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ORIGINAL ARTICLE

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Influence of Soaking Duration and Temperature on the Physicochemical and Functional Properties of a Tunisian Chickpea Cultivar

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ABSTRACT

Background: Chickpea (*Cicer arietinum L.)* is a widely consumed legume renowned for its high nutritional value. However, its utilization is constrained by the presence of antinutritional factors, such as phytates and tannins, which may compromise nutrient bioavailability. Thermal processing, particularly soaking conditions, has been reported as an effective approach for enhancing nutritional quality and reducing antinutritional compounds.

Aims: This study aimed to evaluate the effect of thermal parameters—specifically soaking time and temperature—on the nutritional and antinutritional quality of chickpeas, and to optimize these conditions utilizing a statistical experimental design.

Patients and Methods: A Central Composite Design (CCD) was implemented employing Design-Expert software to assess the combined effects of soaking temperature (20 °C, 40 °C, and 60 °C) and soaking time (6 h, 15 h, and 24 h). Chickpea samples were analyzed for physicochemical properties (dry matter, pH, and conductivity), nutritional components (crude protein and fiber contents), antinutritional factors (phytates and condensed tannins), and functional properties, including swelling and hydration capacities.

Results: Physicochemical parameters remained relatively stable across all thermal treatments. In contrast, soaking at 20 °C for 24 h resulted in increased protein and fiber contents, accompanied by a significant reduction in antinutritional factors, with decreases of 29.68% in phytates and 33.42% in condensed tannin content. Furthermore, functional properties, including swelling and hydration capacities were significantly improved under these optimized conditions.

Conclusions: Optimization of soaking time and temperature constitutes an effective strategy for enhancing the nutritional and functional properties of chickpeas while substantially reducing antinutritional factors. These findings underscore the potential of controlled thermal treatments to improve chickpea quality for food applications.

Keywords: Chickpea; Thermal Treatment; Soaking Parameters; Nutritional Quality; Antinutritional Factors.

Article Information

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1 INTRODUCTION

Legumes, members of the Fabaceae family—including the common bean (*Phaseolus vulgaris*), white bean (*Vicia faba*), pea (*Pisum sativum* L.), lentils (*Lens culinaris*), lupin (*Lupinus* spp.), soybeans (*Glycine max*) and chickpeas (*Cicer arietinum*)—are widely recognized for their exceptional nutritional profile. These legumes are characterized by high levels of starch (40 – 55%), protein (18 – 40%) and dietary fiber (2 – 30%), as well as lower levels of fat (0.4 – 10.0%) predominantly rich in monounsaturated and polyunsaturated fatty acids, essential minerals and vitamins. However, legumes synthesize diverse antinutritional factors (ANFs) that can impair the digestibility and intestinal absorption of dietary proteins and carbohydrates, or function as divalent metal chelators. Furthermore, specific ANFs, such as condensed tannins, may induce toxicological responses or impart an undesirable bitterness to food matrices (Kumar *et al.*, 2022; Samtiya *et al.*, 2020; Semba *et al.*, 2021). These ANFs encompass protease and amylase inhibitors, lectins, tannins, phytic acid, oxalates, polyphenolic compounds, saponins, and α-galacto-oligosaccharides (α-GOS) such as raffinose, stachyose, and verbascose (Das *et al.*, 2022; Kumar *et al.*, 2022; Verma *et al.*, 2021). Although certain ANFs exhibit health-promoting bioactive or phytochemical properties when consumed in moderation, their partial elimination or reduction remains highly desirable to reduce their overarching adverse nutritional impacts (Cid-Gallegos *et al.*, 2022; Corzo-Rios *et al.*, 2020; Corzo-Ríos *et al.*, 2022; Martín-Cabrejas, 2019).

To this end, various processing methodologies have been evaluated to reduce or eradicate these ANFs, including soaking, thermal treatments, germination, and fermentation (Corzo-Ríos *et al.*, 2020; Corzo-Ríos *et al.*, 2021; Corzo-Ríos *et al.*, 2022; Das *et al.*, 2022). Among these post-harvest pre-treatments, soaking represents one of the most ubiquitous interventions due to its operational simplicity, economic viability, and efficacy in improving hydration and functional properties (Khattab & Arntfield, 2009). Historically, implemented to minimize subsequent cooking times, soaking initiates seed rehydration, thereby activating endogenous hydrolytic enzymes. This hydrothermal operation simultaneously facilitates the leaching of water-soluble, hydrophilic ANFs (such as phytates, tannins, protease inhibitors, α-GOS, and saponins) into the aqueous medium, effectively decreasing their concentration (Das *et al.*, 2022; Samtiya *et al.*, 2020).

The influx of water disrupts the seed coat matrix, softens cell-wall polysaccharides, and optimizes subsequent cooking, extraction, or milling. Process temperature and duration constitute the critical parameters governing hydration kinetics: lower temperatures favor gradual hydration and preserve structural integrity, whereas elevated temperatures accelerate water mass uptake at the risk of inducing partial solubilization of structural polysaccharide and thermal modification of storage proteins (Kaptso *et al.*, 2008). Furthermore, the cross-interaction between soaking duration and temperature can influence the final techno-functional attributes of the seed, including swelling index, hydration capacity, and textural characteristics. Precise modulation of these operational conditions yields a reproducible mechanism to modify the physicochemical characteristics of chickpeas, presenting a strategic lever for both domestic culinary preparations and large-scale industrial food formulations (Thakur *et al.*, 2021).

Nevertheless, systematic investigations outlining the interactive effects of soaking duration and temperature remain scarce. To the best of our knowledge, no published literature has optimized these specific operational conditions for Tunisian Kabuli cultivars, presenting a prominent gap in empirical knowledge. Evaluating physical indicators, such as electrical conductivity and swelling capacity, provides critical insights into these underlying structural dynamics: electrical conductivity serves as an indirect measure of cell membrane integrity and the corresponding efflux of intracellular solutes, whereas swelling kinetics reflect water absorption dynamics that directly govern subsequent softening, textural attributes, and the overall sensory acceptability of chickpea-derived food products. Elucidating these relationships allows for the fine-tuning of processing parameters to maximize both nutritional benefits while optimizing culinary attributes (Johnny *et al.*, 2015).

On the other hand, extensive agronomic research has been undertaken in Tunisia to develop novel chickpea cultivars capable of mitigating contemporary agricultural challenges, including shifting market demands, climate variability, and disease pressure. These selective breeding programs target not only yield optimization but also enhanced grain quality, boost stability, and the promotion of sustainable cropping practices in addition to increasing production (Amri *et al.*, 2016). Within this developmental framework, the winter chickpea cultivar 'Rebha' (FLIP03-27C-A1) stands out as a significant agronomic milestone. This variety ensures stable production under adverse environmental conditions by combining tolerance to major viral infections with robust resistance to Fusarium wilt (*Fusarium oxysporum*) and partial resistance to Ascochyta blight (*Ascochyta rabiei*). Moreover, because of its erect growth habit, 'Rebha' exhibits an excellent yield potential (20 – 28 q ha^{-1}), broad agro-ecological adaptation across northern and central territories, and optimal compatibility with mechanical harvesting. Its relatively high hundred-seed weight (42 – 44 g per 100 seeds) enhances its commercial value, making it positioning this cultivar as a pivotal asset for increasing smallholder revenue and strengthening the sustainability of the Tunisian legume sector (Amri *et al.*, 2016).

Consequently, the primary objective of the present study was to systematically evaluate the impact of various soaking parameters (durations and temperature) on the nutritional and antinutritional profiles of chickpea seeds belonging to the 'Rebha' cultivar. This research aims to optimize the time–temperature interaction to maximize the retention of beneficial macronutrients and phytochemicals while concurrently minimizing specific antinutritional factors such as phytates and condensed tannins. Beyond its fundamental scientific contribution, this study seeks to offer the agri-food industry with empirically validated pre-processing conditions designed to elevate product quality, functional value, and marketability, while simultaneously translating these findings into actionable guidelines to optimize chickpea digestibility and nutritional bioaccessibility at the domestic level.

2 Material and Methods

2.1 Plant Material and Sampling

The plant matrix evaluated in this study comprised Kabuli-type chickpea seeds (*Cicer arietinum* L.) belonging to the 'Rebha' cultivar (pedigree registration code: FLIP03-27C-A1). This specific winter chickpea variety was jointly released in 2015 by the National Institute of Agronomic Research of Tunisia (INRAT), the Center of Regional Agricultural Research (CRRGC), and the International Center for Agricultural Research in the Dry Areas (ICARDA), originating from the complex lineage pedigree

(X98TH86 / [ILC267 x FLIP89-4C) x HB-1] x S95345) (Amri *et al.*, 2016). The seed samples were provided by the Field Crops Laboratory at INRAT, where they were harvested in July 2022 and preserved under dry, dark controlled storage conditions until subsequent processing and analysis.

2.2 Hydrothermal Soaking Pre-treatments

Seed hydration kinetics were regulated by adjusting two core operational variables: soaking duration and process temperature. The chickpea seeds were subjected to various time-temperature combinations as dictated by the experimental matrix Table 1, generated via Design-Expert software (version 11.1.2.0, Stat-Ease Inc., Minneapolis, MN, USA). The specific operational condition of 24 hours at 25.85 °C corresponds to the optimized treatment identified at the conclusion of this study rather than a baseline processing protocol. To maintain structural and biochemical integrity, all downstream physical, nutritional, and antinutritional characterizations were performed immediately post-hydration on wet matrices without any preliminary hot-air or freeze-drying steps. Consequently, all mass-dependent analytical data are explicitly expressed on an "as-is" wet weight basis, reflecting the true composition of the seed in its processed state.

Table 1. Experimental Matrix of the Central Composite Design Employed for Thermal Treatment Optimization

Experiences	Time (hours)	Temperature / Actual Values (°C)
1	15.0	40.0
2	6.0	20.0
3	24.0	20.0
4	15.0	40.0
5	15.0	40.0
6	24.0	40.0
7	15.0	11.7
8	15.0	40.0
9	15.0	40.0
10	15.0	68.3
11	2.3	40.0
12	6.0	60.0
13	27.7	40.0

2.3 Physicochemical Characterizations

Fluctuations in total dry matter (DM) content were tracked to evaluate solute migration and organic matter dissolution induced by the soaking treatments. These measurements were carried out utilizing a halogen moisture analyzer (Model MA 210.R, RADWAG, Radom, Poland) by exposing a 3 g crushed sample aliquot to a constant temperature of 150° C for 10 min, under atmospheric pressure.

Potentiometric determination of pH was conducted as a function of soaking duration and temperature according to the standard method of the Association of Official Analytical Chemists (Method 943.02; AOAC, 1995) to measure changes in hydrogen ion activity within the seed matrix.

To evaluate electrolyte leakage and relative mineral dissolution dynamics into the soaking medium, the electrical conductivity kinetics of the chickpea seeds were monitored following the method described by Aubert (1978). Post-hydration, the seeds were gently drained, and the conductivity of the decanted soaking water was immediately determined using a digital conductivity meter (Model ADWA AD3000 Instruments, Szeged, Hungary).

2.4 Functional Factors

The hydration capacity, also referred to as the rate of water absorption, was determined according to the method of Zia *et al.* (2007). A batch of 100 pre-weighed seeds was immersed in distilled water at a fixed 1:10 (w/v) ratio at room temperature (22.2 °C) for 30 min. Consequently, the hydration capacity was calculated by taking the difference in weight before and after soaking and dividing it by the number of seeds as follows:

$$Hydration\ capacity = \frac{P1 - P0}{N}$$

With:

- *P_o represents the mass of the grains prior to hydration (g).*
- *P_1 represents the mass of the grains post-hydration (g).*
- *N denotes the total number of seeds (N = 100)*

The swelling capacity was evaluated using the method established by Zia *et al.* (2007). Aliquots of 100g of seed samples were submerged in 1000 mL of distilled water for a designated duration. The swelling capacity was calculated based on the net volume difference per seed via the following equation:

$$Swilling\ capacity\ (mL\ H2O/seed) = \frac{V_1 - V_o}{N}$$

With:

- *V_o denotes volume of the grains prior soaking.*
- *V_1 denotes Volume of the grains post-soaking.*
- *N represents the total seed count.*

2.5 Quantitative Nutritional Essays

Total nitrogen and crude protein content were quantified via the classical macro-Kjeldahl digestion protocol (Kjeldahl, 1883), applying a standard nitrogen-to-protein conversion factor of 6.25. Conversely, temporal variations in the concentration of soluble protein content were measured via the colorimetric Bradford assay (Bradford, 1976), employing a UV-Visible spectrophotometer (Shimadzu Corp., Kyoto, Japan) at an absorbance wavelength of 595 nm. Crude fiber fraction was isolated employing the method outlined by Pádua *et al.* (2004). Subsequently, the rate of variation depending on soaking parameters was monitored.

2.6 Quantification of Antinutritional Factors

Because raw chickpea seeds contain specific antinutritional factors (ANFs) that can impair protein digestibility and restrict micronutrient bioaccessibility, profiling the degradation kinetics of these compounds under different soaking parameters is required to optimize the processing matrix.

The concentration of condensed tannins was determined via the vanillin-HCl reagent assay (Schofield *et al.*, 2001) and the reduction of these compounds was monitored based on the soaking parameters. Phytic acid (phytate) concentrations were quantified via an adapted indirect colorimetric method (Ellis *et al.*, 1977). This involved a titration of the sample extract with a 0.05% ferric chloride ($FeCl_3$) solution prepared in 0.6% HCl, employing sodium salicylate as the colorimetric indicator. The analytical assay was executed on clear supernatants collected further centrifugation of a chickpea flour and HCl homogenate, followed by tracking the modifications in phytate levels after treatment. All analytical tests were performed in triplicate (n=3).

2.7 Experimental Design

To establish the optimal time and temperature for soaking chickpea seeds, preliminary tests were conducted across three distinct soaking durations: 6 hours, 15 hours, and 24 hours. The chickpeas were soaked in distilled water at a fixed 1:10 (w/v) ratio, and a constant baseline temperature of 20 °C. This initial phase enabled the establishment of the upper and lower limits required to construct the central composite response surface design within Design-Expert software (version 11.1.2.0).

The software proposed a matrix consisting of 13 distinct experimental combinations (detailed in Table 1), exploring a processing time range of 6 – 24 h and a process temperature domain of 20 – 60 °C. A central composite design (CCD) under Response Surface Methodology (RSM) was employed, relying on a quadratic model to guide the experiments:

$$Y = \beta 0 + \sum_{i=1}^{k} \beta iXi + \sum_{i=1}^{k} \beta iiXi^2 + \sum_{i<j}^{k} \beta ijXiXj + \in$$

With:

- *Y denotes actual response*
- *X denotes time-reduced factors centered*
- *β_0 represents original ordinate*
- *β_i: represents coefficients of the linear effects of the factors X_i*
- *β_{ii} represents coefficient of the quadratic effects of factors X_i^2*
- *β_{ij}: represents coefficients of the interactions between X_i and X_j*
- *ε: represents random error*
- *k: represents number of factors studied*

The evaluated dependent variables (responses) within the experimental optimization framework comprised electrical conductivity (R_1): pH (R_2), crude fiber content (R_3), crude protein content (R_4); dry matter content (R_5); swelling capacity (R_6), hydration capacity (R_7); condensed tannin content (R_8): and phytic acid/phytate content (R_9).

2.8 Statistical Analysis

All experiment trials were executed at least in duplicate, with analytical determinations performed in triplicate. Quantitative data are expressed as means ± standard deviation (SD). Statistically significant differences among the treatment means were determined via Tukey's post-hoc honest significant difference (HSD) test employing SPSS software (version 20.0, IBM Corp., Armonk, NY, USA) and Design-Expert (version 11.1.2.0), with the threshold of statistical significance established at $p < 0.05$.

3 Results & discussion

3.1 Physicochemical Fluctuations as a Function of Soaking Parameters

3.1.1 Dynamics of Dry Matter Loss

The influence of soaking duration and immersion temperature on the dry matter (DM) content of chickpeas is illustrated in Figure 1. An increase in both operational variables precipitated a statistically significant ($p < 0.05$) reduction in DM, declining from an initial baseline down to a range of 93.37% to 62.24% following a 24-h hydration period at 60 °C.

This progressive mass reduction in chickpea dry matter with extended soaking time and higher temperatures is

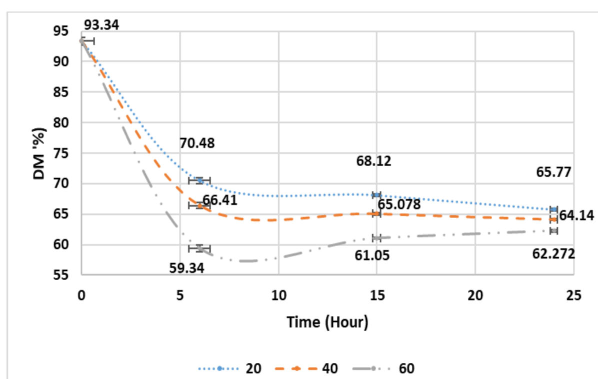

Figure 1. Effect of Soaking Time and Temperature on Dry Matter Content

primarily governed by concurrent mechanisms of water imbibition and the concomitant mass transfer of highly soluble intracellular constituents into the surrounding aqueous medium (Lima *et al.*, 2014). As hydration progresses, the relative ratio of water to total seed mass expands, mathematically diluting the dry matter fraction. Elevated temperatures accelerate this phenomenon by altering cell wall polysaccharide arrays and enhancing the diffusion coefficients of low-molecular-weight solutes, including free mono- and oligosaccharides, soluble proteins, phytates, structural salts, and various organic compounds (Mukherjee *et al.*, 2018).

From an industrial standpoint, this 31% net reduction in DM presents a critical operational trade-off. Processing facilities must optimize the structural benefits of accelerated rehydration and minimized downstream thermal cooking energy against the collateral loss of valuable soluble nutrients and process yield. Retaining nutritional value while attaining desirable functional characteristics can be achieved with the utilization of techniques such as recovering and soaking water or improving soaking conditions to avoid leaching (Martín-Cabrejas, 2019).

3.1.2 Dynamics of Internal pH

Potentiometric profiling of the chickpea matrices revealed distinct pH kinetics under varying time-temperature configurations, as illustrated in Figure 2. A notable variation in the pH kinetics was observed. An escalation in processing temperature from 20 °C to 40 °C, and ultimately to 60 °C, induced a statistically significant drop in pH ($p < 0.05$). At a baseline temperature of 20 °C, extending the soaking duration did not significantly perturb the matrix pH ($p > 0.05$), which corresponded to a stability in chickpea acidity. Conversely, at 40 °C and 60 °C, prolonged immersion triggered a pronounced, time-dependent acidification ($p < 0.05$).

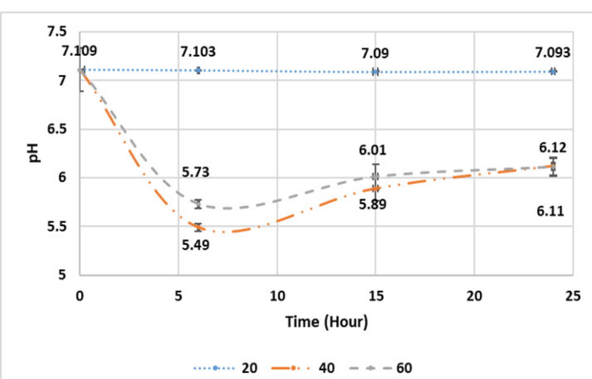

Figure 2. Effect of Soaking Time and Temperature on Chickpea pH Matter Content

This hyper-acidification pathway at elevated temperatures is driven by the thermal acceleration of native enzymatic and chemical reactions in chickpeas, which catalyzes the liberation of free organic acids into the cellular matrix (Mukherjee *et al.*, 2018).

Moreover, prolonged soaking periods allow for more complete hydration and water diffusion within the chickpeas, which promotes the release of soluble compounds. As soaking progresses, the pH of the seeds may decrease due to the accumulation of acidic compounds, reflecting an increase in acidity rather than alkalinity. This shift can influence flavor, microbial stability, and suitability for subsequent processes such as fermentation, as higher acidity can enhance safety and affect taste. Minerals such as carbonates and bicarbonates may also leach into the soaking water, but their buffering effect is generally insufficient to counteract the overall acidification during prolonged soaking. (Bayram *et al.*, 2004).

3.1.3 Electrical Conductivity Responses

The electrical conductivity of the chickpea seed matrices displayed an inverse relationship with soaking duration and temperature, exhibiting a significant drop ($p < 0.05$) from an unsoaked baseline of 9.57 $mS.cm^{-1}$ down to 0.507 $mS.cm^{-1}$ following 24 h of hydrothermal exposure at 60 °C (Figure 3).

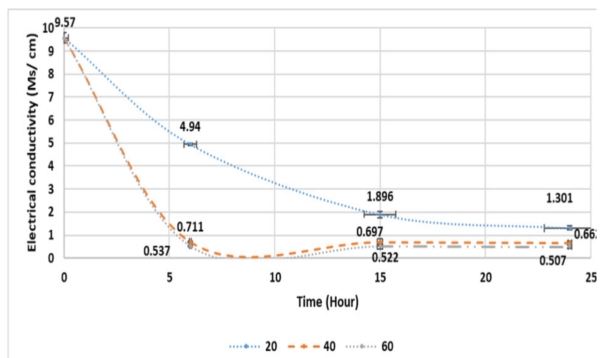

Figure 3. Effect of Soaking Time and Temperature on Electrical Conductivity

This phenomenon can be attributed to the extended soaking time and elevated temperature facilitating a more thorough hydration of the chickpeas, thereby enhancing water absorption and the hydration of cellular structures (Kaur & Prasad, 2023). As a result, the electrical conductivity measured directly on the chickpea seeds decreases, reflecting a relative dilution of ions within the seeds as water penetrates the cellular matrix.

Additionally, during the soaking process, soluble substances in the chickpeas, such as salts and additional electrolytes, may leach into the soaking solution. Nonetheless, with prolonged soaking or higher temperatures, these soluble compounds might continue to diffuse from the chickpeas into the soaking solution, leading to a decrease in electrical conductivity due to the reduction of ions in the solution (Kheto, 2024).

On the other hand, extended soaking times and elevated temperatures can enhance the efficiency of the osmotic process, where water moves into the chickpeas to balance the osmotic pressure between the chickpeas and the soaking solution. This influx of water reduces the number of ions present in the soaking solution, resulting in a further decrease in electrical conductivity (Bayrem *et al.*, 2004).

3.2 Nutritional Profile and Macronutrient Dynamics

3.2.1 Crude and Soluble Protein Alterations

Preserving the structural and nutritional integrity of the protein fraction by minimizing unintended denaturation represents a critical parameter in legume pre-processing. As summarized in Table 2, the total crude protein content of the chickpea seeds displayed divergent behavior depending on the thermal regime. Under ambient conditions (20 °C), the relative protein proportion expanded progressively over time, rising from an initial value of 22.48% to 31.82% after 24 h of immersion. Conversely, hydrothermal treatments at elevated temperatures induced a severe, statistically significant reduction ($p < 0.05$) in total protein content, dropping to 21.71% at 40°C and falling drastically to 1.71% at 60°C.

The observed decrease in total protein content over time at elevated temperatures of 40°C and 60°C suggests that higher temperatures may accelerate processes leading to protein degradation or loss. Elevated temperatures heavily accelerate proteolytic enzyme activity, which may degrade proteins within the chickpeas over time (Lei *et al.*, 2021). Furthermore, hydrothermal stress induces induce structural unfolding and aggregation, which destroys protein solubility profiles and causes massive leaching of these degraded nitrogenous compounds into the soaking water (Mukherjee *et al.*, 2018).

Parallel evaluations of the readily extractable soluble protein fraction under ambient hydration (20 °C) revealed a significant ($p < 0.05$) chronological decline, dropping from 0.280 ± 0.001 $\mu g.\mu L^{-1}$ to 0.249 ± 0.003 $\mu g.\mu L^{-1}$ then to 0.228 ± 0.001 µg/µL, and finally to 0.219 ± 0.002 µg/µL after 24 h of soaking, translating to an apparent denaturation and immobilization rate of 21.79%. While crude protein determination captures the total nitrogenous pool, tracking changes in the soluble protein fraction provides a highly sensitive index of structural modifications and conformational integrity within the food matrix (Agume *et al.*, 2017; Lima *et al.*, 2014).

3.2.2 Crude Fiber Structural Modifications

The structural fiber profile was similarly sensitive to the processing environment (Table 2). At a baseline temperature of 20°C, the measurable crude fiber content exhibited an upward trend over time, ascending from 20.92% to 41.6%. Conversely, prolonged immersion at 40°C and 60°C induced substantial reductions, dropping to 19.41% and 7.45%, respectively.

Table 2. Percentage of Total Protein Content Evolution Depending on the Soaking Parameters

	Soaking temperatures (°C)					
Soaking time (Hours)	20		40		60	
	Protein	Fiber	Protein	Fiber	Protein	Fiber
0	22.48 ± 1.30[a]	20.92 ± 1.02[c]	22.48 ± 1.30[a]	20.92 ± 1.02[a]	22.48 ± 1.30[a]	20.92 ± 1.02[a]
6	27.00 ± 0.70[b]	22.75 ± 0.41[b]	26.03 ± 0.66[b]	20.99 ± 0.62[a]	8.10 ± 0.09[a]	10.97 ± 0.81[b]
15	28.16 ±0.44[c]	23.28 ± 0.43[b]	22.26 ± 0.38[b]	19.87 ± 0.42[a]	4.74 ± 0.04[a]	9.02 ± 0.75[c]
24	31.82 ± 0.47[c]	41.60 ± 0.87[a]	21.71 ± 0.11[b]	19.41 ± 0.74[a]	1.71 ± 0.01[a]	7.45 ± 0.63[d]

Note: Measurements are expressed in mean± standard deviation. Different lowercase letters (a, b, c, and d) in a row indicate significant differences between samples at a 95% confidence level.

The relative enrichment in protein concentration observed at 20°C suggests that prolonged soaking facilitates more complete hydration, resulting in the swelling of the seeds and a higher concentration of proteins. Additionally, during the soaking process, certain soluble components, such as carbohydrates, may dissolve and leach into the soaking water, which further contributes to the concentration of proteins within the seeds (Urga & Gebretsa, 1993). Furthermore, soaking chickpeas partially inactivate antinutrients, thereby enhancing the availability of protein for digestion and absorption (El-Adawy *et al.*, 2000).

The observed increase in fiber content at 20°C suggests that soaking at this temperature enhances the separation of fiber from the chickpea cells, promoting an increase in cellulose, hemicellulose, and lignin content (Vasishtha *et al.*, 2011). The significant depletion observed at elevated temperatures such as 40°C and 60°C, indicates that these conditions may negatively impact fiber stability and integrity, potentially causing structural changes or degradation of fiber components. This marked decrease suggests thermal degradation and partial breakdown of polysaccharide structures under prolonged exposure to elevated temperatures. Additionally, elevated temperatures can lead to the degradation of hemicellulose, which is a heteropolymer recognized for its low heat resistance (Vasishtha *et al.*, 2013). This structural breakdown disrupts cell wall integrity and leads to the partial solubilization of complex carbohydrates into the aqueous phase (Dong *et al.*, 2019).

Furthermore, temperature and pH conditions during soaking may affect endogenous enzymatic activities, such as pectinases and other cell wall-modifying enzymes, which can aid in the breakdown of structural polysaccharides and alter the fiber profile. Comparable findings have been described in legumes where thermal and hydration treatments cause structural changes to cell wall components and alter the distribution of soluble and insoluble dietary fiber (Boye *et al.*, 2010). Degradation of chickpeas can impair the physiological benefits of dietary fiber, including intestinal health and modulation of post-prandial glycemic responses, while also affecting the structural integrity and texture. These findings highlight the need of adjusting soaking conditions to preserve dietary fiber functionality and nutritional quality in industrial and culinary applications (Rehinan *et al.*, 2004).

3.3 Evaluation of Antinutritional Factors

3.3.1 Kinetics of Condensed Tannin Depletion

As illustrated in Figure 4, the condensed tannin content of the chickpea seeds decreased significantly ($p < 0.05$) as a function of both increasing process temperature and soaking duration. At an initial baseline of 129 mg.100 g^{-1} DM, ambient soaking (20 °C) for 24 h reduced the tannin concentration to 85.94 mg.100g^{-1} DM, representing a gradual passive solubilization into the aqueous phase. Escalating the hydration temperature to 40°C markedly accelerated this mass transfer kinetic, lowering the retention level to 26.55 mg.100g^{-1} DM following 24 h. Similarly, soaking at 60°C maximized this degradation curve, with terminal tannin levels dropping to 9.88 mg.100g^{-1} DM.

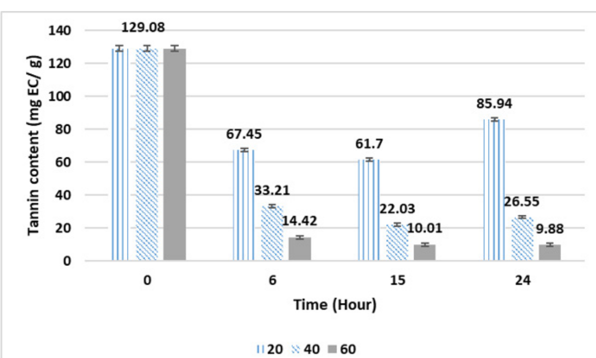

Figure 4. Influence of Soaking Parameters on Tannin Content

The observed decrease in tannin content with increasing temperature and soaking duration indicates that tannins in chickpeas are prone to solubilization and leaching into the soaking water, particularly under sustained thermal stress (Plahar *et al.*, 1997). This phenomenon may result from the disruption of tannin-protein complexes and the degradation of tannin structures due to elevated temperatures and prolonged soaking, associated with the leaching of polyphenols into the soaking water (Kheto *et al.*, 2024).

These findings suggest that modifying the soaking temperature and duration could be effective strategies to reduce the tannin content in chickpeas. Thus, tannins are known to impart astringency and bitterness to legumes, which can negatively affect palatability. By reducing tannin levels through optimal soaking conditions, chickpeas' sensory quality can be considerably improved, making them more appealing to consumers. This effect is particularly relevant for ready-to-cook food or industrial applications where flavor and texture are significant factors in consumer preference. Thus, in addition to enhancing nutritional quality, soaking improves the flavor and overall acceptability of chickpeas.

3.3.2 Phytic Acid De-phosphorylation Dynamics

Mirroring the degradation profiles of the tannin fraction, the phytic acid (phytate) concentration exhibited a systematic, time- and temperature-dependent decline as illustrated in Figure 5. Following a 24-h soaking cycle, the residual phytate content decreased from its baseline value down to 614.41 mg.100 g^{-1} DM at 20°C to 358.41 mg.100 g^{-1} DM at 40°C, and reached a minimum of 323.04 mg/100 g^{-1} DM at 60°C.

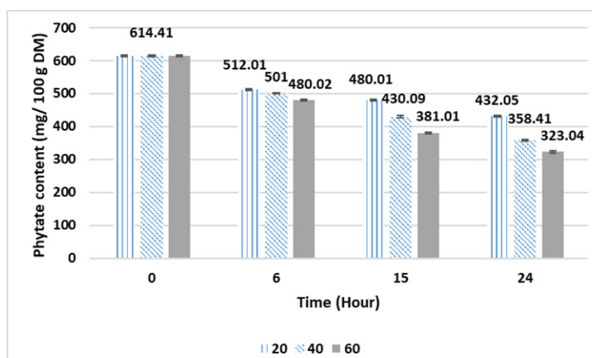

Figure 5. Influence of Soaking Parameters on Phytate Content

Across all temperatures, there is a consistent trend of decreasing phytate levels with longer soaking durations. This indicates that phytates continue to solubilize and leach into the soaking water over time, thereby reducing their levels in the chickpeas (Lestienne *et al.*, 2005). This suggests that phytates are prone to solubilization and leach into the soaking water, particularly at elevated temperatures. This phenomenon likely results from the breakdown of phytate complexes and structures during soaking (Coffigniez *et al.*, 2019). Consequently, the findings indicate that modifying the soaking temperature and duration can significantly impact the reduction of phytate content in chickpeas, potentially enhancing essential mineral bioavailability and decreasing anti-nutrient levels in chickpea-based products (Kheto *et al.*, 2024).

3.3 Functional Properties and Hydric Kinetics

Swelling and Hydration Dynamics

The prolongation of soaking duration induced a statistically significant increase ($p < 0.05$) in the swelling capacity of the chickpea seeds. Under ambient conditions (20 °C), the swelling capacity expanded from an initial value of 2.2 ± 0.001 mL H_2O / seed at 6 hours to a maximum threshold of 3.2 ± 0.002 mL H_2O / seed following 24 h of immersion. An analogous, statistically significant upward trend was recorded during soaking at both 40 °C and 60 °C.

The hydration capacity mirrored this evolutionary pattern, rising significantly from 0.38 ± 0.001 at 6 hours to 0.45 ± 0.001 at the termination of the 24-hour soaking cycle (20 °C). This strong positive correlation between prolonged immersion time and the twin indices of swelling and hydration capacities implies that extended durations facilitate deeper water mass transfer into the chickpea seeds, thereby enhancing their hydration and swelling phenomena (Kaur & Prasad, 2023).

This progressive imbibition process is fundamentally governed by osmosis. Water migrates along a chemical potential gradient from the external medium (lower solute concentration) into the dense intracellular environment of the seeds (higher solute concentration), resulting in the swelling as the seeds absorb water (Lei *et al.*, 2021).

Conversely, elevation in soaking temperature leads to a severe statistically significant decrease in both swelling capacity and hydration capacities ($p < 0.05$). For instance, the swelling capacity collapsed from 2.2 ± 0.001 mL H_2O / seed under the 6-hour / 20 °C configuration down to 0.39 ± 0.001 mL H_2O / seed when processed at 60 °C. Likewise, the hydration capacity dropped from 0.38 ± 0.001 to 0.29 ± 0.001 under identical thermal acceleration.

This reduction in swelling and hydration capacities attributable to increased soaking temperatures can be elucidated by multiple factors:

- *Cellular Disintegration*: Prolonged exposure to elevated temperatures can result in structural alterations within chickpeas, including the disintegration of cell walls internal structural components, compromising the cellular compartmentalization required to sustain osmotic pressure and full physical expansion (Coffigniez *et al.*, 2019).
- *Seed Coat Modification*: Thermal stress can induce structural reordering or denaturation of proteins embedded within the seed coat, reducing its native hydrophilic permeability and restricting the initial rate of water ingress (Coffigniez *et al.*, 2019).
- *Matrix Compaction*: Within the seed, high-temperature fields induce partial protein denaturation and subsequent macromolecular aggregation. Although thermal energy initially facilitates water diffusion and hydrates the free hydroxyl groups of starch, the concurrent disruption of native starch granule organization favors the development of highly cross-linked, compact intermolecular networks. This rigid starch-protein matrix restricts the spatial expansion of starch granules and significantly diminishes the net water-retention capacity of the seed tissue (Kaur et Prasad, 2023; Sayar *et al.*, 2001).

3.4 Response Surface Optimization and Varietal Influence

Multi-Response Desirability Optimization

A multi-response surface optimization framework was executed to define the ideal time-temperature processing scale. The optimization vectors were configured to simultaneously minimize antinutritional components (condensed tannins and phytic acid) while maximizing nutritional density (crude protein and fiber contents). The analysis of variance (ANOVA) revealed that all experimental combinations exerted statistically significant effects ($p < 0.05$) across the entire pool of dependent variables (crude protein, fiber, tannin, phytate, electrical conductivity, pH, dry matter, swelling, and hydration capacity).

The optimal soaking conditions identified in this study could potentially be associated with the intrinsic properties of the chickpea type employed. Given that the 'Rebha' chickpea is a Tunisian chickpea cultivar created to adapt to local environmental circumstances, particularly water scarcity, it may possess structural characteristics that could protect the seed under harsh conditions, such as changes in seed coat structure and permeability, which can affect water absorption during soaking (Amri *et al.*, 2016). The rate of water intake in legumes is highly regulated by the seed coat's physical characteristics and water permeability. In this regard, the optimal soaking temperature and time identified in the current study may reflect 'Rebha' chickpea seeds particular hydration behavior.

As a result, the optimized soaking parameters observed in this investigation may improve processing efficiency while also reflecting the unique physical properties of this regionally adapted variety. This demonstrates how the agronomic characteristics of climate-resilient crops can influence their technological performance during food processing, ultimately connecting crop adaptation tactics to their potential for food applications.

As modeled by the predictive software, the global optimal processing window was established at a temperature of 25.85 °C for 24 hours, yielding a composite desirability score of 0.616 (Figure 6). This overall desirability indicates a compromise among the many responses examined in the model.

In multi-response mathematical optimization, achieving the absolute individual optimum of each for each target variable under a singular operational regime is rarely feasible—particularly when the dependent variables exhibit divergent or antagonistic kinetic vectors. Therefore, the anticipated ideal conditions should be viewed as the best global balance that concurrently satisfies the desired criteria, rather than maximizing each response separately.

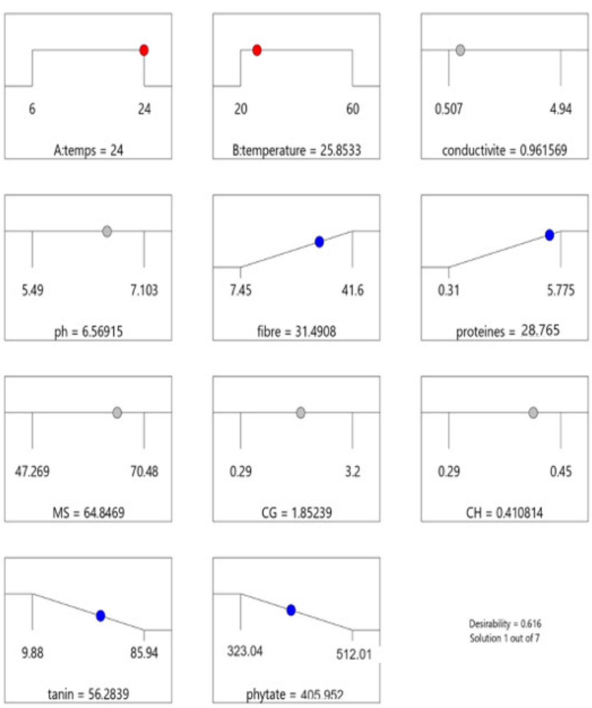

Figure 6. Desirability Function Approach and Optimum Time and Temperature Scale of Soaking

To validate the statistical adequacy of the predictive model, verification trials were conducted under these optimized conditions. The resulting empirical values demonstrated strong statistical alignment with the predicted values ($p > 0.05$), confirming the robustness, accuracy, and geometric adequacy of the model within the evaluated experimental domain.

Genotypic and Agro-Ecological Influences on Hydration Behavior

The optimized soaking and processing parameters identified in this study may be influenced by genotypic differences among chickpea cultivars. Genotypic variability has been demonstrated to influence various physical and physiological characteristics of chickpea seeds, potentially affecting hydration behavior during soaking. Several chickpea cultivars in Tunisia, including *Béja 1*, *Neyer*, *Bochra*, and *Kasseb*, have been extensively studied in agronomic studies, revealing significant heterogeneity in seed characteristics, yield components, and drought tolerance among genotypes (Khamassi *et al.*, 2014).

This inter-varietal divergence directly influences technological behaviors; previous kinetic modeling via the Peleg equation confirms that water absorption rates and saturation thresholds are strictly genotype-dependent and vary widely across cultivars as a function of temperature (Shafaei *et al.*, 2016). As a result, while our optimum circumstances are consistent with general trends in the literature, the specific values achieved in this study most likely reflect the inherent properties of the Tunisian cultivar investigated, such as seed coat structure and content. This emphasizes the significance of considering varietal attributes when determining appropriate processing parameters, as well as the importance of adjusting soaking and treatment conditions to the genotype to achieve correct end-use quality.

3.5 Practical and Industrial Implications

Domestic Culinary Efficiency

The hydric and techno-functional characteristics optimized in this study exhibit immediate, practical relevance for domestic culinary preparation. Soaking facilitates water penetration into the seed structure, softens the cotyledon tissues and reduces the cooking time required to get an acceptable texture. Previous research has revealed that properly hydrating legumes enhances cooking efficiency and results in a softer, more acceptable texture after cooking (Wood & Grusak, 2007).

From a practical perspective, chickpea matrices with higher moisture and swelling capacities cook faster and more consistently. This operational efficiency translates to markedly reduced boiling times and decreased domestic energy consumption. For the end consumer, optimizing soaking conditions represents a straightforward strategy to enhance both the sensory quality and the preparation efficiency, preserving traditional culinary practices while optimizing resource utilization.

Industrial Food Formulations and Techno-Functional Applications

Beyond domestic applications, the industrial food sector is increasingly exploiting the functional characteristics of chickpea-derived products. Chickpea flour is recognized for its potential techno-functional properties, including robust water-binding capacities, excellent emulsification properties, and the ability to drive clean-label matrix formation in complex food systems (Boukid, 2021). These characteristics make chickpea-based ingredients attractive for the development of novel, plant-based food items.

From this perspective, the optimized flour developed from the 'Rebha' cultivar constitutes a highly viable ingredient endowed with specific functional properties. Enhanced hydration and swelling kinetic properties are particularly advantageous across a diverse spectrum of food processing applications. For instance, chickpea flour is increasingly incorporated into gluten-free bakery formulations, where it optimizes dough rheology, crumb texture, and nutritional value (Kaur *et al.*, 2015). Likewise, legume-based components are gaining popularity in plant-based meat replacements, where their protein and water-binding abilities facilitate to create desired textures (Kyriakopoulou *et al.*, 2021).

Furthermore, chickpea-derived ingredients can function as natural stabilizing agents or clean-label texturizers due to their capacity to immobilize water and stabilize food matrices. Given the accelerating market demand for sustainable, plant-based alternatives, optimizing the techno-functional traits of chickpea flour discloses novel pathways for its integration into contemporary food systems. Ultimately, the systematic optimization of soaking conditions not only improves chickpeas culinary performance but may increase the functional and industrial potential of chickpea-derived flours for a variety of food applications.

4 Conclusions

The present study systematically elucidated the interactive effects of soaking duration and temperature on the physicochemical, techno-functional, nutritional, and antinutritional profiles of the climate-resilient Tunisian chickpea cultivar 'Rebha'. By systematically investigating these variables, we aimed to identify optimal conditions that effectively reduce the levels of anti-nutritional factors, such as phytates and condensed tannins, which are recognized to inhibit some nutrient absorption and can negatively impact consumer health. Concurrently, this investigation focuses on enhancing the nutritional profile of chickpeas, by increasing their crude protein and fiber content. Both customers and the food sector may clearly benefit from optimizing soaking conditions. Soaking increases mineral availability and protein digestibility by lowering antinutritional chemicals, which ultimately raises the nutritional value of chickpeas. This can also result in faster cooking times and improved acceptability for consumers. By providing these insights, the study offers valuable recommendations for the food sector to enhance the quality, consistency, and processing conditions of meals prepared through chickpeas.

In conclusion, this investigation underscores the necessity of calibrating post-harvest processing frameworks to the specific genotypic profiles of locally adapted, climate-resilient crop varieties. This approach successfully bridges the gap between agronomic adaptation strategies and functional food innovations, offering valuable recommendations to enhance the quality, consistency, and commercial value of chickpea-derived products.

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