Evaluation of soaking and germination interest: Comparative study of the chemical and phytochemical composition between three species of food legumes listed in the Moroccan official catalog

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Abstract- Despite their health advantages, food legumes have a limited impact on human health due to a variety of factors, including low protein and starch digestibility, low mineral bioavailability, and high antinutritional factors.

This study compared the nutrients and antinutrients of different varieties of food legumes before and after soaking and germination treatment. A fluctuation between the chemical and phytochemical composition results was observed. Due to species*variety*treatment interaction, indeed, the species and varieties that are the subject of the present study act differently with respect to the treatments carried out. In fact, sugars and proteins content increased significantly in the germination-treated varieties, following a decrease during the soaking treatment. Raw chickpeas had the greatest amount of total and reducing sugars, respectively, with maximum amounts of 1.30 g and 4.45 g of glucose equivalent per gram of dry matter.

Raw, soaked, and germinated fava bean varieties have greater average crude fiber rates than chickpea and lentil varieties, respectively, with 9.83%, 10.24%, and 10.16%. It was found that germination treatment improved the nutritional properties of the studied food legumes. Additionally, the treatments also resulted in the reduction of total phenolic compounds, tannins, and flavonoids contents. However, the changes varied depending on the variety and species of food legumes.

Overall, this work demonstrated that germination positively affects the nutritional composition, leading to the reduction of antinutritional factors in the studied food legumes. They prove to be the most appropriate method to take advantage of the benefits of food legumes.

Keywords: Fava bean, chickpea, lentil, soaking, germination, antinutritional factors.

I. INTRODUCTION

Legumes belong to the Fabaceae / Leguminosae family. They are one of the most important crops due to their nutritional quality for human nutrition and also for their various agro-environmental benefits for the maintenance of production systems in arid and semi-arid areas [20].
Food legumes (fava beans, chickpeas, and lentils) are ranked second in importance after cereals in Morocco. Despite their various agronomic [22], economic and nutritional interests [7].

The cultivation of legumes is becoming more important in the world due to their nutritional quality. They are good sources of proteins, starch, fibers, vitamins, minerals, and bioactive compounds. They make an important contribution to human nutrition in many countries [12].

Although characterized by high levels of protein and nutritional value, their use is limited by the presence of certain antinutritional / antiphysiological, / toxic substances; among these are phytates, polyphenols, enzyme inhibitors (trypsin, chymotrypsin, and a-amylase), and hemagglutinins [3]. Indeed, legumes must be treated before consumption [30]. Moreover, the presence of these antinutritional factors in legumes is reduced to varying degrees depending on the method of their preparation and their properties. Indeed, several legume processing methods have been designed for this purpose, including soaking, germination, decortication, fermentation, and cooking [31]. However, these methods greatly influence the nutritional value at different levels by eliminating or reducing certain undesirable components. These methods play an important role, as they also influence the bioavailability of nutrients and improve the digestibility and nutritional value of legumes. In this context, the present study was established with the aim of studying the effect of soaking and germination on the nutritive and antinutritive components of three species of common food legumes listed in the official Moroccan catalog, notably: fava bean (*Vicia faba*), lentil (*Lens culinaris*), and chickpea (*Cicer arietinum*). Thus, to evaluate how these three species act with the treatments carried out following a comparative analysis.

II. MATERIALS AND METHODS

1. Sampling

This study focuses on different varieties of three species of food legumes registered in the official Moroccan catalog, which represent:
Six varieties of Fava beans (*Vicia faba*): "FB1= Karabiga"; "FB2= Abfai 321"; "FB3= De Fes"; "FB4= Aguadulce"; "FB5= Alfai 317"; "FB6= Lobab".
Eight varieties of lentils, (*Lens culinaris*): "LN1 = Zaaria"; "LN2 = Abda"; "LN3 = Hamria"; "LN4 = Bichet"; "LN5 = L24"; "LN6 = L56"; "LN7 = L4605"; "LN8 = Chakouf".
Seven varieties of chickpeas, (*Cicer arietinum*): "CP1 = Mobarak"; "CP2 = Arifi"; "CP3 = Douyet"; "CP4 = Farihane";

"CP5: Garbonzo"; "CP6 = Rizki"; "CP7 = Zahour".

2. Sample preparation

Firstly, 50 g of each sample was weighed for the three stages "T_0 = raw," "T_1 = soaked," and "T_2= germinated" and then finely ground in a grinder with a 1 mm mesh sieve.

3. Soaking

Different varieties of chickpeas, lentils, and fava beans were soaked by immersing them in distilled water for 24 hours at room temperature. Then rinsed after draining the soaking water and crushed after freeze-dried, then stored until analysis.

4. Germination

Germination was performed according to [8]. Briefly, 200 g of seeds were soaked in 1000 mL of 0.07% sodium hypochlorite for 30 minutes at room temperature. The disinfected seeds were washed with distilled water until reaching a neutral pH to ensure the absence of traces of residual NaClo. Then, these seeds were soaked in 1000 mL of distilled water for 24 hours.

Hydrated seeds were placed in germination trays covered with a sterile, moist cloth while maintaining regular hydration. Seeds germinated at room temperature (30–35 °C) with humidity (98% relative humidity), which was maintained by sprinkling water for 15 minutes at 2-hour intervals. After 48 hours, the germinated seeds were washed with distilled water, freeze-dried, then ground and kept until analysis.

5. Chemical composition analysis

5.1. Determination of dry matter, ash , protein, and crude fiber content

Dry matter, ash, protein, and crude fiber were determined by the methods of the AACC [1].

5.2. Estimation of total sugars and reducing sugars contents

Total sugars contents were estimated using phenol-sulfuric acid method [9], at optical density 490 nm. However, reducing sugars were determined according to the method described by Miller [15], using dinitrosalicylic acid (DNS) reagent at optical density 575 nm using a Shimadzu-2450 UV–Vis spectrophotometer. The results of these two reactions are expressed in milligrams of glucose equivalent per gram of dry matter.

6. Extraction and dosage of bioactive molecules

6.1. Phenolic extracts preparation

Phenolic compound extraction was carried out in batch mode in a shaker agitator in order to ensure intensive agitation conditions and control the temperature. A mass of 1 g was introduced into 50 mL tubes. Then a volume of 40 mL of the acetone-water (50%) solvent was added. The tubes were brought to a temperature of 40 °C. Contact between solid and solvent was maintained for 4 hours. After the extraction, the solid-liquid separation was done by centrifugation at 3000 rpm for 15 minutes. Finally, the extracts were filtered, recovered, and stored at -20°C.

6.2. Determination of total phenolic compounds (TPC)

According to the Folin-Ciocalteu spectrophotometric technique [25], the total phenolic content of the food legume extract is determined. Sodium carbonate is used to neutralize the reaction after the Folin-Ciocalteu reagent has oxidized the extracts. The absorbance of the resulting blue color was measured at 760 nm. A standard gallic acid curve was used to compare the measurement. This led to the measurement of the total phenolic content as micrograms of gallic acid equivalents per gram of dry matter (µg GAE/g DM).

6.3. Determination of flavonoids content

The total flavonoid content (TFC) was determined according to the method of Lamaison and Carnat [19]. Quercetin was used to establish the standard curve. The TFC was expressed in micrograms of quercetin equivalent per gram of dry matter (µg QE/g DM).

6.4. Determination of tannins content

The dosage of condensed tannins in legume extracts is carried out according to the vanillin method in an acid medium [18]. This method is based on the ability of vanillin to react with condensed tannin units in the presence of an acid to produce a colored complex measured at 550 nm. Briefly, a volume of 100 µL of the crude extract is added to 1500 µL of the vanillin/methanol solution (4%, m/v), then mixed using a vortex. Then, 750 µL of concentrated hydrochloric acid (HCl) is added. The mixture obtained is left to react at room temperature for 20 min. Absorbance is measured at 550 nm against a blank.
A calibration curve is produced in parallel under the same operating conditions using catechin as a positive control. The results obtained are expressed in micrograms of catechin equivalent per gram of dry matter (µg CE/g DM).

III. STATISTICAL ANALYSIS

All determinations were performed in triplicate. The results are expressed as the mean. They were subjected to a multiple comparison of the means (LSD test) using SAS software version 9.1. At $p < 0.05$, the differences are considered significant.

IV. RESULTS AND DISCUSSION

1. Chemical composition results

The chemical composition of studied legumes, namely the seeds of LN, FB, and CP, in the raw, soaked, and germinated states, is given in Tables 1, 2, and 3, respectively.

The average dry matter content of raw FB, LN, and CP varieties was 89.08%, 90.90%, and 89.45%, respectively, while soaked varieties had averages of 96.14%, 94.35%, and 96.52%, respectively. Also, the germinated ones have 92.41%, 92.43%, and 94.39, respectively. Hence, it is deduced that the moisture content in the different raw legume varieties was higher than those treated by soaking and germination. Thus, the rapid absorption of moisture by dry legumes is influenced by the structure of the seed [17].

The different varieties of FB, CP, and LN presented relatively similar ash content, in the order of (2.74%, 3.07%, and 2.27%) at T_0; (2.90%, 2.60%, and 2.57%) at T_1; and (2.88%, 2.54%, and 2.73%) at T_2, respectively. In addition, the average crude fiber content of the different fava bean, chickpea, and lentil varieties is around (9.83% $_{at\ T0}$, 10.24% $_{at\ T1}$, and 10.16% $_{at\ T2}$); (4.53% $_{at\ T0}$, 4.07% $_{at\ T1}$, 3.86% $_{at\ T2}$); and (4.87% $_{at\ T0}$, 4.95% $_{at\ T1}$, 5.61% $_{at\ T2}$), respectively. Indeed, the crude fiber content of the different studied legumes, varied from 2.72% to 12.33%. It is largely explained by the proportion of integuments in the seed. However, the average rates recorded by the raw, soaked, and germinated fava bean varieties 9.83%, 10.24%, and 10.16%, respectively—are relatively higher than the rates given by different varieties of chickpea and lentil. From which we deduce that the bean is characterized by a slightly higher rate of crude fiber compared to other studied legumes.

On the other hand, the crude fiber content showed a non-significant difference ($P>0.05$) between the lentil varieties and the treatments carried out. While a significant difference ($P<0.05$) occurred between the bean varieties and the soaking and germination treatment, indeed, a highly significant difference ($P<0.01$) was noted following the interaction of the chickpea varieties and the treatments carried out.

Germination produced changes, causing a slight increase in the level of crude fiber in the seeds of studied legumes. This increase was reported in the literature [13]. Germination also caused a significant decrease in crude fiber content in a few varieties. These changes in fiber content can be attributed to the fact that part of the seed fiber was enzymatically solubilized during seed germination [10].

As for the protein contents in the FB, CP, and LN varieties, they were in the order of (27.25%, 21.95%, and 29.77%) at T_0; (27.10%, 28.71%, and 29.0%) at T_1; and (27.68%, 22.18%, and 30.14%) at T_2, respectively. Indeed, the protein content data presented in Tables 1, 2, and 3 indicate that there was a highly significant difference between the CP, FB, and LN varieties and the treatments performed. Indeed, germination resulted in a slight increase in protein content. This could be due to the synthesis of hydrolytic enzymes during various germination events or to a change in composition following the degradation of other seed constituents [5]. A similar trend in the protein content of flaxseeds, fenugreek, soybeans, and cereals was previously reported during germination [16].

However, other studies in the literature [27, 29] have shown different results. They found that the total protein content decreased after germination. Thus, the decline in protein content in some varieties is probably related to proteolysis, which overtakes protein synthesis in growing sprouts [23]. Indeed, the comparison of the three legumes species that are the subject of this study revealed that they have a very similar chemical composition and are rich in proteins.

Table 1: Nutritional quality parameters (g/100g DM) for lentil varieties

P	**%DM**			**%Ash**			**%CF**			**%Proteins**		
T / V	**T_0**	**T_1**	**T_2**	**T_0**	**T_1**	**T_2**	**T_0**	**T_1**	**T_2**	**T_0**	**T_1**	**T_2**
LN1	90.86^{bd}	94.75^{b}	93.37^{b}	1.96^{ab}	2.50^{c}	2.57^{ab}	4.41^{cd}	4.60^{de}	5.00^{a}	29.04^{d}	30.15^{b}	28.82^{de}
LN2	90.85^{bd}	94.79^{b}	93.79^{ab}	2.47^{a}	2.71^{a}	2.88^{ab}	6.01^{a}	5.11^{abcd}	6.34^{a}	29.14^{cd}	30.09^{b}	31.86^{b}
LN3	90.95^{bc}	93.23^{e}	92.18^{d}	1.83^{b}	2.52^{c}	2.79^{ab}	5.21^{b}	5.29 ^{ab}	5.72^{a}	31.84^{a}	30.69^{ab}	32.61^{a}
LN4	90.76^{cd}	95.48^{a}	93.56^{b}	2.26^{ab}	2.56^{c}	2.70^{ab}	4.37^{cd}	5.53 ^{a}	5.82^{a}	28.10^{e}	25.75^{d}	28.20^{e}
LN5	91.34^{a}	93.17^{e}	91.37^{e}	2.39^{ab}	2.51^{c}	2.96^{a}	5.08^{bc}	5.28 ^{abc}	5.46^{a}	28.88^{d}	28.32^{c}	29.06^{cd}
LN6	91.09^{ab}	94.43^{c}	92.86^{c}	2.42^{ab}	2.60^{bc}	2.72^{ab}	5.47^{ab}	4.40^{e}	6.00^{a}	31.08^{a}	31.37 ^{a}	31.75^{b}
LN7	90.68^{d}	93.54^{d}	94.08^{a}	2.53^{a}	2.69$^{\mathbf{ab}}$	2.73^{ab}	4.03^{d}	4.61^{cde}	5.09^{a}	29.86^{bc}	30.49^{ab}	29.72^{c}
LN8	90.70^{cd}	95.47^{a}	88.24^{f}	2.34^{ab}	2.51^{c}	2.51^{b}	4.40^{cd}	4.78^{bcde}	5.47^{a}	30.24^{b}	25.16^{d}	29.10^{cd}
T*V		***			Ns			ns			***	
LSD	0.27	0.25	0.46	0.61	0.10	0.40	0.74	0.67	1.59	0.77	1.11	0.66

P : Parameters ; V : Varieties ; T : Treatment ; LN : Lentils ; DM : Dry matter ; CF : Crude fiber ; T*V : Treatment*variety interaction ; LSD : Least significant difference; $^{a, b, c\cdots}$: Different letters within the same column are significantly different ($P<0.05$) ; ns : Not significant difference; *** : Very highly significant.

Table 2: Nutritional quality Parameters (g/100 g DM) for fava bean varieties

P	%DM			%Ash			%CF			%Proteins		
T / V	T_0	T_1	T_2	T_0	T_1	T_2	T_0	T_1	T_2	T_0	T_1	T_2
FB1	88.97^{bc}	95.92^{c}	94.66^{a}	2.85^{b}	3.24^{a}	4.04^{a}	10.34^{ab}	9.93^{a}	9.94^{a}	27.29^{ab}	29.37^{a}	28.05^{b}
FB2	89.15^{ab}	96.16^{bc}	93.73^{ab}	2.89^{b}	2.82^{c}	3.15^{ab}	9.36^{ab}	10.04^{a}	10.19^{a}	28.46^{a}	28.80^{b}	28.24^{ab}
FB3	88.94^{bc}	96.26^{b}	93.39^{b}	2.64^{c}	2.84^{c}	3.18^{ab}	8.92^{ab}	10.18^{a}	10.68^{a}	27.65^{ab}	27.22^{d}	28.15^{ab}
FB4	89.51^{a}	96.67^{a}	90.63^{c}	3.06^{a}	2.86^{c}	2.67^{b}	9.37^{ab}	9.55^{a}	9.33^{a}	26.34^{bc}	25.70^{e}	26.30^{d}
FB5	88.69^{c}	96.18^{b}	93.29^{b}	2.68^{c}	2.70^{d}	2.94^{ab}	10.84^{a}	12.33^{a}	10.09^{a}	25.26^{c}	23.47^{f}	26.87^{c}
FB6	89.22^{ab}	95.65^{d}	88.80^{d}	2.31^{d}	2.96^{b}	1.28^{c}	10.13^{ab}	9.42^{a}	10.76^{a}	28.54^{a}	28.04^{c}	28.48^{a}
T*V		***			***			*			***	
LSD	0.38	0.24	1.17	0.08	0.05	1.26	1.86	3.39	1.85	1.36	0.33	0.38

P: Parameters; V: varieties; T: Treatment; FB: Fava bean; DM: Dry matter; CF: Crude fiber; T*V: Treatment*variety interaction; LSD: Least significant difference; a, b, c…: Different letters within the same column are significantly different ($P < 0.05$); ***: Very highly significant; *: Significant.

Table 3: Nutritional quality parameters (g/100gDM) for chickpea varieties

P	%DM			%Ash			%CF			%Proteins		
T / V	T_0	T_1	T_2	T_0	T_1	T_2	T_0	T_1	T_2	T_0	T_1	T_2
CP1	89.25^{b}	95.93^{e}	94.00^{ab}	2.64^{de}	2.77^{a}	2.65^{ab}	3.94^{b}	4.15^{a}	5.23^{a}	21.57^{c}	21.43^{d}	21.04^{bc}
CP2	89.78^{a}	96.64^{c}	94.43^{ab}	2.49^{e}	2.50^{a}	2.70^{a}	4.15^{ab}	4.15^{a}	3.79^{bcd}	19.40^{d}	19.06^{e}	20.71^{c}
CP3	89.26^{b}	96.65^{c}	93.84^{b}	2.81^{c}	2.75^{a}	2.33^{c}	4.85^{ab}	4.53^{a}	4.34^{bc}	23.33^{b}	23.99^{a}	22.24^{bc}
CP4	89.02^{b}	97.21^{a}	94.66^{ab}	2.68^{cd}	2.61^{a}	2.73^{a}	5.07^{a}	3.58^{a}	3.53^{cde}	21.82^{c}	22.77^{c}	20.98^{c}
CP5	89.16^{b}	96.87^{b}	94.44^{ab}	2.75^{cd}	2.84^{a}	2.51^{b}	5.02^{a}	3.73^{a}	2.94^{e}	21.49^{c}	23.67^{ab}	25.90^{a}
CP6	89.73^{a}	96.16^{d}	94.28^{ab}	4.47^{a}	2.45^{a}	2.17^{c}	4.56^{ab}	3.97^{a}	4.44^{ab}	24.43^{a}	23.20^{bc}	22.74^{b}
CP7	89.95^{a}	96.17^{d}	95.08^{a}	3.70^{b}	2.29^{a}	2.70^{a}	4.12^{ab}	4.41^{a}	2.72^{e}	21.60^{c}	23.02^{bc}	21.63^{bc}
T*V		***			***			**			***	
LSD	0.30	0.19	0.84	0.16	0.61	0.18	1.06	1.43	0.86	0.57	0.72	1.70

P: Parameters; V: Varieties; T: Treatment; CP: Chickpea; DM: Dry matter; CF: Crude fiber; T*V: Treatment*variety interaction; LSD: Least significant difference; $^{a, b, c…}$: Different letters within the same column are significantly different ($P < 0.05$); ns: Not significant difference; ***: Very highly significant; **: Highly significant.

2. Total and reducing sugar content

Figure 1 below indicates the changes in the total and reducing sugar contents during the two technological treatments carried out. Indeed, soaking and germination of legume seeds can improve starch digestibility and reduce the level of raffinose family oligosaccharides by up to 100% through the release of α-galactosidase [4].

Germination decreased the mean total sugars content from 1.02 mg GE/g DM at T_0 to 0.77 mg GE/g DM in chickpea varieties. The same goes for lentils, with a decrease from 0.75 mg GE/g DM at T_0 to 0.60 mg GE/g DM. Moreover, by comparing all the varieties of studied food legumes, the highest total sugars levels were observed in raw chickpeas. After germination, the content of total sugars decreased in most varieties of chickpeas and lentils, except fava beans. However, the content of reducing sugars in fava bean varieties decreased slightly with soaking, while it increased with germination. Generally, soaking caused a significant decrease in the total and reducing sugars contents. The total and reducing sugars contents varied considerably, depending on the treatments carried out, the variety, and the species of legume. Moreover, by comparing all the studied varieties, the most dominant total and reducing sugars contents were observed in raw chickpeas (CP1 = 1.30 mg GE/g DM and CP7 = 4.45 mg GE/g DM, respectively).

It is therefore clear from these data that soaking is also a metabolic process that precedes the onset of the metabolic changes that occur during germination. Similarly, Urbano et al [28] suggested that the sprouting process causes metabolic changes in legume seeds, in which the storage of carbohydrates in the form of starch and oligosaccharides is hydrolyzed, causing sugars levels to increase. In addition, Martin-Cabrejas et al [14] also suggested that during germination, the activity of α-galactosidase increases, causing the breaking of α-1, 6-galatosidic bonds, thus increasing the content of total sugars.

However, Vidal-Valverde et al [30] explained that during germination, carbohydrates were used as an energy source for embryonic growth, following the increased activity of ß-amylase, which hydrolyzes starch into simple carbohydrate [26]. This might explain the changes in carbohydrate content after germination.

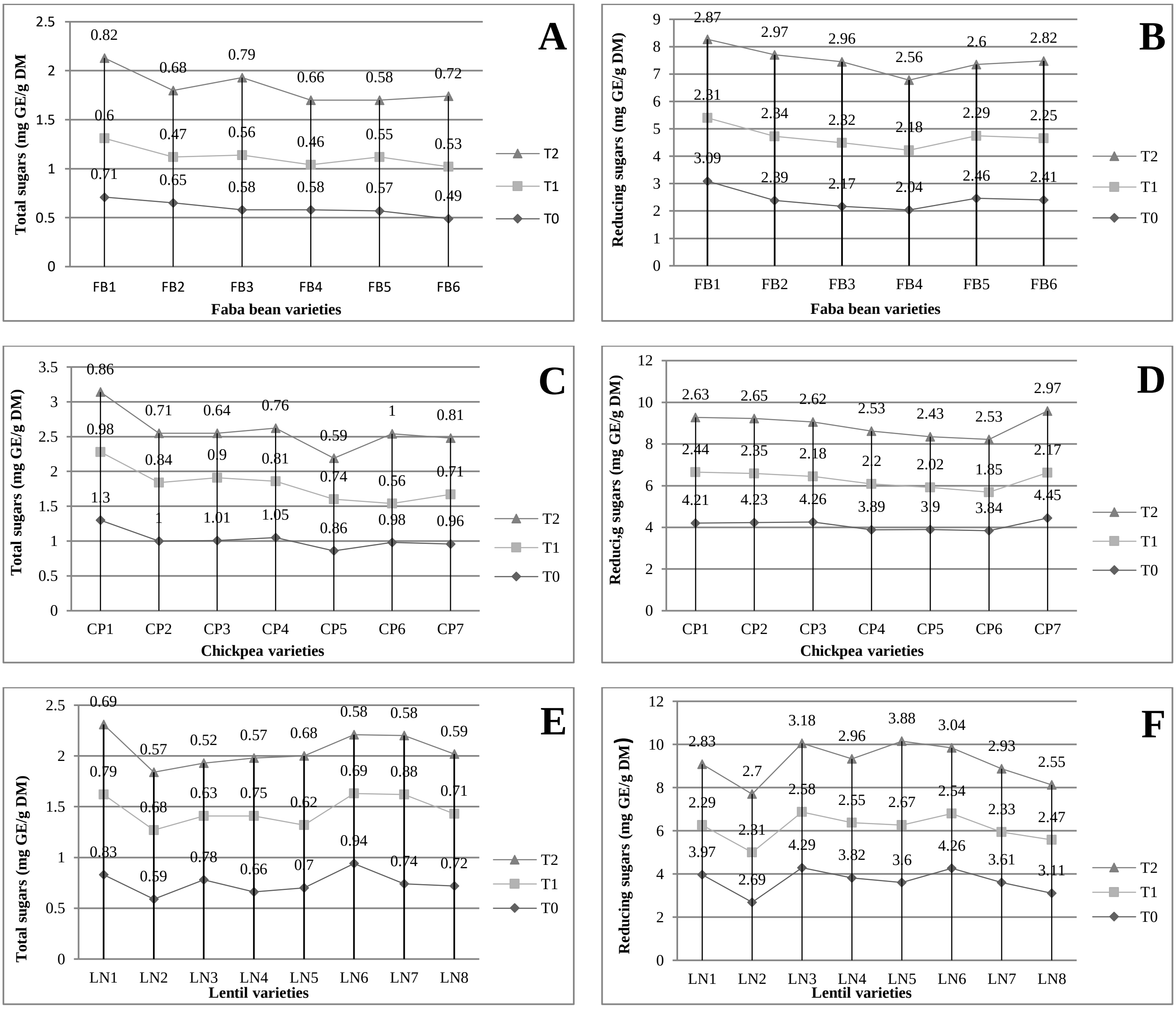

Figure 1: Total and reducing sugars content in faba bean, chickpea and lentil varieties appeared in the graphs A, B, C, D, E and F
T_0 : raw seeds; T_1 :soaked seeds) ; T_2 :germinated seeds ; LN : lentil ; CP : chickpea ; FB : faba beans ; mg GE/g DM : milligrams of glucose equivalent per gram of dry matter.

3. Bioactive compounds contents

The contents of total phenolic compounds (TPC), tannins, and flavonoids in the CP, FB and LN varieties, during the three stages T_0, T_1 and T_2, are presented in tables 4, 5, and 6.

Raw seeds of LN and FB had the highest contents of TPC, tannins, and flavonoids against chickpea seeds. That had the lowest corresponding compounds. Although the different raw lentil varieties contain the highest TPC content with an average content of (7415.44 µg GAE /g DM), the latter was reduced in the following order: (4580.22 µg GAE /g DM) at T_1, and (3499.49 µg GAE /g DM) at T_2. Indeed, these results also showed that there was no significant difference ($p > 0.05$) in the interaction between the different lentil varieties and the performed treatments. Indeed, Aguilera et al [2] reported that lentil seeds exhibited a strong decrease in TPC content during the germination periods.

The different germinated chickpea varieties recorded a higher average TPC content (2919.18 µg GAE/g DM), followed by the soaked ones with an average content of (1301.78 µg GAE/g DM). However, the raw ones showed the lowest average TPC content (1185.98 µg GAE/g DM). Germination altered the TPC content of chickpea and lentil, due to higher polyphenol oxidase activity and thicker seed coat which reduced water uptake [6].

The average levels of TPC in the different fava bean varieties, at T_0, T_1, T_2 were respectively in the order of 4418.08 µg GAE/g DM; 4785.47 µg GAE/g DM; and 3820.99 µg GAE/g DM, respectively. Soaking resulted in an increase in TPC in the fava bean varieties.

However, the soaking and germination processes significantly decreased the flavonoids content. This decrease during the germination process was reported by Salem et al [24] for field beans, chickpea, lentil, and fenugreek seeds. Indeed, the flavonoid contents of 247.36 µg QE/g DM and 165.40 µg QE/g

DM were reported for raw and soaked fava beans, respectively. Consequently, the flavonoids content was significantly reduced in the fava beans treated by the soak. However, germination caused an increase in this content to (269.94 µg QE/g DM).

In addition, the flavonoids content of the different lentil varieties was affected by the treatments ($p < 0.01$). Similarly, a very highly significant difference ($p < 0.001$) was observed between the flavonoid contents of the different bean varieties and the treatments carried out. Thus, the average tannins content of lentil seeds at T_0, T_1, and T_2 decreased in the order of 18.16; 10.54; and 4.62 µg EC/g DM, respectively. Following the statistical analysis, the tannins contents of the different lentil varieties were affected by the treatments carried out ($p < 0.05$). However, no significant difference ($p > 0.05$) was observed between the average tannins and flavonoids contents of the different chickpea varieties and the performed treatments.

The highest tannin levels were found in raw bean varieties (29.49 µg EC/g DM). A decrease was observed in the varieties treated by soaking and germination, with an average content that varied from 10.45 µg CE/g DM to 4.98 µg CE/g DM, respectively.

Based on the above interpretations, it was concluded that legumes exhibit considerable variation in their TPC, tannins, and flavonoids content. The obtained results showed great variability in the different studied legumes. The soaking and sprouting treatments bring about significant changes in the bioactive compounds of food legume seeds.

The increase in TPC was likely attributable to the biosynthesis of phenolic compounds during seed germination and the release of phenolic compounds bound to the cell wall [21].

The influence of germination on total phenolic compounds content has been studied in many food legumes seeds. Also, other studies have shown that germination can gradually accumulate soluble phenolics in germinated seeds compared to raw seeds. However, several studies have also reported a decrease in soluble phenolic compounds in germinated seeds [12]. This contradiction may be partly associated with the results expressed in dry or wet weight if we consider that the water content during germination increases gradually [11].

Table 4: Effect of soaking and germination on the levels of bioactive molecules in chickpea varieties

P	TPC			tannins			flavonoids		
T / V	T_0	T_1	T_2	T_0	T_1	T_2	T_0	T_1	T_2
CP1	1337,07^a	1278,84^b	3222,34^{ab}	24,28^a	11,28^{bc}	2,36^b	369,00^a	289,82^{ab}	470,92^a
CP2	1162,10^a	1250,34^b	2858,20^{ab}	26,58^a	9,19^{bc}	3,76^b	222,02^{ab}	278,70^{ab}	447,60^a
CP3	1445,22^a	1332,99^b	2679,03^{bc}	24,38^a	5,35^c	3,00^b	201,66^b	450,42^a	525,72^a
CP4	1226,69^a	1152,14^b	2148,74^c	45,80^a	13,10^b	5,22^b	360,22^{ab}	108,36^b	264,10^a
CP5	1078,59^a	1796,57^a	2773,34^b	43,56^a	23,14^a	10,16^a	360,40^{ab}	226,42^b	259,79^a
CP6	1049,80^a	1067,60^b	3377,35^a	34,57^a	9,50^{bc}	3,56^b	330,62^{ab}	243,34^{ab}	227,70^a
CP7	1002,40^a	1233,90^b	3375,23^a	32,56^a	11,76^c	2,99^b	285,34^b	286,99^b	481,00^a
V*T		***			ns			ns	
LSD	464,23	280,50	597,58	30,48	6,49	4,04	162,45	223,33	492,80

P: Parameters; V: Varieties; T: Treatment; PC: Chickpea; T*V: Treatment*variety interaction; LSD: Least significant difference; a, b, c…: Different letters within the same column are significantly different ($P < 0.05$); ns: Not significant difference; ***: Very highly significant.

Table 5: Effect of soaking and germination on the levels of bioactive molecules in fava bean varieties

P	TPC			tannins			flavonoids		
T / V	T_0	T_1	T_2	T_0	T_1	T_2	T_0	T_1	T_2
FB1	4901,85^a	4367,57^b	1965,48^{ab}	19,89^a	8,87^{ab}	6,53^a	173,82^b	154,97^a	416,93^a
FB2	3907,27^b	4667,57^{ab}	4705,00^a	28,25^a	15,76^{ab}	2,25^a	198,92^b	124,10^a	381,24^a
FB3	4810,36^a	5715,42^a	4466,93^{ab}	29,02^a	3,93^b	3,03^a	369,54^a	206,39^a	106,36^b
FB4	4370,09^{ab}	5149,29^{ab}	3972,19^{ab}	36,32^a	4,81^b	1,18^a	236,85^b	149,63^a	135,35^b
FB5	4823,92^a	4699,52^{ab}	3979,99^{ab}	36,76^a	17,19^a	8,24^a	234,52^b	165,66^a	309,39^a
FB6	3694,99^b	4113,53^b	3836,34^{ab}	26,70^a	12,17^{ab}	8,64^a	270,49^b	191,65^a	270,37^{ab}
V*T		ns			ns			***	
LSD	829,24	1134,30	2621,70	26,00	11,97	10,65	97,99	138,65	166,86

P: Parameters; V: Varieties; T: Treatment; FB: Fava bean; T*V: Treatment*variety interaction; LSD: Least significant difference; a, b, c…: Different letters within the same column are significantly different ($P < 0.05$); ns: Not significant difference; ***: Very highly significant.

Table 6: Effect of soaking and germination on the levels of bioactive molecules in lentils varieties

P	**TPC**			**tannins**			**flavonoids**		
T / V	**T_0**	**T_1**	**T_2**	**T_0**	**T_1**	**T_2**	**T_0**	**T_1**	**T_2**
LN1	5686,40^{b}	4103,78^{d}	3623,58^{a}	20,54^{ab}	11,53^{b}	4,08^{ab}	407,95^{bc}	443,27^{a}	252,76^{b}
LN2	13938,29^{a}	3978,97^{d}	2798,81^{b}	24,61^{a}	13,07^{ab}	5,14^{ab}	623,69^{a}	388,93^{a}	287,17^{ab}
LN3	7564,60^{ab}	5473,92^{a}	4021,12^{a}	25,89^{a}	18,45^{a}	5,44^{ab}	471,32^{bc}	374,70^{a}	298,69^{ab}
LN4	6875,28^{b}	4280,13^{d}	3363,26^{ab}	16,26^{bc}	10,96^{b}	7,17^{a}	492,87^{ab}	337,94^{a}	380,50^{a}
LN5	7121,74^{ab}	4763,69^{c}	3841,52^{a}	18,90^{ab}	7,69^{bc}	2,49^{b}	447,41^{bc}	367,07^{a}	318,12^{ab}
LN6	7461,48^{ab}	4627,77^{c}	3708,09^{a}	15,14^{bc}	7,31^{bc}	5,42^{ab}	342,52^{c}	339,58^{a}	325,22^{ab}
LN7	6204,97^{b}	5115,46^{b}	3817,67^{a}	13,53^{bc}	10,91^{b}	4,98^{ab}	389,65^{bc}	355,64^{a}	317,46^{ab}
LN8	4470,78^{b}	4298,03^{d}	2821,85^{b}	10,37^{c}	4,41^{c}	2,23^{b}	401,32^{bc}	389,65^{a}	283,31^{b}
V*T		ns			*			**	
LSD	6957,7	327,05	718, 17	7,8258	5,9909	4,4342	132,66	115,01	97,051

P: Parameters; V: varieties; T: Treatment; T*V: Treatment*variety interaction; LSD: Least significant difference; a, b, c…: Different letters within the same column are significantly different (P <0.05); ns: Not significant difference; **: Highly significant;*: Significant.

V. CONCLUSION

The present study demonstrates that soaking and germination significantly affect the nutritional composition of legume seeds and their antinutritional properties.

Various processing methods influenced the main nutrients in these legumes, including protein, carbohydrates, and fiber. Several bioactive compounds were also detected following germination.

A general decrease in the content of soluble compounds was observed in the soaking process, although an increase in the content was observed during germination. Indeed, germination led to an increase in the content of crude protein, total and reducing sugars.

To improve the digestibility of certain nutrients, including carbohydrates and proteins, food legumes should be processed before consumption.

REFERENCES

[1] AACC. (2000). American Association of Cereal Chemists. Approved Methods of the AACC, (10th ed.). Method 44-15A. St. Paul, MN: The Association.

[2] Aguilera, Y., Liébana, R., Herrera, T., Rebollo-Hernanz, M., Sanchez-Puelles, C., Benítez, V., Martín-Cabrejas, M.A. (2014). Effect of illumination on the content of melatonin, phenolic compounds, and antioxidant activity during germination of lentils (Lens culinaris L.) and kidney beans (Phaseolus vulgaris L.). J. Agric. Food Chem. 62, 10736–10743.

[3] Alonso, R., Aguirre, A., Marzo, F. (2000). Effects of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans. Food Chem. 68, 159-165.

[4] Aranda, P., Dostalova, J., Frias, J., Lopez-Jurado, M., Kozlowska, H., Pokorny, J., Urbano, G., Vidal-Valverde, C., Zdyunczyk, Z. (2001) Nutrition. In CL Hedley, ed, Carbohydrates in Grain Legume Seeds. Improving Nutritional Quality and Agronomic Characteristics. CAB International, Wallingford, UK. 61–87.

[5] Bau, H.M., Villaume, C., Nicolas, J.P., Mejean, L. (1997). Effect of germination on chemical composition, biochemical constituents and antinutritional factors of soya bean (Glycine max) seeds. J. Food Sci. Agric. 73, 1-9.

[6] Boesewinkel, F.D., Bouman, F. (1995). The seed: structure and function. In J. Kigel& G. Galili (Eds.), Seed development and germination. CRC Press. 1–24.

[7] Bond, D.A., Laws, D.A., Hawtin, G.C., Saxena, M.C., Stephens, J.H. (1985). Faba bean (Viciafaba L.). In: R.J. Summerfield and E.H. Roberts (Editors). Grain Legume Crops. Collins, London. 199-265.

[8] Donkor, O.N., Stojanovska, L., Ginn, P., Ashton, J., Vasiljevic, T. (2012). Germinated grains e Sources of bioactive compounds. Food Chem. 135, 950-959.

[9] Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F. (1956). Colorimetric method for determination of sugar and related substances. Anal. Chem. 28, 350-356.

[10] El-maki, H.B, Babiker, E.E., El-tinay, A.H. (1999). Changes in chemical composition, grain malting, starch and tannin contents and protein digestibility during germination of sorghum cultivars. Food Chem. 64, 331-336

[11] Guo, X., Li, T., Tang, K., Liu, R.H. (2012). Effect of germination on phytochemical profiles and antioxidant activity of mung bean sprouts (Vigna radiata). J. Agric. Food Chem. 60(44), 11050–11055.

[12] Magalhães, S.C., Taveira, M., Cabrita, A.R., Fonseca, A.J., Valentão, P., Andrade, P.B. (2017). European marketable grain legume seeds: Further insight into phenolic compounds profiles. Food Chem. 215, 177–184.

[13] Martín-Cabrejas, M.A., Ariza, N., Esteban, R.M., Mollá, E., Waldron, K., López-Andréu, F.J. (2003). Effect of germination on the carbohydrate composition of the dietary fibre of peas (Pisum sativum L.). J. Agric. Food Chem. 51, 1254–1259.

[14] Martin-Cabrejas, M.A., Diaz, M.F., Aguilera, Y., Benitez, V., Molla, E. Esteban, R.M. (2008). Influence of germination on the soluble carbohydrates and dietary fiber fractions in non-conventional legumes. Food Chem. 107(3), 1045-1052.

[15] Miller, G.L. (1959). Use of dinitrosaiicyiic acid reagent for determination of reducing sugar. Anal. Chem. 31, 426–428.

[16] Narina, S.S., Hamama, A.A., Bhardwaj, H.L. (2012). Nutritional and Mineral composition of flax sprouts. J. Agric. Sci. 4, 60-66.

[17] Nonogaki, H., Bassel, G.W., Bewley, J.W. (2010). Germination-still a mystery. Plant Sci. 179, 574-581.

[18] Price, M.L, Van scoyoc, S., Butler, L.G. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J. Agric. Food Chem. 1978, 26, 5, 1214–1218.

[19] Quettier-Deleu, C., Gressier, B., Vasseur, J., Dine, T., Brunet, C., Luyckx, M., Cazin, M., Cazin, J.C., Bailleul, F., Trotin, F. (2000). Phenolic compounds and antioxidant activities of buckweat (Fagopyrum esculentum Moench) hulls and flour. J. Ethnopharmacol. 72, 35–42.

[20] Rachwa-Rosiak, D., Nebesny, E., Budryn, G. (2015). Chickpeas-composition, nutritional value, health benefits, application to bread and snacks: a review. Crit. Rev. Food Sci. Nutr. 55(8), 1137-45.

[21] Randhir, R., Lin, Y.T., Shetty, K. (2004). Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mung bean sprouts in response to peptide and phytochemical elicitors. Process Biochem. 39(5), 637–646.

[22] Rochester, I.J., Peoples, M.B., Hulugalle, N.R., Gault, R.R., Constable, G.A. (2001). Using legume to enhance nitrogen fertility and improve soil condition in cotton cropping systems. Field Crops Res. 70, 27-41.

[23] Rodriguez, C., Frias, J., Vidal-Valverde, C., Hernandez, A. (2008). Correlations between some nitrogen fractions, lysine, histidine, tyrosine, and ornithine contents during the germination of peas, beans, and lentils. Food Chem.108 (1), 245-252.

[24] Salem, A.A., El-Bostany, A.N., Al-Askalany, S.A., Thabet, H.A. (2014). Effect of domestic processing methods of some legumes on

phytochemicals content and in vitro bioavailability of some minerals. J. Am. Sci. 10, 276–288.
[25] Singleton, V.L., Rossi, J.A. (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16,144-158
[26] Suda, M., Watanabe, T., Kobayashi, M., Matsuda, K. (1986). Changes in starch content and related enzyme activities during the growth of germinating soybeans. Agric. Biol. Chem. 50(12), 3195- 3196.
[27] Torres, A., Frias, J., Granito, M., Vidal-Valverde, C. (2007). Germinated Cajanuscajan seeds as ingredients in pasta products: Chemical, biological and sensory evaluation. Food Chem. 101(1), 202-211.
[28] Urbano, G., Lopez-Jurado, M., Frejnagel, S., Gomez-Villalva, E., Porres, J.M., Frias, J., Vidal-Valverde, C., Aranda, P. (2005). Nutritional assesment of raw and germinated pea (Pisum Sativum L.) protein and carbohydrate by in vitro and in vivo techniques. Nutrition 21(2), 230-239.
[29] Veluppillai, S., Nithyanantharajah, K., Vasantharuba, S. (2009). Biochemical Changes Associated with Germinating Rice Grains and Germination Improvement. Rice Sci. 16(3), 240–242.
[30] Vidal-Valverde, C., Frias, J., Sierra, I., Blazquez, I., Lambein, F., Kuo, Y. (2002). New functional legume foods by germination: effect on the nutritive value of beans, lentils and peas. Eur. Food Res. Technol. 215(6), 472-477.
[31] Yoshiki, Y., Kudou, S., Okubo, K. (1998) Relationship between chemical structures and biological activities of triterpenoid saponins from soybean (Review). Biosci. Biotechnol. Biochem. 62, 2291–2299.

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