Reduction of pulse "antinutritional" content by optimizing pulse canning process is insufficient to improve fat-soluble vitamin bioavailability

Tiffany Antoine, Stéphane Georgé, Alexandre Leca, Charles Desmarchelier, Charlotte Halimi, Sarah Gervais, Fabien Aupy, Grégory Marconot, Emmanuelle Reboul

Reduction of pulse "antinutritional" content by optimizing pulse canning process is insufficient to improve fat-soluble vitamin bioavailability

Tiffany Antoine[1], Stephane Georgé[2], Alexandre Leca[3], Charles Desmarchelier[1], Charlotte Halimi[1], Sarah Gervais[2], Fabien Aupy[2], Grégory Marconot[1] and Emmanuelle Reboul[1]*

[1] Aix Marseille Univ, INRAE, INSERM, C2VN, Marseille, France.

[2] Centre Technique de Conservation des Produits Agricoles, Avignon, France.

[3] Avignon Université, UMR SQPOV Avignon 84914, France.

Address correspondence to:* Emmanuelle Reboul, Center for CardioVascular and Nutrition Research (C2VN), UMR 1263 INSERM/1260 INRA/Aix-Marseille Université, Faculté de Médecine la Timone, 27 boulevard Jean-Moulin, 13385 Marseille Cedex 5 FRANCE. Tel: (+33).4.91.32.42.78; E-mail: Emmanuelle.Reboul@univ-amu.fr

Abbreviated running title: Pulse canning process and fat-soluble vitamin bioavailability

Data described in the manuscript will be made available upon request pending application and approval.

Abstract

Some bioactive compounds found in pulses (phytates, saponins, tannins) display antinutritional properties and interfere with fat-soluble vitamin bioavailability (i.e., bioaccessibility and intestinal uptake). As canned chickpeas are consumed widely, our aim was to optimize the chickpea canning process and assess whether this optimization influences fat-soluble vitamin bioavailability. Different conditions during soaking and blanching were studied, as was a step involving prior germination. Proteins, lipids, fibers, vitamin E, lutein, 5-methyl-tetrahydro-folate, magnesium, iron, phytates, saponins and tannins were quantified. Bioaccessibility and intestinal uptake of vitamin D and K were assessed using in vitro digestion and Caco-2 cells, respectively. Significant reductions of phytate, saponin and tannin contents (-16 to -44%), but also of folate content (up to –97%) were observed under optimized canning conditions compared with the control. However, bioaccessibility and cellular uptake of vitamin D and K remained unaffected after in vitro digestion of test meals containing control or optimized canned chickpeas.

Keywords: Chickpeas; germination; phytates; tannins; saponins; vitamin D; vitamin K; bioaccessibility; Caco-2 cells.

1. Introduction

Pulses display interesting nutritional properties, as they are good sources of proteins, fibers and micronutrients (Margier et al., 2018). Pulses are also a significant source of bioactive compounds such as phytates, saponins and tannins (Margier et al., 2018), the consumption of which demonstrates some interesting lipid-lowering properties. For instance, dietary fibers positively impact postprandial lipemia (Desmarchelier et al., 2019; Lee et al., 2020) and cholesterolemia (Schoeneck & Iggman, 2021). Phytates at high concentrations and tannins were shown to interfere with human lipase activity (Antoine et al., 2021). Finally, saponins have often been described as anti-obesogenic compounds (Marrelli et al., 2016), partly because they also interact with lipase activity (Antoine et al., 2021; Marrelli et al., 2016). However, these compounds can also be considered "antinutritional", as they can negatively interact with micronutrients such as minerals. In particular, phytates and tannins are divalent cation quenchers that can decrease iron and zinc bioavailability (Antoine et al., 2021; Lestienne et al., 2005). Furthermore, we recently showed that fibers, phytates, tannins and saponins from pulses can reduce fat-soluble vitamin bioavailability in vitro, and more precisely that they can modulate the bioavailability of vitamin K (Margier et al., 2019) and vitamin D (Antoine et al., 2021).

Different strategies can be set up to decrease pulses' "antinutritional" content, one of the most straightforward being the optimization of pulse cooking/transformation methods (Margier et al., 2018). As canned pulses are consumed widely, we decided to investigate the optimization of canning process. Chickpeas were selected as the pulse to be studied. The objective of this study was thus i) to identify and optimize the parameters of the canning process that could reduce chickpeas' levels of bioactive compounds displaying antinutritional properties (phytates, saponins and tannins); and ii) assess whether the decreases in phytates, saponins

67 and tannins resulting from canning process optimization were sufficient to improve the
68 bioavailability of vitamins D and K in test meals containing canned chickpeas.

2. Material and methods

2.1. Chemicals

Cholecalciferol (vitamin D_3), phylloquinone (vitamin K_1), γ-tocopherol, retinyl acetate (all >95% pure), phytic acid sodium salt hydrate, soyasaponin I, 4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), catechin 4-(dimethylamino)cinnamaldehyde (DMACA), 2-(N-cyclohexylamino) ethane sulfonic acid (CHES), 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES), α-amylase from bacillus sp., protease from Streptomyces griseus, and 5-methyltetrahydrofolate (5-CH3-H4 folate calcium salt) were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Lutein (>95% pure) was from Extrasynthèse (Genay, France). Chicken pancreas conjugase was from R-Biopharm (Darmstadt, Germany). Isio4 oil was from Lesieur (Asnières-sur-Seine, France). Iron (III) chloride hexahydrate and vanillin (all 99% pure) were from Acros Organics (Noisy-le-Grand, France). Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g/L glucose and trypsin-EDTA (500 and 200 mg/L, respectively), non-essential amino acids, penicillin/streptomycin and phosphate-buffered saline (PBS) were purchased from Life Technologies (Illkirch, France). Fetal bovine serum (FBS) came from PAA (Vélizy Villacoublay, France). Chickpeas (*Cicer arietinum* 'Eldorado'), were produced locally and provided by the GPS France cooperative (Manosque, France). All solvents were HPLC grade and obtained from Carlo Erba (Peypin, France).

2.2. Chickpea preparation

2.2.1. Control canning process

The control canning protocol was devised by the Centre Technique de Conservation des Produits Agricoles (CTCPA, Avignon, France) and implemented in their pilot plant. In brief,

chickpeas were soaked in low-ionized water (hardness titration [HT]=30 °f) at 15 °C at a ratio of 1:3 (Chickpeas:water; w/w) for 14h. Afterwards, immersion blanching was done at 90 °C for 5 min. Chickpeas were then conditioned in cans (200 g), and hot brine (0.5% salt, 70°C) at a ratio of 86:114 (w:w) was added. The cans were then sterilized at 127 °C for 16 min, and subsequently cooled to 30 °C for 10 min.

2.2.2. Preliminary tests

Preliminary tests were performed to address the effect of modifying single parameters. Soaking and blanching parameters were thus modified independently (Table 1). An "antinutritional" compound analysis was conducted after each step, but not on the final canned products.

- *Modification of the soaking step (Table 1)*

Hot water soaking (water at 80°C) was done in a Stephan® Combicut TC System (Stephan Bvba, Nazareth, Belgium) for 2h. The "soft" water (HT=6 °f) was Volvic water (Société des eaux de Volvic – Danone group, Volvic, France) and the "hard" water (HT=184 °f) was Hépar water (Nestlé Waters Vosges, Vittel, France). Chickpeas were soaked at different ratios: 1:3, or 2:3, or 1:10 (chickpeas:water; w:w). An agitation step could be added either with a pump (Yamada, Arlington Heights, IL, USA) during soaking at 15 °C or with paddles in a Stephan® Combicut TC during soaking at 80°C. Water could be replaced at the midpoint of the soaking time. Citric acid (La Littorale - Erbslöh, Servian, France) and sodium bicarbonate (Cérébos, Levallois-Perret, France) were used for a soaking at pH=5 and pH=9, respectively. Finally, a test was done after the addition of a germination step performed before soaking and blanching: chickpeas were immersed for 12 h in water (1:3, w:v), then kept between thick layers of damp cotton cloth and allowed to germinate for 3 days at room temperature (20-25°C).

- *Modification of the blanching step (Table 1)*

Tests on the blanching step were carried out on chickpeas previously soaked via the control process. A longer blanching time was also tested (20 min). Moreover, the immersion blanching was changed into either steaming or cascading. Steaming consisted of a 5-min exposure to steam using a steam oven (Bourgeois, Faverge, France). Cascading was performed using vertically flowing water in a steam-heated autoclave (Auriol, Marmande, France).

2.2.3. Optimization tests on the global process (Table 2)

Once the preliminary tests were analyzed, different processes combining the most efficient soaking and blanching parameters were tested. For reasons of technological practicality, hot soaking was done with a high chickpea-to-water ratio (1:10) for a shorter time (2h). Conditioning and sterilization parameters were not modified. Contrary to the preliminary tests, bioactive compounds were only analyzed in the final products.

2.3. Texture analysis

The texture of the canned chickpeas was analyzed to check whether process modifications induced changes in chickpea texture compared to chickpeas treated with the control process. A multipurpose texture analyzer TA-plus (Lloyd Instruments, Farenham, UK) was used with a method adapted from Chenoll and colleagues (Chenoll et al., 2009). The peak compression force (N) was recorded as the hardness of the chickpeas.

2.4. In vitro digestion experiments

The test meals used for in vitro digestion experiments were made of 3.35 g of canned chickpeas and 100 mg of Isio 4 oil. Isio 4 oil contributed minor amounts of fat-soluble vitamins to the meal, without consequences to data interpretation, as oil was present in all conditions. Each test meal was supplemented with 125 µM of fat-soluble vitamin D or K

dissolved in the oil. The in vitro digestion experiments were conducted as previously described (Malapert et al., 2018). Aliquots of digesta and mixed micelles were taken and stored at −80 °C under a nitrogen atmosphere until analysis. Bioaccessibility was calculated as the percentage of vitamin recovered in mixed micelles divided by the amount of vitamins recovered in the digesta at the end of the digestion.

2.5. Fat-soluble vitamin absorption by intestinal cells in culture

2.5.1. Preparation of micelles from in vitro digestion

Preliminary cytotoxicity tests were conducted. Briefly, Caco-2 cells were seeded and grown for 3 days in 96-well plates, prior to a 1 h-incubation with diluted micelles obtained from in vitro digestion. The cell culture medium was then removed, and a MTT solution (1 mg/mL) was added for 2 h at 37 °C. The supernatant was then replaced by DMSO (100 µL per well) and the absorbance was measured at 540 nm. In accordance with these tests, fat-soluble vitamin micelles obtained from in vitro digestions were diluted in DMEM at a 1:6 ratio (v:v) for further cell experiments (data not shown).

2.5.2. Cell experiments

Caco-2 clone TC7 cells were cultured as previously described (Goncalves et al., 2016). For each experiment, cells were seeded and grown for 2 weeks in 6-well plates (24 mm diameter, 1 µm-pore-size polycarbonate membrane; Becton Dickinson, Le Pont-de-Claix, France). Twelve hours prior to each experiment, the complete medium (i.e., DMEM supplemented with 16% FBS, 1% non-essential amino acids and 1% antibiotics) was replaced by serum-free complete medium. At the beginning of the experiment, cell monolayers received diluted micelles obtained from in vitro digestions. Cells were incubated for 1h at 37 °C with the micelles. Next, cells were washed with 0.5 mL of ice-cold PBS to eliminated adsorbed fat-soluble vitamins, then scraped and collected in 0.5 mL of ice-cold PBS. Vitamin absorption percentage was calculated by dividing the amount of vitamin recovered in cells by the total

amount of vitamin delivered to the cells (i.e., vitamin found in the medium plus vitamin remaining in the cells at the end of the experiment). All samples were stored at −80 °C until analysis.

2.6. Chickpea lyophilization

The canned chickpeas were first lyophilized for 48h in a Cosmos freeze dryer (Cryotec, Saint-Gély-du-Fesc, France) and then ground into powder using a Proline 700W blender (Proline, Paris, France).

2.7. Bioactive compound assays

2.7.1. Phytate assay

Phytates from chickpea powder were analyzed using a spectrophotometric method derived from that published by Dost & Tokul (Dost & Tokul, 2006). Briefly, phytates were extracted from 200 mg of powder with 10 mL of chloridric acid (0.5 M, Fisher Scientific, Saint-Herblain, France) for 1 h at room temperature (20-25°C). The extract was then centrifuged 5 min at 800×g and the supernatant was recovered. One hundred µL of supernatant was added to 0.9 mL of water and 2 mL of iron (III) chloride hexahydrate. This mixture was mixed for 2h and 30 min at 40 °C and centrifuged 5min at 800×g. The absorbance of the supernatant was measured at 480 nm against water using a Shimadzu UV 8000 spectrophotometer (Shimadzu, Marne-la-Vallée, France). The results were expressed as phytic acid equivalent.

2.7.2. Saponin assay

Saponins were analyzed by a spectrophotometric method inspired by the method previously published by Li and colleagues (Li et al., 2010). The first step was the extraction of saponins from the chickpea powder. One gram of flour was added to 5 mL of methanol (80% in water), mixed for 24 h at room temperature (20-25°C) and centrifuged 5 min at 800×g. This step was

repeated twice. Supernatants (0.2 mL) were completed with 0.3 mL of methanol (80% in water), 0.5 mL of vanillin (8% in water), and 5 mL of sulfuric acid (72%). The mixtures were incubated at 60°C for 10 min and placed into an ice bath. The absorbance of the mixtures was measured at 544 nm against methanol (80% in water). The results were expressed as soyasaponin I equivalent.

2.7.3. Tannin assay

Tannins were extracted from 0.12 g of chickpea powder with 12 mL of water. Two milliliters of a water/methanol mixture (1:1; v:v), 4 mL of tannin extract, and 1 mL of DMACA solution were mixed together. After 20 min, samples were centrifuged 5 min at 800×g and the absorbance of the supernatants was measured at 640 nm against a water/methanol mixture (1:1). The results were expressed as catechin equivalent.

2.8. Lipid, protein, fiber and mineral assays

Lipids were extracted from the chickpea powder. Briefly, 0.5 g of powder, 2 mL of distilled water, and 5 mL of ethyl acetate were mixed for 6 min. The extract was centrifuged 15 min at 1100 × g and the supernatant was recovered. This procedure was repeated once with the addition of ethyl acetate. To wash the extract, 3 mL of water and 0.5 mL of ethanol were added to the supernatant, and the mixture was vortexed for 5 min and centrifuged 10 min at 1100×g. Pooled supernatants were evaporated to dryness under nitrogen. Total lipids were measured by weighing the dry extracts.

The analysis of protein, fibers, magnesium and iron analysis was subcontracted to Phytocontrol Agrifood (Nîmes, France), which is accredited to conduct these specific analyses (Cofrac #1-1904).

2.9. Vitamin assay

2.9.1. Vitamin E (γ-tocopherol) and lutein from chickpeas

Vitamin E (γ-tocopherol) and lutein were extracted from 250 mg of chickpea powder using the following method: 1 mL of distilled water was added to the sample. The internal standard (retinyl acetate) was added to the sample in 1 mL of ethanol. The mixture was extracted twice with 4 mL of hexane. After centrifugation (2500×g, 10 min at 4 ◦C), the hexane phase was evaporated to dryness under nitrogen. The dried extract was dissolved in 200 µL of methanol–dichloromethane (65:35, v:v). A final volume of 180 µL was used for HPLC analysis. Fat-soluble vitamins and carotenoids were separated as previously described (Gleize et al., 2012). Luteine and γ-tocopherol were detected at 445 and 298 nm, respectively, and identified by retention time in comparison with pure standards. Quantification was performed using Chromeleon 6.8 software (ThermoFisher Scientific, Villebon sur Yvette, France) to compare the peak area with standard reference curves.

2.9.2. Vitamin D and vitamin K assay from in vitro digestion and cell samples

Vitamin D and vitamin K were extracted from 200 µL of digesta and mixed micelles, and from 500 µL of apical medium and cells. The internal standard was retinyl acetate. Both extraction and HPLC analysis were performed according to Antoine and al. (Antoine et al., 2021) and Goncalves et al. (Goncalves et al., 2014), respectively. Vitamins were identified by spectral analysis and/or retention time and co-injection in comparison with pure standards. Quantification was performed using Chromeleon 6.8 software to compare the peak area with standard reference curves.

2.9.3. Folate assay

Folates from chickpea powder were analyzed by a HPLC method derived from the method published by Ruggeri and colleagues (Ruggeri et al., 1999). Powder (0.75g) was added to 10.5 mL of extraction buffer in ebullition (HEPES buffer, pH 7.85, containing 2% (w:v) sodium ascorbate and 10 mM 2-mercaptoethanol). After flushing the samples with nitrogen, they were heated for 10 min in a boiling water bath and homogenized with an Ultra Turrax device

for 30 s at a speed of 13500 rpm. Extraction buffer was then added (qsp 15 mL). The pH was adjusted to 4.9 and 600 µL of chicken pancreas conjugase, 120 µL of rat serum and 600 µL of α-amylase were added. Samples were incubated under agitation at 37°C for 3 h. The pH was adjusted to 7.0, then 1.2 mL of protease were added, and samples were incubated for 1h as previously described. Enzymes were then inactivated by boiling for 10 min. Samples were centrifuged for 30 min at 2000×g. Supernatants were transferred to a strong anion exchanger (SAX) column (ThermoFisher Scientific) to purify folates (solid phase extraction). A final volume of 20 µL was used for HPLC analysis. The HPLC system comprised a Dionex separation module (P680HPLC Pump and ASI-100 Automated Sample Injector, Dionex, Aix-en-Provence, France) and a Jasco fluorimetric detector (Lisses, France). Analyses were performed on a HypersilTM ODS C18 column (150×4.6 mm; 5µm) coupled with a 10×4 mm C18-5µm HypersilTM ODS guard column (FisherScientific, Illkirch, France) kept at a constant temperature (40 °C). The mobile phase consisted of a gradient of acetonitrile and 30mM phosphate potassium buffer at pH 2.6. The flow rate was 0.8 mL/min. The run time was 35 min. The gradient profile of the mobile phase was set at 5-95% and changed linearly to 17-83% from minute 9 to 30. The mobile phase then changed back to 5-95% from minute 30 to 35. 5-methyltetrahydrofolate, which is the main form of folate found in chickpeas (Ruggeri et al., 1999), was detected at 356 nm after excitation at 290 nm, and identified by retention time compared with a pure standard. Quantification was performed using Chromeleon software to compare the peak area with standard reference curves.

2.10. Statistics

For all groups, data were expressed as mean ± SEM. Statistical analyses were performed using GraphPad Prism software, version 8.4.3 (GraphPad Software, San Diego, California, U.S.A.). Prior to ANOVA (fixed-effects models), data were tested for equality of variances. The distribution of all dependent variables was graphically checked and was considered

normal. Tukey's test was used as a post-hoc test for pairwise comparisons. Values of $p \leq 0.05$ were considered significant.

3. Results

3.1. Chickpea contents in phytates, saponins and tannins can be decreased by modifying canning parameters

3.1.1. Phytate and saponin contents

Figures 1A and 1B show the results of preliminary tests. We observed a significant decrease of phytate content after hot soaking (PT1), mixed soaking (PT7) and acid soaking (PT9) (-36.0 ± 4.9%, -25.0 ± 7.0%, -25.0 ± 3.2%, $p \leq 0.05$, respectively), but also after soaking with low-ionized water (PT4), with a low chickpeas-to-water ratio (PT6) or after water replacement (PT8) (-19.0 ± 2.6%, -18.0 ± 6.3%, -17.0 ± 2.7%, $p \leq 0.05$, respectively) (Figure 1A). Moreover, Figure 1B shows that phytate content was significantly reduced when the chickpeas were blanched by cascading (PT14, -7.0 ± 1.7%, $p \leq 0.05$).

Concerning saponins, there was no significant diminution of their concentrations when soaking and blanching parameters were modified. Saponin content was slightly reduced when soaking was done with low-ionized water (PT4) or with a low chickpeas-to-water ratio (PT6) (-13.0 ± 3.1%, -10.1 ± 1.2%, respectively), but these values remained non-significantly different (Figure 1 A and 1B).

We then explored the phytate and saponin content in canned and sterilized chickpeas after these different parameters were combined. As shown in Figure 1C, phytate and saponin concentrations were both significantly decreased when chickpeas were canned after i) hot soaking with water replacement followed by immersion blanching (T3) (-26.0 ± 1.9%, -29.0 ± 1.7%, $p \leq 0.05$, respectively), ii) hot mixed soaking followed by cascading blanching (T8) (-37.0 ± 1.6%, -35.0 ± 2.2%, $p \leq 0.05$, respectively), and iii) hot soaking with water replacement followed by cascading blanching (T9) (-28.0 ± 0.7%, -39.0 ± 2.4%, $p \leq 0.05$, respectively). Moreover, we observed a significant reduction of saponin content after soaking at 15 °C with

i) low-ionized water and blanching by cascading (T10), ii) agitation blanching by immersion (T5), iii) water replacement and cascading blanching (T12) and vi) germination (T13) before soaking and blanching (-21.0 ± 2.1%, -17.0 ± 2.1%, -16.0 ± 1.7%, -29.0 ± 6.2%, $p \leq 0.05$, respectively). These 7 conditions were chosen for further analysis.

3.1.2. Tannin content

Our results show that tannin content was reduced in 3 conditions among 7 (Figure 1D): i) hot soaking with water replacement followed by immersion blanching (T3), ii) hot soaking with water replacement followed by cascading blanching (T9), and iii) germinated chickpeas (T13) (-44.1 ± 8.6%, -40.0 ± 7.6%, -43.0 ± 6.8%, $p \leq 0.05$, respectively). However, tannin content increased significantly relative to the control when chickpeas were soaked in hot mixed water and blanched by cascading (T8) (+74.0 ± 20.4%, $p \leq 0.05$).

3.2. The texture of optimized canned chickpeas is different from that of control chickpeas

The texture of the different batches of canned chickpeas was analyzed to check if process modifications changed the chickpea texture compared to the control condition. Table 3 shows that there was a significant increase in chickpea hardness after hot soaking followed by immersion blanching: +43.9 ± 3.8% for hot soaking followed by immersion blanching (T1), +48.4 ± 3.8% for mixed and hot soaking followed by immersion blanching (T2) and +41.9 ± 5.2% for hot soaking with water replacement followed by immersion blanching (T3). Moreover, we observed a significant decrease in chickpea hardness after soaking at 15 °C and blanching by cascading: -33.1 ± 3.1% after soaking at 15 °C with low-ionized water followed by cascading blanching (T10) and -26.2 ± 4.1% after mixed soaking at 15 °C followed by cascading blanching (T11).

3.3. Protein, mineral and vitamin contents can be modified after optimized canning processes compared with control canning.

Table 4 shows the concentrations of proteins, fibers, iron and magnesium, lipids and vitamins in the 7 optimized canned chickpeas and in control chickpeas. There was a reduction in protein content after soaking at 15 °C with i) low-ionized water followed by cascading blanching (T10), ii) agitation followed by an immersion blanching (T5) and iii) a germination step (T13) (-35.8 ± 0.1%, -19.5 ± 0.1%, -23.6 ± 0.1%, $p \leq 0.05$, respectively). The magnesium content decreased similarly (-35.9 ± 0.04%, -21.5 ± 0.05%, -27.6 ± 0.06%, $p \leq 0.05$, respectively), but increased after hot soaking when water is replaced, followed by immersion blanching (T3, +12.8 ± 0.05%, $p \leq 0.05$). Moreover, our results show that relative to the control, iron content was increased after hot soaking with i) water replacement and immersion blanching (T3), ii) agitation and cascading blanching (T8) and iii) water replacement and cascading blanching (T9): +30.7 ± 1.1%, +24.5 ± 0.8%, +40.0 ± 1.6%, $p \leq 0.05$, respectively. We also found a higher iron content in chickpeas soaked in water at 15 °C with either i) low-ionized water (T10) or ii) water replacement (T12), both followed by a cascading blanching (+26.7 ± 0.8%, +67.3 ± 1.1%, $p \leq 0.05$, respectively).

Additionally, we noticed that germination induced a decrease in lipid content (-36.1 ± 4.6%, $p \leq 0.05$) (Table 4).

Vitamin E content significantly increased for 4 conditions relative to the control: +94.2 ± 16.7% for hot soaking with water replacement and cascading blanching (T9), +82.1 ± 17.3% for soaking at 15 °C with low-ionized water followed by cascading blanching (T10), +130.2 ± 14.3% for mixed soaking at 15 °C and immersion blanching (T5), and +102.4 ± 11.2% for soaking at 15 °C with water replacement followed by cascading blanching (T12). Conversely, a significant decrease in lutein content occurred in the germination condition relative to the control condition (-63.8 ± 3.8%, $p \leq 0.05$). Finally, 5-methyltetrahydrofolate content decreased

349 significantly for all conditions (from -12.1± 4.7 % to -97.1± 0.1 %), except for a mixed hot
350 soaking followed by a cascading blanching (T8).
351
352 **3.4. Vitamin D and K bioavailability is not improved in meals containing optimized**
353 **canned chickpeas**
354 As shown in Figure 2A, there was no difference in vitamin D and K bioaccessibility between
355 meals containing control chickpea and meals containing optimized canned chickpeas. Similar
356 results were obtained regarding vitamin D and K uptake by differentiated Caco-2 cells (Figure
357 2B).
358

4. Discussion

Pulse components such as phytates, saponins and tannins have previously been shown to modulate fat-soluble vitamin bioavailability (Antoine et al., 2021; Margier et al., 2019). The objective of this study was to find technological solutions to address this negative effect. We thus modified several parameters of the canning process to decrease phytate, saponin and tannin content in chickpeas.

To this aim, we first modified some process parameters independently of each other. Our results indicate that phytate content can be significantly decreased by modifying soaking or blanching parameters. Under the conditions of our study, this is likely due to an improved release of these compounds, caused by the agitation of chickpeas during soaking, the increase of chickpea coat permeability at 80°C (Tabekhia & Luh, 1980), osmosis for a soaking with low-ionized water (Vijayakumari et al., 1996), a low chickpeas-to-water ratio or with water replacement/ cascading. Moreover, phytates can also be degraded by phytase during either hot or acid soaking, as phytase activity is increased by heat (Khokhar & Chauhan, 1986) and optimized at a pH of 4.6 (Wodzinski & Ullah, 1996).

According to our data, saponin content was not significantly reduced relative to the control condition when a single parameter was changed. A mild reduction in saponin content was observed after soaking with low-ionized water and with a low chickpeas-to-water ratio, reflecting previously published results (Shi et al., 2009), but these differences remained non-significant, likely because of the variability of the results. Yoshiki and colleagues showed that saponin content was decreased after blanching for 30 min (Yoshiki et al., 1998). Our blanching conditions (10 or 20 min) may thus not have been long enough.

After performing preliminary tests using single modifications, we then combined the most effective processes (13 conditions compared with the control process). The decreases in

384 phytate and saponin content were greater than those in preliminary tests because of the
385 combinations of different parameters, followed by the use of hot brine and sterilization, in
386 accordance with previous data (Jood et al., 1986; Tabekhia & Luh, 1980).
387 It is noteworthy that no difference in phytate and saponin contents were observed after
388 germination. According to previous results (Kyriakidis et al., 1998)(El-Adawy, 2002),
389 phytates were expected to decrease due to a rise in phytase activity during germination. We
390 hypothesize that we failed to observe a decrease in phytates due to the method used - as we
391 did not perform a purification step, we may also have detected the product of phytic acid
392 hydrolysis (phosphoric acid) together with the remaining phytic acid. Saponin assay may also
393 detect the hydrolysis product of saponins: sapogenin (Hiai et al., 1976; Price et al., 1986)
394 together with remaining saponins. In addition, Ruiz and colleagues showed a reduction in
395 saponin content 6 days after germination (Ruiz et al., 1996), while our germination period was
396 of only 3 days. Ayet and colleagues observed a decrease in saponin content 3 days after
397 germination, but it was followed by an increase after 6 days (Ayet et al., 1997). Different
398 germination times should thus be tested to fully comprehend these phenomena, and other
399 analytical methods such as mass spectrometry detection should be set up for further
400 investigations.
401 Following these intermediate results, 7 conditions inducing the greatest decreases in both
402 phytate and saponin content were ultimately selected to be fully characterized. Changes in
403 texture were observed between these conditions, but further investigations including a sensory
404 analysis are needed to confirm whether consumers would perceive these changes.
405 The first additional compound evaluated were tannins. A decrease in tannin content was
406 observed for the 2 conditions incorporating water replacement during the soaking step. As
407 tannins are water-soluble, they are likely better released by osmosis during soaking when the
408 water is replaced (Deshpande et al., 1986). A decrease in tannin content was also observed

409 after germination. This is consistent with previous works (Khandelwal et al., 2010)(Rao &
410 Deosthale, 1982): polyphenol oxidase activity increases during germination and induces
411 tannin hydrolysis. Conversely, an increase in tannin content occurred after hot (80 °C) mixed
412 soaking and cascading blanching. We suggest this is due to an inactivation of polyphenol
413 oxidase (Buckow et al., 2009).
414 We then checked whether compounds of interest remained unaltered after modifying the
415 canning process. Fiber content was not modified. Although the presence of fibers can
416 decrease vitamin bioavailability (Margier et al., 2019), its preservation is important because
417 most people lack fiber in their diets (Lairon et al., 2003). In accordance with previous data
418 (El-Adawy, 2002), lipid content decreased after germination only, as sprouting chickpeas use
419 lipids for growth. Lutein content also decreased during germination, presumably for the same
420 reason.
421 Water-soluble compounds such as minerals or proteins were expected to decrease when
422 soaking or blanching were optimized. Protein content decreased moderately under 3
423 conditions (immersion blanching, cascading blanching and germination) that likely increased
424 chickpea moisture and softness. These increases may facilitate the release of water-soluble
425 compounds. Our results differ from previous data (Chitra et al., 1996)(El-Adawy, 2002), but
426 authors compared raw chickpeas with germinated chickpeas.
427 Surprisingly, iron content significantly increased under 4 conditions (as did magnesium
428 content in 1 condition). We suggest that this effect may be due either to i/ lower phytate
429 content, leading to fewer quenched cations, or to ii/ greater disruption of the chickpea matrix,
430 which may favor mineral extraction prior analysis. Vitamin E content also increased under 4
431 conditions. Much as with iron, we suggest that greater softening of the vegetal walls would
432 make vitamin E more extractable, as has been observed in kidney beans (Margier et al., 2018).
433 Finally, 5-methyltetrahydrofolate contents were reduced after most of the optimized processes

relative to the control process. Although heat does not appear to be the main factor in inducing folate loss (Delchier et al., 2013), folates are known to be highly susceptible to leaching during food transformation processes (Scott et al., 2000).

In a second set of experiments, we assessed vitamin bioavailability after in vitro digestion of test meals made with the different batches of canned chickpeas. We focused especially on vitamin D_3 (cholecalciferol) and vitamin K_1 (phylloquinone), as these vitamins have very different structures and thus may behave differently, as previously observed (Antoine et al., 2021; Margier et al., 2019).

No difference was observed between the different conditions in term of bioaccessibility (i.e., the vitamin amount transferred from the solid phase of the meal to the aqueous phase of the digesta during in vitro digestion). No difference was observed between the different conditions in term of cellular uptake by Caco-2 cells in culture, either. This means that the decreases in phytate, saponin and/or tannins contents in optimized canned chickpeas were not important enough to modulate micelle formation, vitamin incorporation in mixed micelles or vitamin membrane transporter functioning. In a previous study, we observed significant differences in terms of vitamin K bioaccessibility and bioavailability when we compared household cooked chickpeas vs canned chickpeas (Margier et al., 2019). The decreases in phytate, saponin, tannin and fiber content were of -24%, -4%, -17,5% and -22%, respectively. We suggest that the difference between the two studies may be due partly to a decrease in fiber content not observed in the present study.

In conclusion, we identified different soaking and blanching parameters in the present study that promoted decreases in phytate, tannin and saponin contents of canned chickpeas (-16 to -44%). Further experiments are required to validate these data in other chickpea varieties and/ or in other pulse species. Additionally, when using these chickpeas in tests meals, we did not observe any improvement in the bioaccessibility and the cellular uptake of vitamins D and K

as compared with the control conditions. Although further modification of the canning process could lead to greater decreases in bioactive amounts, these modifications may be more expensive and less environmentally friendly. We thus suggest that upstream solutions, such as improved cultivar choice, should also be considered.

Acknowledgement: The authors are very grateful to Dr. Youna Hemery (UMR Qualisud, Montpellier, France) for her helpful advices regarding folate assay.

Author contributions: Conceptualization, E.R.; methodology, E.R., S.G., A.L., T.A., C.H.; software, T.A; validation, E.R. and T.A.; formal analysis, T.A., F.A., C.H., GM, SG; writing—original draft preparation, E.R.; writing—review and editing, all authors; funding acquisition, E.R. and S.G. All authors have read and agreed to the published version of the manuscript.

Funding: T. Antoine received a doctoral fellowship from INRAE (National Research Institute for Agriculture, Food and Environment), CTCPA (Technical Center for the Conservation of Agricultural Products) and C2VN (Center for CardioVascular research and Nutrition).

Conflicts of interest: The authors declare no conflict of interest.

480 **References:**

481

482 Antoine, T., Icard-Vernière, C., Scorrano, G., Salhi, A., Halimi, C., Georgé, S., Carrière, F.,
483 Mouquet-Rivier, C., & Reboul, E. (2021). Evaluation of vitamin D bioaccessibility and
484 mineral solubility from test meals containing meat and/or cereals and/or pulses using in vitro
485 digestion. *Food Chemistry*, *347*, 128621. https://doi.org/10.1016/j.foodchem.2020.128621

486 Ayet, G., Burbano, C., Cuadrado, C., Pedrosa, M. M., Robredo, L. M., Muzquiz, M., Cuadra,
487 C. de la, Castaño, A., & Osagie, A. (1997). Effect of Germination, under Different
488 Environmental Conditions, on Saponins, Phytic Acid and Tannins in Lentils (Lens culinaris).
489 *Journal of the Science of Food and Agriculture*, *74*(2), 273-279.
490 https://doi.org/10.1002/(SICI)1097-0010(199706)74:2<273::AID-JSFA800>3.0.CO;2-L

491 Buckow, R., Weiss, U., & Knorr, D. (2009). Inactivation kinetics of apple polyphenol oxidase
492 in different pressure–temperature domains. *Innovative Food Science & Emerging*
493 *Technologies*, *10*(4), 441-448. https://doi.org/10.1016/j.ifset.2009.05.005

494 Chenoll, C., Betoret, N., & Fito, P. (2009). Analysis of chickpea (var. "Blanco Lechoso")
495 rehydration. Part I. Physicochemical and texture analysis. *Journal of Food Engineering*,
496 *95*(2), 352-358. https://doi.org/10.1016/j.jfoodeng.2009.05.014

497 Chitra, U., Singh, U., & Venkateswara Rao, P. (1996). Phytic acid, in vitro protein
498 digestibility, dietary fiber, and minerals of pulses as influenced by processing methods. *Plant*
499 *Foods for Human Nutrition*, *49*(4), 307-316. https://doi.org/10.1007/BF01091980

500 Delchier, N., Ringling, C., Le Grandois, J., Aoudé-Werner, D., Galland, R., Georgé, S.,
501 Rychlik, M., & Renard, C. M. G. C. (2013). Effects of industrial processing on folate content
502 in green vegetables. *Food Chemistry*, *139*(1), 815-824.
503 https://doi.org/10.1016/j.foodchem.2013.01.067

504 Deshpande, S. S., Cheryan, M., Salunkhe, D. K., & Luh, B. S. (1986). Tannin analysis of food
505 products. *C R C Critical Reviews in Food Science and Nutrition*, *24*(4), 401-449.
506 https://doi.org/10.1080/10408398609527441

507 Desmarchelier, C., Borel, P., Lairon, D., Maraninchi, M., & Valéro, R. (2019). Effect of
508 Nutrient and Micronutrient Intake on Chylomicron Production and Postprandial Lipemia.
509 *Nutrients*, *11*(6), 1299. https://doi.org/10.3390/nu11061299

510 Dost, K., & Tokul, O. (2006). Determination phytic acid in wheat and wheat products by
511 reverse phase high performance liquid chromatography. *Analytica Chimica Acta*, *558*, 22-27.
512 https://doi.org/10.1016/j.aca.2005.11.035

513 El-Adawy, T. A. (2002). Nutritional composition and antinutritional factors of chickpeas
514 (Cicer arietinum L.) undergoing different cooking methods and germination. *Plant Foods for*
515 *Human Nutrition*, *57*(1), 83-97. https://doi.org/10.1023/A:1013189620528

516 Gleize, B., Steib, M., André, M., & Reboul, E. (2012). Simple and fast HPLC method for
517 simultaneous determination of retinol, tocopherols, coenzyme Q(10) and carotenoids in
518 complex samples. *Food Chemistry*, *134*(4), 2560-2564.
519 https://doi.org/10.1016/j.foodchem.2012.04.043

520 Goncalves, A., Margier, M., Roi, S., Collet, X., Niot, I., Goupy, P., Caris-Veyrat, C., &
521 Reboul, E. (2014). Intestinal Scavenger Receptors Are Involved in Vitamin K1 Absorption.
522 *Journal of Biological Chemistry*, *289*(44), 30743-30752.
523 https://doi.org/10.1074/jbc.M114.587659

524 Goncalves, A., Margier, M., Tagliaferri, C., Lebecque, P., Georgé, S., Wittrant, Y., Coxam,
525 V., Amiot, M.-J., & Reboul, E. (2016). Pinoresinol of olive oil decreases vitamin D intestinal
526 absorption. *Food Chemistry*, *206*, 234-238. https://doi.org/10.1016/j.foodchem.2016.03.048

527 Hiai, S., Oura, H., & Nakajima, T. (1976). Color reaction of some sapogenins and saponins
528 with vanillin and sulfuric acid. *Planta Medica*, *29*(2), 116-122. https://doi.org/10.1055/s-
529 0028-1097639

530 Jood, S., Chauhan, B. M., & Kapoor, A. C. (1986). Saponin content of chickpea and black
531 gram : Varietal differences and effects of processing and cooking methods. *Journal of the*
532 *Science of Food and Agriculture*, *37*(11), 1121-1124. https://doi.org/10.1002/jsfa.2740371111

533 Khandelwal, S., Udipi, S. A., & Ghugre, P. (2010). Polyphenols and tannins in Indian pulses :
534 Effect of soaking, germination and pressure cooking. *Food Research International*, *43*(2),
535 526-530. https://doi.org/10.1016/j.foodres.2009.09.036

536 Khokhar, S., & Chauhan, B. M. (1986). Antinutritional factors in Moth Bean (Vigna
537 aconitifolia) : Varietal Differences and Effects of Methods of Domestic Processing and
538 Cooking. *Journal of Food Science*, *51*(3), 591-594. https://doi.org/10.1111/j.1365-
539 2621.1986.tb13887.x

Kyriakidis, N. B., Galiotou-Panayotou, M., Stavropoulou, A., & Athanasopoulos, P. (1998). Increase in Phytase Activity and Decrease in Phytate During Germination of Four Common Legumes. *Biotechnology Letters*, *20*(5), 475-478. https://doi.org/10.1023/A:1005488112582

Lairon, D., Bertrais, S., Vincent, S., Arnault, N., Galan, P., Boutron, M.-C., & Hercberg, S. (2003). Dietary fibre intake and clinical indices in the French Supplementation en Vitamines et Minéraux AntioXydants (SU.VI.MAX) adult cohort. *Proceedings of the Nutrition Society*, *62*(1), 11-15. https://doi.org/10.1079/PNS2002210

Lee, D. P. S., Low, J. H. M., Chen, J. R., Zimmermann, D., Actis-Goretta, L., & Kim, J. E. (2020). The Influence of Different Foods and Food Ingredients on Acute Postprandial Triglyceride Response : A Systematic Literature Review and Meta-Analysis of Randomized Controlled Trials. *Advances in Nutrition (Bethesda, Md.)*, *11*(6), 1529-1543. https://doi.org/10.1093/advances/nmaa074

Lestienne, I., Besançon, P., Caporiccio, B., Lullien-Péllerin, V., & Tréche, S. (2005). Iron and zinc in vitro availability in pearl millet flours (Pennisetum glaucum) with varying phytate, tannin, and fiber contents. *Journal of Agricultural and Food Chemistry*, *53*(8), 3240-3247. https://doi.org/10.1021/jf0480593

Li, J., Zu, Y.-G., Fu, Y.-J., Yang, Y.-C., Li, S.-M., Li, Z.-N., & Wink, M. (2010). Optimization of microwave-assisted extraction of triterpene saponins from defatted residue of yellow horn (Xanthoceras sorbifolia Bunge.) kernel and evaluation of its antioxidant activity. *Innovative Food Science & Emerging Technologies*, *11*(4), 637-643. https://doi.org/10.1016/j.ifset.2010.06.004

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*(11), 1653. https://doi.org/10.3390/nu10111653

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*(11), 1801323. https://doi.org/10.1002/mnfr.201801323

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 :

571 Characterization of Pulses Frequently Consumed in France and Effect of the Cooking
572 Method. *Nutrients*, *10*(11), 1668. https://doi.org/10.3390/nu10111668

573 Marrelli, M., Conforti, F., Araniti, F., & Statti, G. A. (2016). Effects of Saponins on Lipid
574 Metabolism : A Review of Potential Health Benefits in the Treatment of Obesity. *Molecules*
575 *(Basel, Switzerland)*, *21*(10), E1404. https://doi.org/10.3390/molecules21101404

576 Price, K. R., Curl, C. L., & Fenwick, G. R. (1986). The saponin content and sapogenol
577 composition of the seed of 13 varieties of legume. *Journal of the Science of Food and*
578 *Agriculture*, *37*(12), 1185-1191. https://doi.org/10.1002/jsfa.2740371206

579 Rao, P. U., & Deosthale, Y. G. (1982). Tannin content of pulses : Varietal differences and
580 effects of germination and cooking. *Journal of the Science of Food and Agriculture*, *33*(10),
581 1013-1016. https://doi.org/10.1002/jsfa.2740331012

582 Ruggeri, S., Vahteristo, L. T., Aguzzi, A., Finglas, P., & Carnovale, E. (1999). Determination
583 of folate vitamers in food and in Italian reference diet by high-performance liquid
584 chromatography. *Journal of Chromatography A*, *855*(1), 237-245.
585 https://doi.org/10.1016/S0021-9673(99)00674-3

586 Ruiz, R. G., Price, K., Rose, M., Rhodes, M., & Fenwick, R. (1996). A preliminary study on
587 the effect of germination on saponin content and composition of lentils and chickpeas.
588 *Zeitschrift Für Lebensmittel-Untersuchung Und Forschung*, *203*(4), 366-369.
589 https://doi.org/10.1007/BF01231075

590 Schoeneck, M., & Iggman, D. (2021). The effects of foods on LDL cholesterol levels : A
591 systematic review of the accumulated evidence from systematic reviews and meta-analyses of
592 randomized controlled trials. *Nutrition, Metabolism, and Cardiovascular Diseases: NMCD*,
593 *31*(5), 1325-1338. https://doi.org/10.1016/j.numecd.2020.12.032

594 Scott, J., Rébeillé, F., & Fletcher, J. (2000). Folic acid and folates : The feasibility for
595 nutritional enhancement in plant foods. *Journal of the Science of Food and Agriculture*, *80*(7),
596 795-824. https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<795::AID-
597 JSFA599>3.0.CO;2-K

598 Shi, J., Xue, S. J., Ma, Y., Li, D., Kakuda, Y., & Lan, Y. (2009). Kinetic study of saponins B
599 stability in navy beans under different processing conditions. *Journal of Food Engineering*,
600 *93*(1), 59-65. https://doi.org/10.1016/j.jfoodeng.2008.12.035

601 Tabekhia, M. M., & Luh, B. S. (1980). Effect of Germination, Cooking, and Canning on
602 Phosphorus and Phytate Retention in Dry Beans. *Journal of Food Science*, *45*(2), 406-408.
603 https://doi.org/10.1111/j.1365-2621.1980.tb02631.x

604 Vijayakumari, K., Siddhuraju, P., & Janardhanan, K. (1996). Effect of soaking, cooking and
605 autoclaving on phytic acid and oligosaccharide contents of the tribal pulse, Mucuna
606 monosperma DC. ex. Wight. *Food Chemistry*, *55*(2), 173-177. https://doi.org/10.1016/0308-
607 8146(95)00081-X

608 Wodzinski, R. J., & Ullah, A. H. (1996). Phytase. *Advances in Applied Microbiology*, *42*,
609 263-302. https://doi.org/10.1016/s0065-2164(08)70375-7

610 Yoshiki, Y., Kudou, S., & Okubo, K. (1998). Relationship between Chemical Structures and
611 Biological Activities of Triterpenoid Saponins from Soybean. *Bioscience, Biotechnology, and*
612 *Biochemistry*, *62*(12), 2291-2299. https://doi.org/10.1271/bbb.62.2291

613

614

615 **Figure legends**

616

617 **Figure 1: Bioactive compound contents in canned chickpeas.**

618 ***A:*** *Phytates and saponin content under different soaking conditions after preliminary tests.* ***B:***
619 *Phytate and saponin content under different blanching conditions after preliminary tests*.
620 During preliminary tests (PT), canning process parameters were modified independently.
621 Phytate (white bars) and saponin (black bars) contents were analyzed in chickpea powder
622 after each step, but not in the final product. Soaking was done with hot (80°C), acidic (pH 5),
623 alkaline (pH 9), mixed, low- (HT = 6 °f) and high-ionized (HT = 184 °f) water, but also in
624 different chickpeas-to-water ratios (2:3 or 1:10), with water replacement and for a longer time
625 (24h). Blanching was tested for a longer time (20 min) and with different methods (cascading
626 or steaming). Data are expressed as mean ± SEM (n = 3).
627 ***C:*** *Phytate and saponin content in canned chickpeas.* Different processes combining the most
628 efficient soaking and blanching parameters were tested (T). Contrary to preliminary tests,
629 phytate (white bars) and saponin (black bars) contents were analyzed on the final product
630 powders, after conditioning and sterilization. Data are expressed as mean ± SEM (n = 9).
631 ***D:*** *Tannin content in canned chickpeas.* Once optimized conditions (low phytate and saponin
632 content) were chosen, tannins were analyzed in chickpea powders. Data are expressed as
633 mean ± SEM (n = 9).
634 A difference of $p \leq 0.05$ was considered significant. Cp: Control Process; Germ: Germination.
635 dm = dry matter.

636

637

638 **Figure 2. Vitamin D and K bioavailability in the presence of optimized chickpeas**

A: Vitamin D and K bioaccessibility. Test meals were made with the different canned chickpeas and supplemented with vitamin D or vitamin K. Bioaccessibility was calculated as the percentage of the vitamins recovered in mixed micelles divided by the vitamin amount recovered from the digesta at the end of the digestion.

B: Vitamin D and K intestinal absorption. Mixed micelles obtained from in vitro digestions were delivered to Caco2-TC7 cells after appropriate dilution. The apical side and the well monolayers were collected after 1h of incubation. The vitamin absorbed was calculated as the percentage of the vitamins recovered from the cells divided by the total vitamin amount delivered to the cells .

Vitamin D: white bars; vitamin K: black bars; CP: Control Process; Germ: Germination. Data are expressed as mean ± SEM (n = 6). A difference of $p \leq 0.05$ was considered significant.

Tables

Table 1: Chickpea soaking and blanching parameters for preliminary experiments

	SOAKING												BLANCHING				
Parameters	**CP**	**PT 1**	**PT 2**	**PT 3**	**PT 4**	**PT 5**	**PT 6**	**PT 7**	**PT 8**	**PT 9**	**PT 10**	**PT 11**	**Parameters**	**CP**	**PT 12**	**PT 13**	**PT 14**
Temperature (°C)	15	**80**	15	15	15	15	15	15	15	15	15	15	**Temperature (°C)**	90	90	90	90
Time (h)	14	14	**24**	14	14	14	14	14	14	14	14	14	**Time (min)**	5	**20**	5	5
Water hardness (°f)	30	30	30	**184**	**6**	30	30	30	30	30	30	30	**Mode**	Immersion		**Steaming**	**Cascading**
Chickpeas-to-water ratio	1:3	1:3	1:3	1:3	1:3	**2:3**	**1:10**	1:3	1:3	1:3	1:3	1:3	**-**	**-**	**-**	**-**	**-**
Agitation	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**Yes**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Water replacement	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**Yes**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Additive	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**Acid**	**Base**	**-**	**-**	**-**	**-**	**-**	**-**
Germination	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**Yes**	**-**	**-**	**-**	**-**	**/**

Different preliminary tests (PT) were done by modifying single soaking and blanching parameters of the control process (CP).

Table 2: Optimization tests on global process

		Control	T 1	T 2	T 3	T 4	T 5	T 6	T 7	T 8	T 9	T 10	T 11	T 12	T 13
Soaking	**Temperature (°C)**	15	**80**	**80**	**80**	15	15	15	**80**	**80**	**80**	15	15	15	15
	Time (h)	14	**2**	**2**	**2**	14	14	14	**2**	**2**	**2**	14	14	14	14
	Water hardness (°f)	30	30	30	30	**6**	30	30	30	30	30	**6**	30	30	30
	Chickpeas-to-water ratio	1/3	**1/10**	**1/10**	**1/10**	1/3	1/3	1/3	**1/10**	**1/10**	**1/10**	1/3	1/3	1/3	1/3
	Mixing	No	No	**Yes**	No	No	**Yes**	No	No	**Yes**	No	No	**Yes**	No	No
	Water replacement	No	No	No	**Yes**	No	No	**Yes**	No	No	**Yes**	No	No	**Yes**	No
	Additive	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	Germination	No	No	No	No	No	No	No	No	No	No	No	No	No	**Yes**
Blanching	**Temperature (°C)**	90													
	Time (min)	5													
	Mode	Immersion							Cascading						Immer-sion
Conditioning in a can (200 g), and hot brine (0.5% salt, 70 °C) at a ratio of 86:114 (w:w)															
Autoclave sterilization at 10 rpm and 127 °C for 6 min															

Different tests (T) based on the results of preliminary tests and combining the most efficient soaking and blanching parameters were performed. Six soaking conditions were defined, followed either by immersion blanching or cascading blanching. Conditioning and sterilization parameters remained unchanged.

664 Table 3. Canned chickpea texture

665

	Texture Maximal applied force (N)
Control	9.58 ± 0.5
T1	13.77 ± 0.4 *
T2	14.02 ± 0.4*
T3	13.60 ± 0.5*
T4	8.33 ± 0.4
T5	8.60 ± 0.6
T6	12.10 ± 0.9
T7	10.14 ± 0.3
T8	10.00 ± 0.4
T9	9.50 ± 0.4
T10	6.53 ± 0.3*
T11	7.07 ± 0.4*
T12	8.50 ± 0.55

666

667 A compression force was applied on the pulses using an Uniaxial Microsystem TA XT texture
668 analyzer to determine the texture (compression forces are positively correlated with seed
669 hardness). Data are expressed as mean ± SEM (n = 30). A difference of $p \leq 0.05$ was
670 considered significant.

671

672

673

674 **Table 4. Protein, lipid, fiber, mineral, fat-soluble vitamin and folate content in canned**

675 **chickpeas**

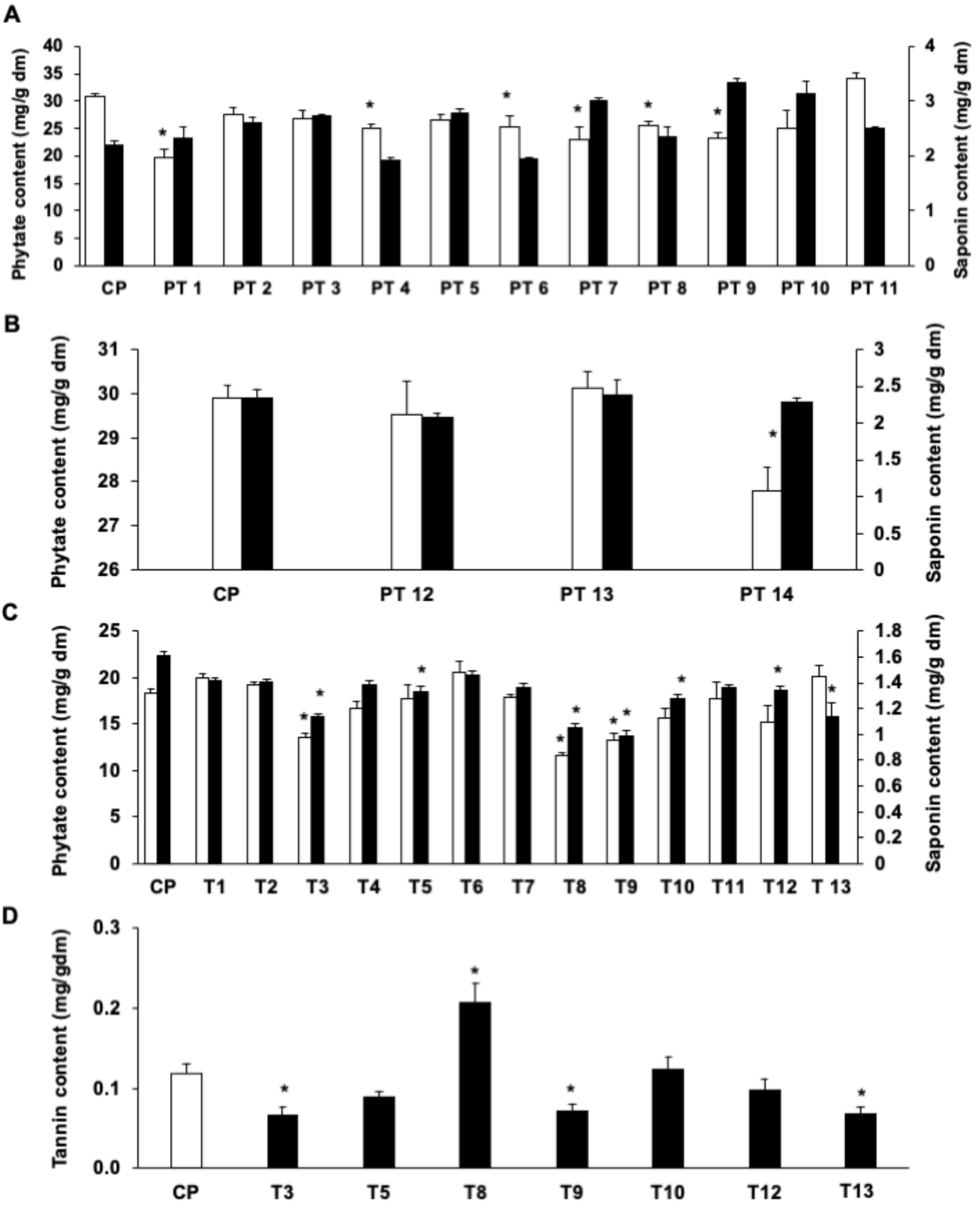
	Control process	T3	T5	T8	T9	T10	T12	T13
Proteins (g/100g dm)	23.1 ± 1.0	23.5 ± 1.1	18.6 ± 0.9*	21.4 ± 0.1	22.2 ± 1.0	14.8 ± 0.7*	22.1 ± 1.0	17.6 ± 0.8*
Total lipids (g/100g dm)	5.0 ± 0.1	4.8 ± 0.2	6.4 ± 1.6	6.0 ± 0.6	5.40 ± 0.05	5.1 ± 0.1	4.0 ± 0.3	3.2 ± 0.2*
Total fibers (g/100g dm)	14.9 ± 2.0	15.7 ± 1.9	14.8 ± 2.1	16.6 ± 2.0	14.1 ± 1.9	16.6 ± 2.0	14.9 ± 2.01	14.6 ± 1.9
Vitamin E (mg/100g dm)	2.2 ± 0.4	3.0 ± 0.3	5.2 ± 0.3*	3.1 ± 0.1	4.3 ± 0.4*	4.1 ± 0.4*	4.6 ± 0.3*	2.3 ± 0.3
Lutein (µg/100g dm)	226.7± 36.0	198.1 ± 20.8	321.4 ± 34,1	158.7 ± 5.1	140.7 ± 18.8	255.3 ± 38.5	220.0 ± 17.1	82.1 ± 8.6*
5-methyl-tetrahydro-folate (µg/100g dm)	32.1 ± 9.6	6.4 ± 1.4*	0.9 ± 0.1*	28.2 ± 1.5	1.8 ± 0.3*	3.5 ± 0.8*	1.8 ± 0.4*	4.9 ± 1.3*
Magnesium (mg/100g dm)	131.6 ± 9.5	148.5 ± 10.7*	103.3 ± 7.3*	122.9 ± 8.9	140.7 ± 10.1	84.4 ± 6.0*	116.6 ± 8.3	95.2 ± 6.7*
Iron (mg/100g dm)	32.2 ± 1.7	42.0 ± 1.6*	32.5 ± 1.7	40.0 ± 1.7*	45.0 ± 3.2*	40.7 ± 1.7*	53.8 ± 3.5*	27.2 ± 1.9
Dry matter (g/ 100g)	34.2	35.7	33.9	34.9	35.5	34.4	33.4	35.7

676

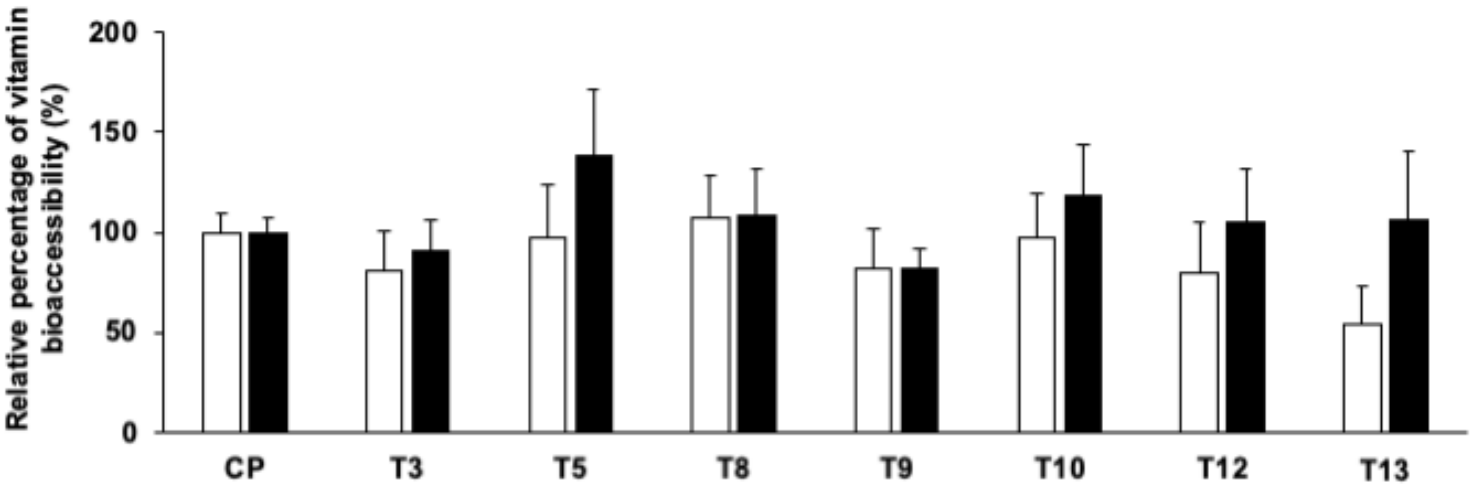
677 The nutritional characterization of canned chickpeas was performed on chickpeas processed
678 according to either the control process (CP) or our 7 optimized conditions (See Table 2). dm =
679 dry matter. Data are expressed as mean ± SEM (n = 3). A difference of $p \leq 0.05$ was
680 considered significant.

681

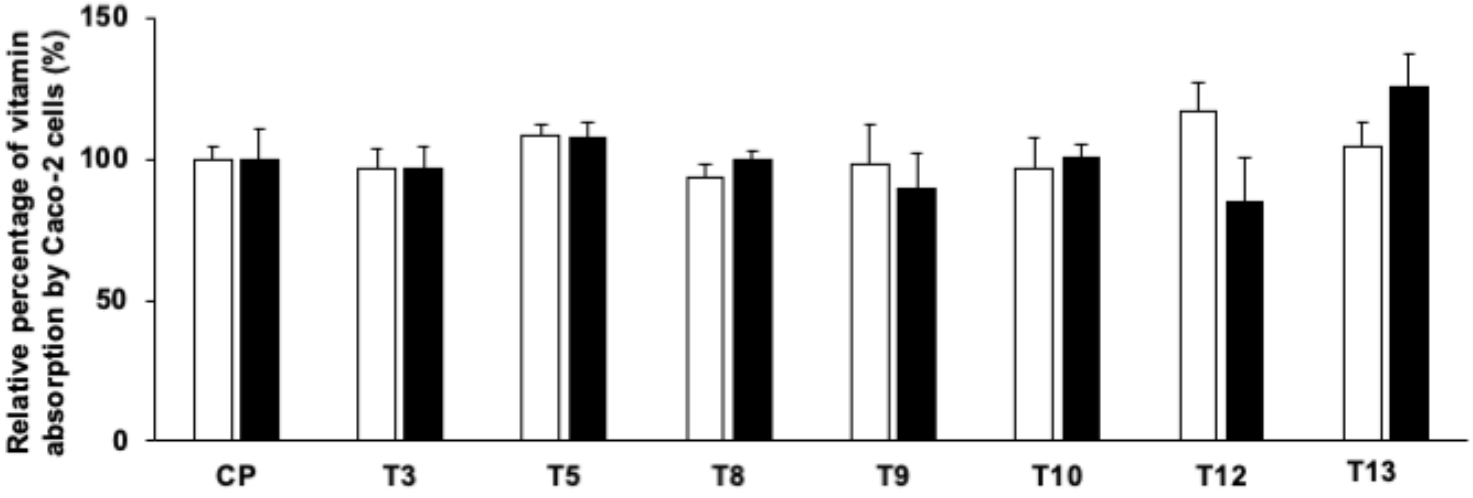
682


A
Phytate content (mg/g dm)
Saponin content (mg/g dm)
CP
PT 1
PT 2
PT 3
PT 4
PT 5
PT 6
PT 7
PT 8
PT 9
PT 10
PT 11
B
Phytate content (mg/g dm)
Saponin content (mg/g dm)
CP
PT 12
PT 13
PT 14
C
Phytate content (mg/g dm)
Saponin content (mg/g dm)
CP
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
T11
T12
T 13
D
Tannin content (mg/gdm)
CP
T3
T5
T8
T9
T10
T12
T13

A


Relative percentage of vitamin bioaccessibility (%)
200
150
100
50
0
CP
T3
T5
T8
T9
T10
T12
T13

B


Relative percentage of vitamin absorption by Caco-2 cells (%)
150
100
50
0
CP
T3
T5
T8
T9
T10
T12
T13