerals, thus rendered inaccessible for absorption, or to interact with digestive enzymes (Samtiya et al., 2020), hindering nutrient or FSM digestion process.

The chickpea seed ability to synthesize secondary metabolites is driven by several parameters including the genetic background of a specific chickpea variety, as well as environmental factors such as temperature, rainfall or soil type (Nikolopoulou et al., 2006; Ashokkumar et al., 2014; Saini and Keum, 2018). Processing of the seeds, for instance at home by soaking followed by cooking, enables softening and better digestibility, decreases antinutrient contents, but also modifies the seed composition in FSM (Ruiz et al., 1996; El-Adawy, 2002). However, there is a lack of study addressing specifically whether the processing step preserves the seed composition profile.

The objectives of this study were first to characterise the FSM and antinutrient content of chickpea seed samples of two varieties grown in two French regions over two consecutive years, before and after a soaking/cooking process, and then to evaluate the impact of these factors (i.e., variety, growing location and year) on FSM bioaccessibility after chickpea incorporation into a FSM-rich test meal.

Material and Methods

Material

Raw seed samples of 2 chickpea varieties (Flamenco and CDC Orion), each cultivated in 2 French regions (Vendée and Puy de Dôme) over two consecutive years (2023 and 2024), were generously provided by Terres Inovia. A 20-g-aliquot of raw seeds was freeze-dried over 48 h (Christ Alpha Mannifold Freeze-dryer, Fischer Scientific, Illkirch, France) prior to being milled for 5 min in a mortar grinder to obtain a fine flour (Pulverisette 2, Frisch, Idar-Oberstein, Germany). Another 30-g aliquot was processed as previously described by Margier et al., 2018, with the following adaptations. Seeds were soaked 12 h in distilled water (1:5; seed:water; w:w), at room temperature. They were drained and subsequently cooked covered 2 h in mineral water (1:4; seed:water; w:w) at low heat. After drainage, seeds were cooled and kept in the fridge, before being either use within a week or freeze-dried and ground 15 s to flour using the mortar grinder. These two flour aliquots are referred to as “raw” and “cooked” chickpea flour, respectively.

Except from the lycopene taken from Extrasynthèse (Genay, France), all other chemicals were purchased from Sigma Aldrich (Saint-Quentin-Fallavier, France) and solvents from Carlo Erba reagents (Peypin,

France). Soyasaponin I was kindly provided by CTCPA. Canned tomato pulp, red bell pepper and Isio4 oil were purchased from a local supermarket (Intermarché, Marseille, France).

In vitro digestion

Static *in vitro* digestion was based on previously published model (Malapert et al., 2018; Alvarado-Ramos et al., 2024). The FSM-rich recipe used as a test meal was made of 3 g cooked chickpeas, 1.5 g cooked red bell peppers, 1.5 g canned tomato pulp and 100 mg oil enriched with 1 µg/g cholecalciferol. The buccal phase was simulated by a mechanical grinding of the recipe in 16 mL NaCl 0.9%, 1 min at 10 000 rpm (Ultra-Turrax dispenser, IKA, Staufen, Germany) and an incubation of the mixture with 1.25 mL artificial saliva ($NaHCO_3$ 62 mM, NaCl 15 mM, KCl 6.4 mM, $CaCl_2.2H_2O$ 3 mM, K_2HPO_4 6 mM, 2.16 mg mucin /mL, 13 mg α-amylase /mL, pH7), 10 min at 37°C, 190 rpm in a water bath. This phase was followed by stomachal and duodenal phases, for which the sample pH was adjusted to 4 with HCl 1M and 7 with $NaHCO_3$ 0.9M, respectively. These two phases were independently mimicked by the addition of 1 mL pepsin (40 g/L HCl 0.1M) for stomachal and a mix of 4.5 mL pancreatin (3 g/L) and 2 mL bile (12 g/L $Na_3C_6H_5O_7$ 0.1M) for duodenal phase, with incubations of 30 min at 37°C, 190 rpm. At the end of the digestion, an aliquot was collected and the rest of the digesta was centrifugated 1.2 h at 10°C, 1962 g. The supernatant was passed through 0.8 µm and 0.2 µm pore-size filters. Both digesta aliquot and filtrate, corresponding to the micellar phase, were kept at -80°C before further analysis.

Saponin assay

100 mg raw or cooked chickpea flour was combined with 1 mL MeOH 80%. The mixture was agitated during 24 h and saponins were recovered in the supernatant after a centrifugation 10 min at 4°C, 4415 *g*. This step was repeated over 3 days and supernatants were pooled. Then, saponins contained in 6.6 µL supernatant, diluted with 10 µL MeOH, were oxidised during 10 min at 60°C with 16.6 µL ethanolic vanillin 8% under acidic condition obtained with 166.6 µL sulfuric acid 12M. The reaction resulted in a green coloration and the absorbance at 544 nm was measured on a plate reader (Ensight, PerkinElmer, Villebon-sur-Yvette, France) and compared to a calibration curve of soyasaponin I.

Tannin assay

The tannin content was assayed from 120 mg raw or cooked chickpea flour. Tannins were extracted by mixing the flour 30 min with 12 mL distilled water and then recovered in the supernatant after a centrifugation 10 min at 4°C and 4415 *g*. Then, 114 µL of the supernatant, diluted with 57 µL MeOH 50%, were mixed with 29 µL 4-dimethyl amino cinnamaldehyde solution (720 µg/mL 1:5 MeOH/HCl 6M), 20 min, at room temperature. At the end of the reaction, the absorbance was measured at 644 nm and tannin content was calculated according to the absorbance of a catechin calibration curve.

Phytate assay

To extract phytates from raw or cooked chickpea flour, 100 mg was mixed with 5 mL HCl 0.5M during 1 h at room temperature. The solvent containing the extracted phytates was recovered after a centrifugation 10 min at 4°C, 4415 *g*. 30 µL of the supernatant, diluted 10 times, was incubated with 600 µL thiocyanate-iron complex solution ($FeCl_3$ 75 µg/mL, NH_4SCN 37.5 mg/mL, nitric acid 0.2%) during 2.5 h at 40°C. Once cooled, the mix was centrifugated 5 min at 4°C, 4415 *g* and the supernatant absorbance was measured at 480 nm. The value was compared to a calibration curve obtained with a phytic acid standard.

Fat-soluble micronutrient analyses

FSM extraction and quantification were adapted from previously published methods (Margier et al., 2018; Alvarado-Ramos et al., 2024).

FSM were extracted from either 500 µL micellar phase or digesta or 250 mg raw or cooked chickpea flour dissolved in 1 mL distilled water. The aqueous phase was combined with 500 µL ethanolic solution containing an internal standard, either retinyl acetate for tocopherol and carotenoid quantification or deuterated cholecalciferol for that of vitamin D (quantified only in *in vitro* digestion fluids). FSM were recovered in 2 mL hexane after a 10 min agitation followed by a centrifugation 10 min at 4°C, 1363 *g*. This step was repeated twice and supernatants were pooled and dried under nitrogen. Dried extracts for carotenoid and tocopherol quantification were directly resuspended in 200 µL MeOH/DCM (65/25; v/v). Extracts for cholecalciferol quantification were submitted to a derivatisation step, consisting of two 10-min agitations with 50 µL 4-Phenyl-1,2,4-triazole-3,5-dione solution (4 mg/mL acetonitrile) and one of 5 min with 20 µL distilled water. Once dried, this extract was resuspended in 100 µL acetonitrile.

Carotenoid and tocopherol separation and quantification were carried out on a Thermo Scientific Dionex Ultimate 3000 HPLC system. The device was equipped with a guard and YMC-Pack C30 columns (250 x 4,6 mm; 5 µm) thermostatted at 40°C, a Dionex Ultimate DAD-3000 detector and linked to Chromeleon 7.2. Compounds were separated in the column with a MeOH/MTBE/H_2O gradient (1 mL/min; 32 min). Tocopherols, retinyl acetate and carotenoids were detected at UV-VIS wavelengths of 290, 325 and 450 nm respectively, except for lycopene detected at 472 nm. They were subsequently identified and quantified at their respective absorbance wavelength based on their retention time and peak areas compared to a pure standard of these FSM and the recovery of the internal standard. Cholecalciferol separation and quantification was achieved by LC-$HRMS^2$ with instrumentation and chromatography conditions described by Bonnet et al., 2019.

Statistical analysis

Theoretical FSM initial amount in the recipe was calculated as the sum of the FSM content of each ingredient. Digesta and micelle FSM content was calculated as the total FSM amount found in the respective digestion fluid. FSM bioaccessibility was calculated as the amount of FSM quantified in the micellar phase over that in the digesta, expressed as a percentage.

Unless stated, the results are expressed as mean ± SEM of 4 technical replicates. Statistical analyses were performed on GraphPad Prism 10.4.2. Three-way ANOVAs were applied for antinutrient and FSM contents, on data passing ($p > 0.05$) normality of residuals with Shapiro-Wilk's test and heteroscedasticity with Spearman's test. If the later was not passed ($p < 0.05$), three-way ANOVA was performed on log transformed data. Pearson coefficient was used for correlation assessment. For *in vitro* digestion results, Kruskal-Wallis tests were applied with Dunn's multiple comparison test. Principal Component Analyses were performed using the function PCAshiny of Factoshiny package on RStudio.

Results

Variations in weather conditions

For both growing locations, 2023 was characterised by lower total rainfall compared to 2024, accompanied with lower humidity, whereas solarization and temperature amplitude were higher than in 2024. Each year, Puy de Dôme cumulated less rainfall and was on average more humid than Vendée, as well as more solarized. Puy de Dôme was also each year in average warmer with higher temperature amplitude as depicted by an average minimal temperature lower than Vendée (Table 1).

Table 1

Year differentiations in temperature, rainfall, humidity and solarization for the growing locations Data were obtained from meteo.data.gouv.fr French monthly basic climatological database. For Puy de Dôme and Vendée, Clermont-Ferrand (registered site n°63113001) and La Roche-sur-Yon (registered site n°85191003) were respectively chosen as the closest (< 50 km) positions providing all information. For total rainfall and solarization, data are the sum of cumulative measures over 12 months. Temperatures and humidity values are presented as yearly averages calculated from monthly averages.

	2023		**2024**	
	Puy de Dôme	**Vendée**	**Puy de Dôme**	**Vendée**
Total rainfall (mm)	514.6	943.0	715.2	1037.1
Average maximal temperature (°C)	19.3	18.3	18.2	17.2
Average minimal temperature (°C)	8.7	9.4	8.4	9.0
Average temperature amplitude (°C)	10.6	9.0	9.7	8.1
Average humidity (%)	67.7	79.1	74.2	84.4
Total solarization (h)	2091.7	2046.6	1841.1	1752.8

Effect of variety, year, location and cooking on chickpea FSM profile

All carotenoid contents of raw chickpeas were markedly impacted by the year of cultivation (Table 2).

Table 2

Proportion of the total variation in chickpea compound content explained by the factors and their interactions in raw chickpea flour Three-way ANOVA results expressed as the proportion (%) of the total variation in chickpea compound content (mg or µg/100g raw DM) explained by the factors Variety (V), Year (Y), Growing location (G) and their interactions. Asterisks or the mention "ns" indicate respectively a significant or insignificant impact of the column title source of variation (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001). $ indicates that the data for the compound were log transformed.

Compound	Variety	Year	Growing Location	V x Y	V x G	Y x G	V x Y x G
Saponins	40.75 ****	20.42 ****	11.25 **	ns	ns	ns	ns
Tannins	32.76 ****	ns	34.46 ****	ns	ns	ns	ns
Phytates	ns	ns	ns	ns	ns	ns	ns
Lutein$	12.71 ***	35.01 ****	2.858 *	ns	3.971 *	36.53 ****	ns
Zeaxanthin$	8.920 ***	39.55 ****	2.606 *	ns	4.678 *	34.20 ****	ns
α-carotene	ns	45.30 ****	ns	ns	ns	10.19 **	ns
β-carotene$	5.699 **	26.95 ****	41.34 ****	ns	8.861 ***	ns	5.155 **
α-tocopherol$	ns	29.29 ****	24.14 ***	ns	ns	10.69 **	ns
γ-tocopherol$	8.170 *	27.90 ***	ns	ns	ns	8.626 *	ns

Both xanthophyll (i.e., lutein and zeaxanthin) contents varied similarly among chickpea samples, and interactions between variety or year with growing location were observed (Table 2). The highest amounts were found in chickpeas cultivated in Puy de Dôme in 2023, while lowest amounts were measured in chickpeas cultivated in the same region in 2024 (up to 255.6 ± 37.5 and 48.7 ± 6.1 µg/100g DM and down to 59.0 ± 4.3 and 13.4 ± 1.0 µg/100g DM for lutein and zeaxanthin respectively; Fig. 1A-B). The same trends remained after processing, although the content was increased on the dry matter basis (up to + 256% for lutein and + 335% for zeaxanthin; Fig. 1G-H). The contents in these two carotenoids were highly correlated in raw material ($p < 0.0001$; Fig. 3A and 3B) and this remained similar in processed material ($r = 0.804$, $p = 0.016$ and $r = 0.781$, $p = 0.022$ for lutein and zeaxanthin, respectively; Table 3). In raw chickpeas, these two xanthophylls were negatively associated with saponin content ($p < 0.05$; Fig. 3A), but once processed, these correlations were no longer observed (Fig. 3B).

Table 3
Antinutrient and FSM profile conservation after chickpea processing

	Compound	Pearson r	p-value
Antinutrients	Saponins	0.471	0.239
	Tannins	-0.655	0.078
	Phytates	0.295	0.478
Xanthophylls	Lutein	0.804	0.016 *
	Zeaxanthin	0.781	0.022 *
Carotenes	α-carotene	0.235	0.576
	β-carotene	0.786	0.021 *
Vitamin E	α-tocopherol	0.631	0.093
	γ-tocopherol	0.923	0.001 **

Pearson correlation coefficients (r) between raw (n = 8) and cooked (n = 8) antinutrient and FSM chickpea content on DM, and associated p-value. Asterisks indicate respectively a significant correlation (*p < 0.05; **p < 0.01).

Unlike xanthophylls, which exhibited between one another consistent and correlated patterns in both raw and cooked samples, α- and β-carotenes did not demonstrate such a relationship. Specifically, in both raw and cooked materials, there was a lack of significant correlation between these two carotenes (Fig. 3A-B). In fact, the variation in raw chickpeas was differently explained by the agronomic factors and their interactions, β-carotene being sensitive to most parameters compared to α-carotene mostly impacted by the year (Table 2). The highest α-carotene content was found in the chickpea CDC Orion cultivated in Vendée in 2023 (17.0 ± 0.8 µg/100g DM; Fig. 1C), whilst chickpea containing the highest content of β-carotene was the same variety cultivated in Puy de Dôme in 2024 (47.8 ± 2.4 µg/100g DM; Fig. 1D). On the other hand, for a same variety cultivated the same year, most of those grown in Vendée displayed lower amounts of both α- and β-carotenes compared to their variety-counteracts cultivated in Puy de Dôme (Fig. 1C-D). Except in few samples, processing induced a decrease (up to -63%) in α- and β-carotene contents (Fig. 1I-J). Whilst α-carotene profile was not conserved after processing, β-carotene profile remained similar (r = 0.786, p = 0.021; Table 3). Remarkably, negative correlations with antinutrients between α-carotene content before and after processing were observed (Fig. 3A-B). Conversely to α-carotene, β-carotene was positively correlated to tocopherols (Fig. 3A-B).

γ-tocopherol was the most abundant fat-soluble micronutrient in chickpeas, with levels ranging from 2.5 ± 0.3 to 7.1 ± 0.9 mg/100g DM, followed by α-tocopherol with values ranging from 0.2 ± 0.0 to 0.9 ± 0.1 mg/100g DM (Fig. 1E-F). Besides the impact of the year of culture itself, which accounted for most of the variation in raw chickpea tocopherol contents, an interaction year/growing location also explained a part of the differences for both α- and γ- forms (Table 2). In 2023, the amounts of the two forms were

clearly higher in varieties cultivated in Puy de Dôme compared to those cultivated in Vendée, conversely to 2024 where the difference was less pronounced for α-tocopherol and in the other way for γ-tocopherol (Fig. 1E-F). After processing, the dry matter content increased from 116 to 689% for α-tocopherol and 71 to 248% for γ-tocopherol (Fig. 1K-L). The profile in α-tocopherol tended to be conserved after the processing ($r = 0.631$, $p = 0.093$) and remained similar for γ-tocopherol ($r = 0.923$, $p = 0.001$) (Table 3). A positive correlation tended to appear between the two tocopherol forms in raw chickpeas ($r = 0.643$, $p = 0.085$) but not when looking at cooked content, which could be attributed to the above-mentioned discrepancy between raw and cooked α-tocopherol profile (Fig. 3A-B).

The dry matter content of processed chickpeas was about 30% (Table 3), which reflects the grain hydration during processing. The ultimate FSM quantity for a 100-g portion of cooked chickpea is globally lower than 100-g portion of raw chickpea when considering the water content (data not shown).

Effect of variety, year, location and cooking on chickpea antinutrient profile

Saponin contents of raw chickpeas ranged from 351.6 ± 24.2 to 489.7 ± 38.7 mg/100g DM in 2023 and 404.2 ± 9.1 to 617.1 ± 14.0 mg/100g DM in 2024 for both years (Fig. 2A). These contents were affected by the three main factors (i.e., variety, year and growing location) but not by their interactions (Table 2). Chickpea processing decreased saponin content on a dry matter basis, from 35% to 57% for CDC Orion variety cultivated in Puy de Dôme in 2023 and 2024, respectively (Fig. 2A and 2D). The saponin profile in raw chickpeas tended to correlate with the saponin profile in cooked chickpeas (Table 3).

Interestingly, tannin content was inversely correlated with saponin content in raw chickpeas ($r = -0.743$, $p = 0.035$; Fig. 3A). Indeed, in contrast to saponins, CDC Orion cultivated in Vendée displayed the highest tannin amount both years with 55.8 ± 4.0 and 54.9 ± 2.9 mg/100g DM in 2023 and 2024 respectively, compared to Flamenco cultivated in Puy de Dôme both years, that accounted 32.3 ± 2.1 and 34.3 ± 1.0 mg/100g DM (Fig. 2B). The variety and the growing location, but not the cultivation year, explained a major part of the total tannin variation (Table 2). Surprisingly, the processing step appeared to highly impact tannin profiles, as a negative correlation close to significance appeared between raw and cooked chickpeas in terms of tannin content ($r = -0.655$, $p = 0.078$; Table 3). This observation was also noticed with other chickpea batches (unpublished data). In turn, the negative correlation between saponin and tannin contents in raw material was no longer noticeable and tended towards the opposite direction in processed material (Fig. 3B). Both augmentations and reductions in tannin contents were observed after processing, with for instance + 60% and − 60% tannins in cooked compared to raw chickpeas for Flamenco seeds cultivated in Puy de Dôme and Vendée in 2023, respectively (Fig. 2E).

For phytates, no significant differences were observed between the different raw chickpeas, with values ranging from 3124.8 ± 116.3 to 4097.4 ± 458.2 mg/100g DM (Fig. 2C), highlighting no effect of main factors and their interactions (Table 2). No correlation between raw and cooked profiles was observed (Table 3), with differences in dry matter content ranging from − 28% to + 3% for CDC Orion cultivated in Puy de Dôme and Vendée in 2023, respectively (Fig. 2F).

When antinutrient content was expressed on a fresh weight basis, accounting for dry matter values ranging from 28.7% to 32.0% following cooking (Table 4), a reduction in all antinutrient levels was observed from raw to cooked chickpeas, with average reductions of -82%, -65% and – 72% for saponins, tannins and phytates, respectively (data not shown).

Table 4
Dry Matter content of chickpeas after processing

Variety	Year	Growing Location	Dry Matter
Flamenco	2023	Puy de Dôme	31.9
		Vendée	29.0
	2024	Puy de Dôme	31.8
		Vendée	30.6
CDC Orion	2023	Puy de Dôme	32.0
		Vendée	28.7
	2024	Puy de Dôme	31.4
		Vendée	29.1

Dry Matter (%) of processed chickpeas.

FSM content from chickpea-containing recipe to micellar content

Six FSM were correctly identified in all *in vitro* digestion fractions (i.e., ingredients, digesta and micelles): α- and γ-tocopherols, zeaxanthin, β-carotene, lycopene and cholecalciferol (Fig. 4). Chickpeas used in the recipes contained distinct amount of α- and γ-tocopherols, zeaxanthin and β-carotene. Therefore, the proportion of these FSM brought by each ingredient differed depending on the chickpea used in the test meal (Table 5) and the total theoretical amount varied as well **(**Fig. 4A**)**. At the end of the digestion, the amount of γ-tocopherol, zeaxanthin and α-tocopherol found in the digesta correlated with the initial amount present in test meals, and significant differences between test meals were observed ($p = 0.0018$, $p = 0.0001$ and $p = 0.0011$, respectively; Fig. 4A and 4B). Accordingly, no significant difference was observed in the digesta for β-carotene ($p = 0.2105$), lycopene ($p = 0.1102$) and cholecalciferol ($p = 0.3756$) that were mostly brought by red bell pepper, tomato and supplemented oil in the test meals and were thus brought in similar amounts (Fig. 4A and 4B).

Table 5
Proportion of the FSM provided by the ingredients of the *in vitro* digestion recipe

Xanthophylls	**FSM**	**Tomato**	**Red bell pepper**	**Chickpea**	**Oil**
	Lutein	**8–25**	**0**	**75–92**	
	Zeaxanthin	**1–2**	**20–49**	**50–80**	
Carotenes	Lycopene	99.8–99.9	0.16–0.17	0	
	α-carotene	6–7	89–91	2–4	
	β-carotene	16–18	78–82	1–6	
Vitamin E	α-tocopherol	4–5	49–65	30–46	
	γ-tocopherol	0	0	100	
Vitamin D	cholecalciferol				100

The proportion (%) of FSM provided in the FSM-rich meal by each ingredient. Values are expressed as the minimum and the maximum depending on the chickpea FSM richness.

Significant differences between test meals were observed for all six FSM in terms of micellar amounts (Fig. 4C). The amount of micellar γ-tocopherol, which was exclusively provided by chickpeas, significantly correlated with the amount found in the digesta ($R = 0.8571$, $p = 0.0065$; Table 6). For the two other micronutrients for which the chickpea batch influenced the initial and digesta amount, i.e., zeaxanthin and α-tocopherol, the amount in micelles also tended to correlate with the amount found in the digesta ($R = 0.6662$, $p = 0.0712$ and $R = 0.5725$, $p = 0.1381$, Table 6). In contrast, for β-carotene, lycopene and cholecalciferol, no correlation was observed (Table 6). For γ-tocopherol bioaccessibility, there was a significant test-meal effect ($p = 0.0380$, Fig. 4D), but multiple comparison tests did not reveal significant pairwise differences. For the other FSM, the absence of correlations between micelles and digesta FSM amounts were thus due to variation in FSM bioaccessibility (Fig. 4D).

Table 6
Correlation between fat-soluble micellar and digesta amount

Compound	Pearson r	p-value
γ-tocopherol	0.8571	0.0065 **
Zeaxanthin	0.6662	0.0712
α-tocopherol	0.5725	0.1381
β-carotene	0.5505	0.1574
Lycopene	0.3148	0.4476
Cholecalciferol	-0.0077	0.9856

Pearson correlation coefficients (r) between digesta amount (n = 8) and micellar amount (n = 8) for FSM identified in in vitro digestion of FSM-rich recipes containing the chickpea batches, and associated p-value. Asterisks indicate respectively a significant correlation (**p < 0.01).

Relation between antinutrient content, variety, year, growing location and FSM bioaccessibility

Two PCA analyses were conducted in order to first determine whether chickpea antinutrient content could be linked to FSM bioaccessibility results and then to appreciate whether chickpea variety, year or growing location could also explain the results.

As observed by the distribution of variables in Fig. 5A, phytate and total antinutrient contents tended to be inversely associated with most FSM bioaccessibility values. However, these associations remained insignificant.

Agronomic factor describing chickpea batches didn't significantly discriminated the FSM bioaccessibility values, as observed by the superposing ellipses for variables Year, Variety and Growing Location in Fig. 5B. Bioaccessibility values obtained with the variety CDC Orion were relatively dispersed compared to those obtained with Flamenco.

Discussion

As part of a diversified diet, chickpea pulses can participate to the intake of several FSM (Padhi et al., 2017; Margier et al., 2018). This is therefore interesting to maximise the potential of these seeds by identifying the most suitable agronomic conditions not only to increase the FSM intrinsic quantity in the seed, but also to play in favour of a high bioaccessibility. It was hypothesised that a favourable chickpea profile would be characterised by a low amount of antinutrients. In fact, antinutrients present in the seed can alter both the bioaccessibility of these endogenous FSM and that of FSM brought by other food items of the meal (Margier et al., 2019; Antoine et al., 2023; Antoine et al., 2023; Alvarado-Ramos et al., 2024).

Here, we first reported the impact of agronomic factors on FSM chickpea content.

As previously observed in different varieties, zeaxanthin and lutein contents, highly correlated to one another, were more impacted by the effect of cultivar rather than by the environment (Ashokkumar et al., 2014). This is not surprising because the expression of genes implicated in carotenoid biosynthesis can vary depending on chickpea cultivar (Rezaei et al., 2016), genetic effect overcoming other agronomic traits (tillage, fertilization) over 3 years (Abbo et al., 2010). Similarly, γ-tocopherol - the main form of vitamin E found in chickpea and in most pulses (Boschin and Arnoldi, 2011), was greatly impacted by the cultivar. Such effect did not exist for the α-form, which was around 10 times less concentrated in the seeds. Accordingly, a study reported the effect of the genotype on both tocopherols in chickpeas, with an effect less marked for the α-form (Gül et al., 2008).

The cultivation year also had a great influence on the seed content in FSM. Such year effect had already been highlighted for chickpea proximate composition (Nikolopoulou et al., 2006). In the case of our study, the effect of the year may also be due to differences in postharvest period (Saini and Keum, 2018). However, it was previously reported that at low water activity, carotenoid loss was negligeable after 24 week-storage at room temperature (Kantha et al., 1987), so the impact of the postharvest period may be minimal.

The interaction between the year and growing location that appeared for most FSM suggests that the meteorological conditions (i.e., rainfall, temperature, solarization...) specific to the cultivation area and year constitute a great modulator of FSM biosynthesis. In fact, carotenoid synthesis is also driven by stress and maturation stage undergone by the seed (Smolikova and Medvedev, 2015). For instance, zeaxanthin cycle, a reversible processus by which this carotenoid is converted to other carotenoid depending on the light level to dissipate energy, is involved in heat tolerance in chickpea (Demmig-Adams and Adams, 1996; Kumar et al., 2020). In this line, we observed that xanthophyll amounts were higher in 2023, when the total solarization and average maximal temperature were greater in both growing locations.

Processing to enable eating can modify the composition of the seed and therefore the initial FSM profile of the pulse (Margier et al., 2018). Tocopherol and xanthophyll contents were both increased on a dry matter basis after processing, but decreased when expressed as fresh matter. This increase on dry weight was also noted for lentil cultivars after domestic cooking (Zhang et al., 2014). This could be attributed to the loss of soluble solids during cooking, which would increase the concentration of these compounds on a dry matter basis (Wang et al., 2010), or to a better extractability due to cell membrane disruption (Zhang et al., 2014). In contrast, carotene contents were reduced considering both dry and fresh weights. Despite these changes in total amount in the seed, there were significant correlations between the FSM contents of raw and cooked chickpeas. This result highlights that the change in FSM content is proportional to the initial amount in raw material. Therefore, selecting cultivars with high FSM content in raw material is likely to provide FSM-rich chickpeas when cooked, at least following a domestic cooking process.

Several evidences suggested that saponins, tannins and phytates display a negative impact on FSM bioaccessibility (Jenkins and Atwal, 1994; Margier et al., 2019; Margier et al., 2019; Antoine et al., 2022; Alvarado-Ramos et al., 2024). The initial content in these antinutrients can also be modulated by agronomic factors (Nikolopoulou et al., 2006) and by processing (Margier et al., 2018; Antoine et al., 2022). In particular, it was previously mentioned that rainfall could be the driver of tannin quantity (Oluwatosin, 1999; Nikolopoulou et al., 2006). This meteorological parameter differed quite widely between locations and yearly in our study as well. However, conversely to the inverse correlation between wet season and tannin content previously observed in cowpea (Oluwatosin, 1999), it seemed that Puy de Dôme, for which both years was characterised by lower rainfall amount and humidity provided lower tannin-rich chickpeas. In the chickpeas described in this study, phytate content did not discriminate the batches and the agronomic factors did not explain any differences. This does not exclude that in other environmental conditions and varieties tested, the effect of these factors would not occur, as previously observed (Wu et al., 2016). It was previously reported that cooler temperatures of temperate summers might lead to the production of lower phytate in lentil (Thavarajah et al., 2010). However, in our study, the differences in temperatures between the studied growing locations and years did not lead to such observations. On the other hand, as previously observed (Rao and Deosthale, 1982; Nikolopoulou et al., 2006; Ruggeri et al., 2017), we noted that varietal and growing location factors explained the differences in saponins and tannins between batches, with a proportional difference depending on the year for saponins. Saponin and tannin biosynthesis in the seed starts from a same precursor as FSM, the isopentenyl pyrophosphate originating from the mevalonate (MVA) or methyl erythritol phosphate (MEP) pathways (Smolikova and Medvedev, 2015; Lauer et al., 2026). Therefore, one might expect their amounts to be interrelated in chickpeas. It was observed that bitter (i.e., saponin-rich) quinoa genotype contained lower amounts of lutein and zeaxanthin to a lesser extent compared to a non-bitter one (i.e. saponin-poor), and a moderate negative correlation between saponins and carotenoids was observed (Lauer et al., 2026). Interestingly, we observed this same negative correlation in raw chickpeas, for the main carotenoid zeaxanthin and lutein.

Consistently with the literature, on dry and fresh matter basis, saponin and phytate contents were decreased after the soaking and cooking process (El-Adawy, 2002). However, none of the cooked contents were correlated with raw amount. Surprisingly, tannin content even tended to be negatively correlated. This could be linked to a difference in tannin loss (Avanza et al., 2013) or hydrolysis of larger phenolic compounds (Kumar et al., 2024) during processing. We observed this same tendency in other chickpea batches (unpublished data).

Finally, we conducted an *in vitro* digestion of test meals containing the different chickpea batches to mimic a vegetal-based meal rich in FSM. The idea was to evaluate whether at the end of the digestion, micellar phase, which contains absorbable FSM, would be representative of the initial FSM amount in the recipe, depending on the chickpea used, or if bioaccessibility would impact the resulting amount. Chickpea batches influenced the initial amount of γ-tocopherol, zeaxanthin and α-tocopherol amount, and to a lesser extent that of β-carotene. At the end of the digestion, the digesta content resembled the initial amount. However, the resulting amount in the micellar phase was significantly consistent with the

digesta amount only for γ-tocopherol, the FSM in higher initial quantity. This result suggests that some factors present in chickpeas, the unique ingredient different between the meals, impacted FSM bioaccessibility.

Our hypothesis was that antinutrient content would be negatively associated with bioaccessibility values, as it was observed previously (Margier et al., 2019; Antoine et al., 2023; Alvarado-Ramos et al., 2024). However, no such association was significantly observed, probably due to the antinutrient profiles of the different chickpea batches, which remained close, especially in terms of phytate content. In line with this result, we previously observed that an optimization of chickpea canning process resulting in a decrease of -37% in saponin content and − 35% in phytate content was insufficient to improve vitamin D bioaccessibility (Antoine et al., 2022). In comparison in this study, once cooked, the highest differences between chickpea batches were of -18.5% and − 25.7% for saponins and phytates, respectively. Moreover, other components discriminating the chickpea batches may explain the differences in bioaccessibility. For instance, fibres, phytosterols or the other fat-soluble micronutrients can modulate the bioaccessibility of FSM (Borel, 2003; Goncalves et al., 2011; Dube et al., 2018; Reboul et al., 2026). The quantity of these substances are likely to be modulated as well by agronomic factors in chickpeas (Nikolopoulou et al., 2006).

In conclusion, we confirmed that agronomic conditions can modulate the intrinsic quantity of some antinutrients. Deeper characterisation is needed to understand how specific aspects in the growing location and climate yearly drive chickpea content, temperature and rainfall being probably some of the influent factors. Regarding FSM, although it seems complicated to predict their level in chickpeas as they depend on specific conditions each year, a high FSM content in raw seeds will likely lead to higher absorbable FSM levels. Overall, it thus appears that yearly climatic conditions can significantly modify both the nutritional and antinutritional profiles of chickpeas - even for the same variety grown in the same location - complicating seed selection for nutritional traits. Given the variability in antinutrient and FSM contents in the seed, we recommend prioritizing other traits, such as resistance to heat stress and pests. Despite we did not find any correlation between antinutrient content and bioaccessibility, we hypothesize that selecting agronomic conditions that promote high FSM or low antinutrient levels may still be beneficial to improve FSM bioaccessibility, if combined with a processing method that effectively reduce antinutritional compounds. Encouraging more frequent but smaller portions of pulses could help limit antinutrient intake while still benefiting from their nutritional value (RAC-SFN, 2024).

Declarations

Data availability

All data have been deposited at Data INRAE and are available at the following URL: (*URL currently under validation*).

Acknowledgments

The authors thank Terres Inovia for kindly providing chickpea batches and Sylvain Tréguier for reviewing the draft.

Funding

Angélique Berthomé received a PhD fellowship from Région PACA (France) and CTCPA Avignon (France).

Author contributions

ER and SG conceptualised the study and acquired funding. ER was in charge of project administration and supervision. SG, QL, YL, and ER provided resources. ER defined methodologies. AB did formal analyses, investigation, data curation, visualization and statistical analyses. AB and ER validated the data. AB wrote the original draft. ER reviewed and edited the manuscript. All authors contributed to the manuscript and agreed with its final version.

References

1. Abbo S, Bonfil DJ, Kerem Z, Berkovitch Z, Reifen R (2010) Towards enhancing lutein concentration in chickpea, cultivar and management effects. Plant Breeding 129: 407–411
2. Alvarado-Ramos K, Bravo-Nunez Á, Halimi C, Maillot M, Icard-Vernière C, Forti C, Preite C, Ferrari L, Sala T, Losa A, et al (2024) Improving the antinutritional profiles of common beans (Phaseolus vulgaris L.) moderately impacts carotenoid bioaccessibility but not mineral solubility. Sci Rep 14: 11908
3. Antoine T, El Aoud A, Alvarado-Ramos K, Halimi C, Vairo D, Georgé S, Reboul E (2023) Impact of pulses, starches and meat on vitamin D and K postprandial responses in mice. Food Chemistry 402: 133922
4. Antoine T, Georgé S, Leca A, Desmarchelier C, Halimi C, Gervais S, Aupy F, Marconot G, Reboul E (2022) Reduction of pulse “antinutritional” content by optimizing pulse canning process is insufficient to improve fat-soluble vitamin bioavailability. Food Chemistry 370: 131021
5. Antoine T, Icard-Vernière C, Scorrano G, Salhi A, Halimi C, Georgé S, Carrière F, Mouquet-Rivier C, Reboul E (2021) Evaluation of vitamin D bioaccessibility and mineral solubility from test meals containing meat and/or cereals and/or pulses using in vitro digestion. Food Chemistry 347: 128621
6. Ashokkumar K, Tar’an B, Diapari M, Arganosa G, Warkentin TD (2014) Effect of Cultivar and Environment on Carotenoid Profile of Pea and Chickpea. Crop Science 54: 2225–2235
7. Avanza M, Acevedo B, Chaves M, Añón M (2013) Nutritional and anti-nutritional components of four cowpea varieties under thermal treatments: Principal component analysis. LWT - Food Science and Technology 51: 148–157
8. Bohn T (2019) Carotenoids and Markers of Oxidative Stress in Human Observational Studies and Intervention Trials: Implications for Chronic Diseases. Antioxidants 8: 179

9. Bonnet L, Margier M, Svilar L, Couturier C, Reboul E, Martin J-C, Landrier J-F, Defoort C (2019) Simple Fast Quantification of Cholecalciferol, 25-Hydroxyvitamin D and 1,25-Dihydroxyvitamin D in Adipose Tissue Using LC-HRMS/MS. Nutrients 11: 1977
10. Borel P (2003) Factors affecting intestinal absorption of highly lipophilic food microconstituents (fat-soluble vitamins, carotenoids and phytosterols). Clin Chem Lab Med 41: 979–994
11. Boschin G, Arnoldi A (2011) Legumes are valuable sources of tocopherols. Food Chemistry 127: 1199–1203
12. Chaturvedi P, Pierides I, López-Hidalgo C, Garg V, Zhang S, Barmukh R, Bellaire A, Li J, Bachmann G, Valledor L, et al (2024) Natural variation in the chickpea metabolome under drought stress. Plant Biotechnology Journal 22: 3278–3294
13. Demmig-Adams B, Adams WW (1996) The role of xanthophyll cycle carotenoids in the protection of photosynthesis. Trends in Plant Science. doi: 10.1016/S1360-1385(96)80019-7
14. Dube N, Chandra Mashurabad P, Hossain F, Pullakhandam R, Thingnganing L, Kumar Bharatraj D (2018) β-Carotene bioaccessibility from biofortified maize (Zea mays) is related to its density and is negatively influenced by lutein and zeaxanthin. Food & Function 9: 379–388
15. El-Adawy TA (2002) Nutritional composition and antinutritional factors of chickpeas (Cicer arietinum L.) undergoing different cooking methods and germination. Plant Foods Hum Nutr 57: 83–97
16. Fernández-Marín B, Míguez F, Méndez-Fernández L, Agut A, Becerril JM, García-Plazaola JI, Kranner I, Colville L (2017) Seed Carotenoid and Tocochromanol Composition of Wild Fabaceae Species Is Shaped by Phylogeny and Ecological Factors. Front Plant Sci. doi: 10.3389/fpls.2017.01428
17. Ghavidel RA, Prakash J (2007) The impact of germination and dehulling on nutrients, antinutrients, in vitro iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seeds. LWT - Food Science and Technology 40: 1292–1299
18. Goncalves A, Gleize B, Bott R, Nowicki M, Amiot M-J, Lairon D, Borel P, Reboul E (2011) Phytosterols can impair vitamin D intestinal absorption in vitro and in mice. Molecular Nutrition & Food Research 55: S303–S311
19. Gül MK, Egesel CÖ, Turhan H (2008) The effects of planting time on fatty acids and tocopherols in chickpea. Eur Food Res Technol 226: 517–522
20. Jenkins KJ, Atwal AS (1994) Effects of dietary saponins on fecal bile acids and neutral sterols, and availability of vitamins A and E in the chick. The Journal of Nutritional Biochemistry 5: 134–137
21. Jukanti AK, Gaur PM, Gowda CLL, Chibbar RN (2012) Nutritional quality and health benefits of chickpea (Cicer arietinum L.): a review. British Journal of Nutrition 108: S11–S26
22. Kantha SS, Erdman Jr. JW, Simpson KL (1987) Legume carotenoids. C R C Critical Reviews in Food Science and Nutrition 26: 137–155
23. Karalija E, Vergata C, Basso MF, Negussu M, Zaccai M, Grossi-de-Sa MF, Martinelli F (2022) Chickpeas' Tolerance of Drought and Heat: Current Knowledge and Next Steps. Agronomy 12: 2248

24. Kumar G, Perera D, Sudheer KP, Zhang P, Dhital S (2024) Leaching of Phytochemicals from Beans during Hydration, Kinetics, and Modeling. Foods 13: 354
25. Kumar P, Yadav S, Singh MP (2020) Possible involvement of xanthophyll cycle pigments in heat tolerance of chickpea (Cicer arietinum L.). Physiol Mol Biol Plants 26: 1773–1785
26. Landes N, Pfluger P, Kluth D, Birringer M, Rühl R, Böl G-F, Glatt H, Brigelius-Flohé R (2003) Vitamin E activates gene expression via the pregnane X receptor. Biochemical Pharmacology 65: 269–273
27. Landrier J-F, Gouranton E, El Yazidi C, Malezet C, Balaguer P, Borel P, Amiot M-J (2009) Adiponectin Expression Is Induced by Vitamin E via a Peroxisome Proliferator-Activated Receptor γ-Dependent Mechanism. Endocrinology 150: 5318–5325
28. Landrier J-F, Gouranton E, Reboul E, Cardinault N, El Yazidi C, Malezet-Desmoulins C, André M, Nowicki M, Souidi M, Borel P (2010) Vitamin E decreases endogenous cholesterol synthesis and apo-AI-mediated cholesterol secretion in Caco-2 cells. The Journal of Nutritional Biochemistry 21: 1207–1213
29. Lauer LA, Kollmar M, Schmöckel SM, Frank J (2026) Comparative analysis of minerals, carotenoids, and tocochromanols in ripe seeds, immature seeds and tepals of bitter and non-bitter quinoa genotypes. Journal of Food Composition and Analysis 152: 109021
30. Malapert A, Tomao V, Margier M, Nowicki M, Gleize B, Dangles O, Reboul E (2018) β-Cyclodextrin Does not Alter the Bioaccessibility and the Uptake by Caco-2 Cells of Olive By-Product Phenolic Compounds. Nutrients 10: 1653
31. Margier M, Antoine T, Siriaco A, Nowicki M, Halimi C, Maillot M, Georgé S, Reboul E (2019) The Presence of Pulses within a Meal can Alter Fat-Soluble Vitamin Bioavailability. Molecular Nutrition & Food Research 63: 1801323
32. Margier M, Georgé S, Hafnaoui N, Remond D, Nowicki M, Du Chaffaut L, Amiot M-J, Reboul E (2018) Nutritional Composition and Bioactive Content of Legumes: Characterization of Pulses Frequently Consumed in France and Effect of the Cooking Method. Nutrients 10: 1668
33. Nikolopoulou D, Grigorakis K, Stasini M, Alexis M, Iliadis K (2006) Effects of cultivation area and year on proximate composition and antinutrients in three different kabuli-type chickpea (Cicer arientinum) varieties. Eur Food Res Technol 223: 737–741
34. Nour SM, Fernandez MP, Normand P, Cleyet-Marel J-C (1994) Rhizobium ciceri sp. nov., Consisting of Strains That Nodulate Chickpeas (Cicer arietinum L.). International Journal of Systematic and Evolutionary Microbiology 44: 511–522
35. Oluwatosin OB (1999) Genotype × environment influence on cowpea (Vigna unguiculata (L) walp) antinutritional factors: 1 – trypsin inhibitors, tannins, phytic acid and haemagglutinin. Journal of the Science of Food and Agriculture 79: 265–272
36. Padhi EMT, Liu R, Hernandez M, Tsao R, Ramdath DD (2017) Total polyphenol content, carotenoid, tocopherol and fatty acid composition of commonly consumed Canadian pulses and their contribution to antioxidant activity. Journal of Functional Foods 38: 602–611

37. RAC-SFN (2024) Comment concilier nutrition et climat ? Pour la prise en compte des enjeux environnementaux dans le Programme National Nutrition Santé.
38. Rao PU, Deosthale YG (1982) Tannin content of pulses: Varietal differences and effects of germination and cooking. Journal of the Science of Food and Agriculture 33: 1013–1016
39. Reboul E, Gomes AM, Petroni K, Riso P, Toccaceli M, Real CV, Martins AP, Del Bo' C, Martini D, Marino M, et al (2026) Will climate change affect nutrient, micronutrient and bioactive bioavailability? Critical Reviews in Food Science and Nutrition 1–24
40. Rezaei MK, Deokar A, Tar'an B (2016) Identification and Expression Analysis of Candidate Genes Involved in Carotenoid Biosynthesis in Chickpea Seeds. Front Plant Sci. doi: 10.3389/fpls.2016.01867
41. Rodriguez-Concepcion M, Avalos J, Bonet ML, Boronat A, Gomez-Gomez L, Hornero-Mendez D, Limon MC, Meléndez-Martínez AJ, Olmedilla-Alonso B, Palou A, et al (2018) A global perspective on carotenoids: Metabolism, biotechnology, and benefits for nutrition and health. Progress in Lipid Research 70: 62–93
42. Ruggeri R, Primi R, Danieli PP, Ronchi B, Rossini F (2017) Effects of seeding date and seeding rate on yield, proximate composition and total tannins content of two Kabuli chickpea cultivars. Italian Journal of Agronomy 12: 890
43. Ruiz RG, Price KR, Arthur AE, Rose ME, Rhodes MJC, Fenwick RG (1996) Effect of Soaking and Cooking on the Saponin Content and Composition of Chickpeas (Cicer arietinum) and Lentils (Lens culinaris). J Agric Food Chem 44: 1526–1530
44. Saini RK, Keum Y-S (2018) Significance of Genetic, Environmental, and Pre- and Postharvest Factors Affecting Carotenoid Contents in Crops: A Review. J Agric Food Chem 66: 5310–5324
45. Samtiya M, Aluko RE, Dhewa T (2020) Plant food anti-nutritional factors and their reduction strategies: an overview. Food Prod Process and Nutr 2: 6
46. Smolikova GN, Medvedev SS (2015) Seed carotenoids: Synthesis, diversity, and functions. Russ J Plant Physiol 62: 1–13
47. Tang G (2010) Bioconversion of dietary provitamin A carotenoids to vitamin A in humans12345. The American Journal of Clinical Nutrition 91: 1468S-1473S
48. Thavarajah D, Thavarajah P, See C-T, Vandenberg A (2010) Phytic acid and Fe and Zn concentration in lentil (*Lens culinaris* L.) seeds is influenced by temperature during seed filling period. Food Chemistry 122: 254–259
49. Udvardi M, Poole PS (2013) Transport and Metabolism in Legume-Rhizobia Symbioses. Annual Review of Plant Biology 64: 781–805
50. Wang N, Hatcher DW, Tyler RT, Toews R, Gawalko EJ (2010) Effect of cooking on the composition of beans (*Phaseolus vulgaris* L.) and chickpeas (*Cicer arietinum* L.). Food Research International 43: 589–594
51. Wu G, Johnson SK, Bornman JF, Bennett SJ, Singh V, Simic A, Fang Z (2016) Effects of Genotype and Growth Temperature on the Contents of Tannin, Phytate and In Vitro Iron Availability of Sorghum

Grains. PLOS ONE 11: e0148712

52. Zhang B, Deng Z, Tang Y, Chen PX, Liu R, Ramdath DD, Liu Q, Hernandez M, Tsao R (2014) Effect of Domestic Cooking on Carotenoids, Tocopherols, Fatty Acids, Phenolics, and Antioxidant Activities of Lentils (*Lens culinaris*). J Agric Food Chem 62: 12585–12594

Figures

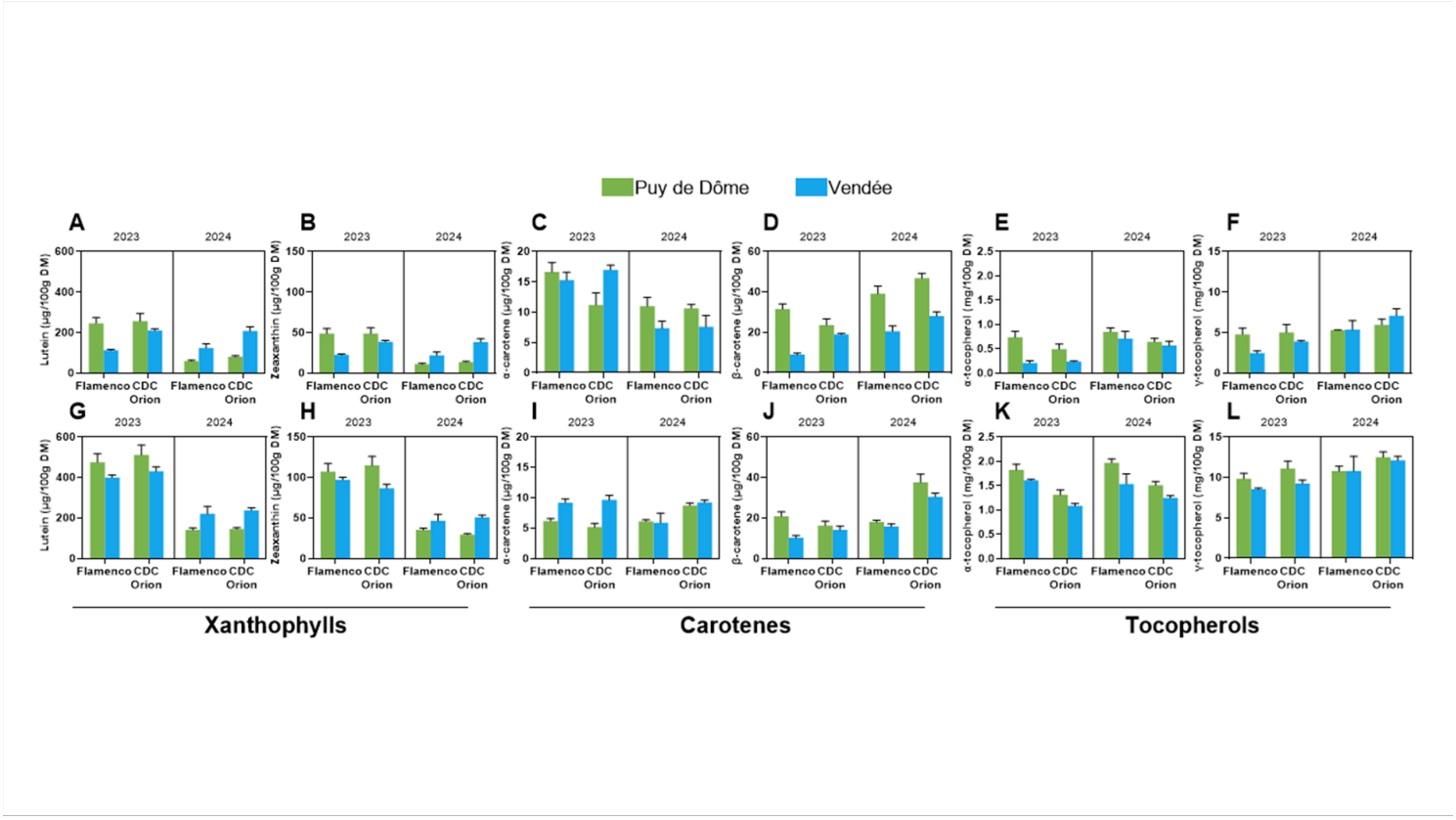


Figure 1

Chickpea FSM content

Effect of variety, growing location and year on the chickpea FSM content. Xanthophylls: (A) Lutein and (B) Zeaxanthin; Carotenes: (C) α-carotene and (D) β-carotene; Tocopherols: (E) α-tocopherol and (F) γ-tocopherol measured in raw chickpea flour. Pannels (G) to (L) correspond respectively to the same FSM measured in cooked chickpea flour. Data are represented as mean ± SEM, (n = 4) and expressed in µg/100g DM for carotenoids and mg/100g DM for tocopherols. DM= Dry Matter

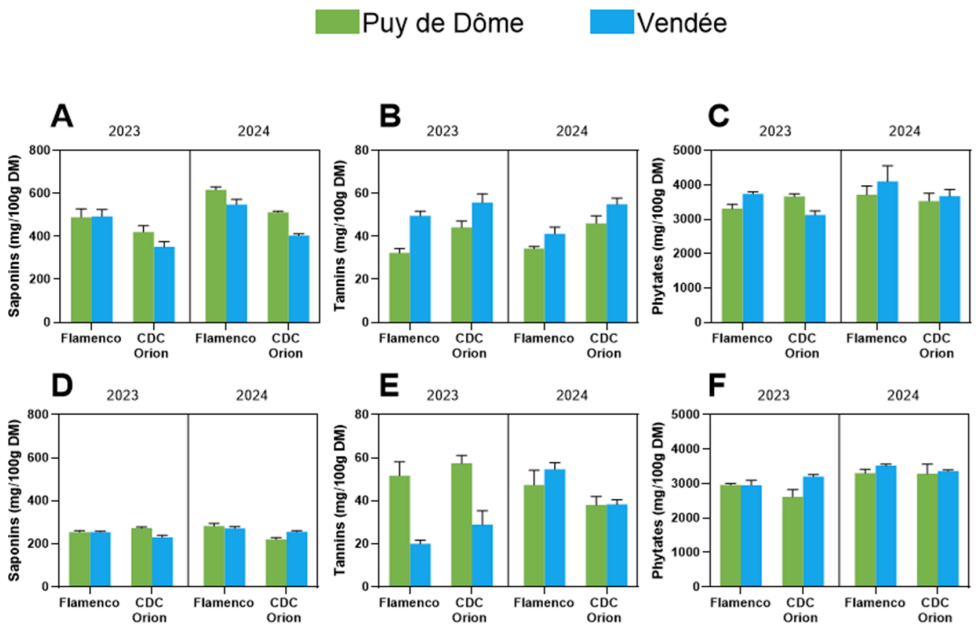


Figure 2

Chickpea antinutrient content

Effect of variety, growing location and year on the chickpea antinutrient content. (A) Saponins, (B) Tannins and (C) Phytates measured in raw chickpea flour. Pannels (D), (E), (F) correspond respectively to the same antinutrients measured in cooked chickpea flour. Data are represented as mean ± SEM, (n = 4) and expressed in mg/100g DM. DM= Dry Matter

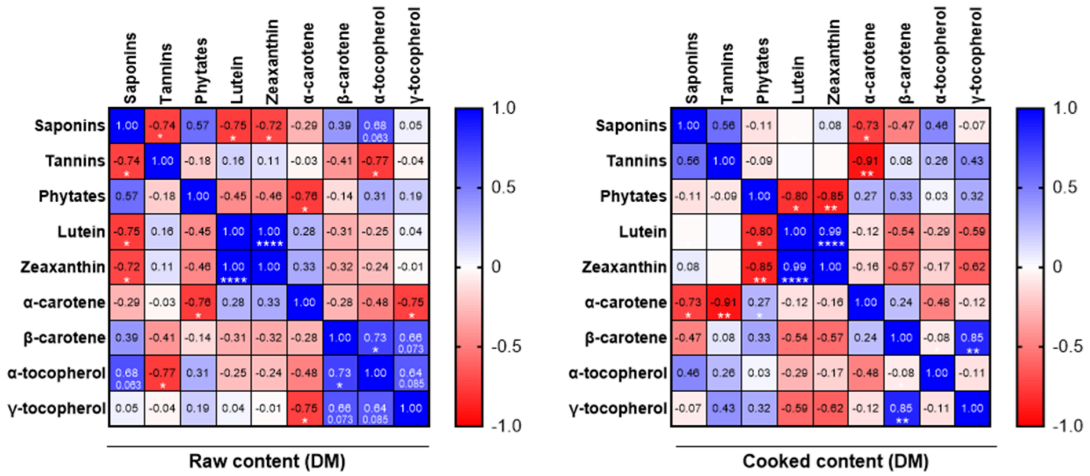


Figure 3

Correlations between secondary metabolites

Pearson correlation matrixes for secondary metabolites (antinutrient and FSM) contents in (A) Raw and (B) Cooked chickpea flour (DM). DM= Dry Matter. Significant p-values (<0.05) or close to significance (p <0.10) are reported underneath the coefficient. *p <0.05; **p<0.01; ***p<0.001; ****p<0.0001.

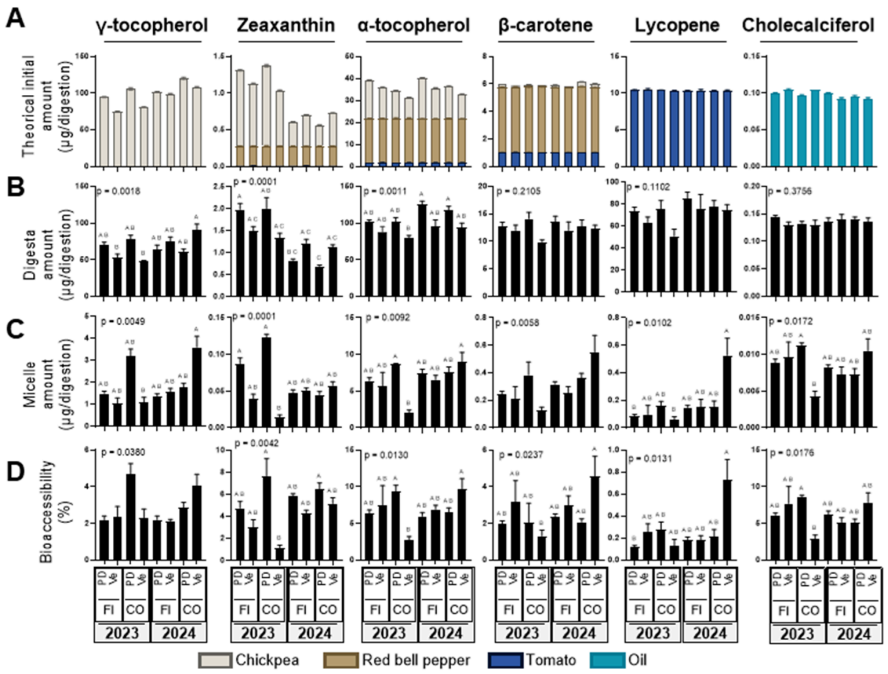


Figure 4

***In vitro* digestion outcomes**

Impact of the incorporation of the 8 different chickpea batches, Flamenco (Fl) or CDC Orion (CO) cultivated in 2023 or 2024, in Puy de Dôme (PD) or Vendée (Ve), on fat-soluble micronutrient fate in an *in vitro* digestion. (A) Theoretical amount in the recipe, (B) Amount in the digesta (C) Amount in the micelles and (D) Bioaccessibility, of γ-tocopherol, zeaxanthin, α-tocopherol, β-carotene, lycopene and cholecalciferol. Amounts are reported as µg/digestion and bioaccessibility in %. Data are represented as mean ± SEM, (n = 2-4). Distinct letters indicate differences between samples ($p < 0.05$).

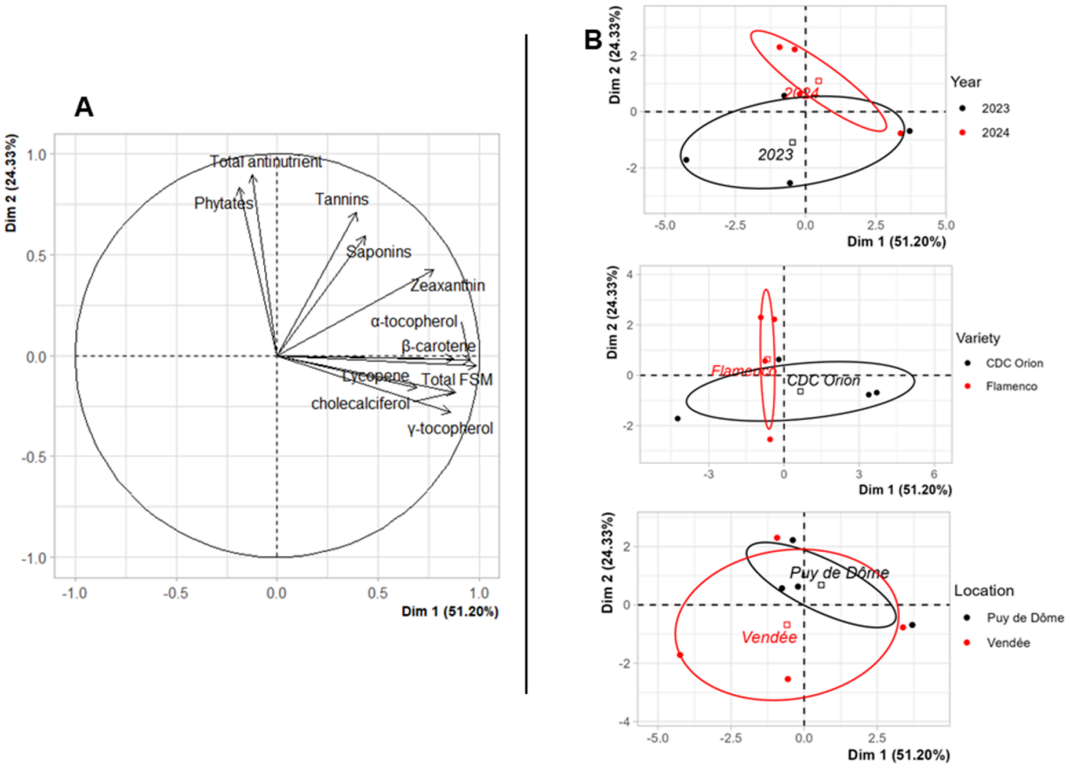


Figure 5

Association between FSM bioaccessibility and antinutrient content or agronomic factors

(A) Relationship between FSM bioaccessibility values and antinutrient content of cooked chickpeas (FM). FM= Fresh Matter. (B) Repartition of chickpea batches according to the FSM bioaccessibility values and their agronomic trait, cultivation year (top panel), variety (middle panel) and growing location (bottom panel).