

Towards Include Preservation of Vitamins in Fenugreek and Carob Seeds by the Instant Controlled Pressure-Drop Process (DIC Process)

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Abstract

Fenugreek and carob seed were germinated in the dark for 4 days and 15 days respectively. The two species are rich in vitamins A, B1, B3, B8. Germination promotes the increase in their concentrations. To ensure preservation and increased bioavailability of the vitamins, it is necessary to apply a DIC of 30s to 400 kPa making it possible to support the cellular expansion and the decontamination of germinated seeds. Vitamin A content in germinated carob seed increased by 82.54%, which is not the case for fenugreek. A decrease in vitamin A concentration of 4 µg/100 g of dry matter. DIC increase vitamin B1, B3 and B8 in germinated fenugreek seeds. A slight decrease of B vitamins in germinated carob seed after treatment with DIC has been noticed. This loss can be explained by the low bioavailability caused mainly by the botanical structures of the seeds.

Keywords

Fenugreek, Carob, Seed, Germination, Preservation, Bioavailability, Vitamin, Instant Controlled Pressure Drop (DIC)

1. Introduction

Germinated seeds are used as a food for their nutritional value or exploited for

decoration [1]. Many varieties are suitable for consumption like the leguminous seeds (bean, fenugreek, alfalfa, lentils, peas, chickpeas, soybean...), the cereals (wheat, corn, millet, barley, rice, buckwheat...), the oilseeds (sesame, sunflower...), the umbelliferous seeds (carrot, celery, celeriac, fennel), the mucilaginous ones (watercress, flax), the cruciferous (mustard, radish, turnip, various cabbages).

The germinated seeds are biogenic foods which are also considered as life generator. Their nutritional qualities are numerous [1]. In addition, germination makes seeds more digestible and the nutrients more easily assimilated by the body. They help the intestinal transit and facilitate digestion.

The germinated seeds contain a reserve of nutrients in dormancy phase able to give a life to the whole plant [2]. The normal plant tissue contains approximately about 90% water while the seed contains less between 5% and 18%. The chemical reaction which takes place during germination makes it possible for the seeds to release from the inhibitors of enzymes. Indeed these enzymes make it possible for the starch presenting in seed to be transformed into simple sugar, to split proteins in amino-acids and the lipids in fatty-acids directly comparable to the level of the small intestine, which increases the digestibility from 50% to 60% and increases the nutritional value [3].

When germination performs an extraordinary molecular transformation, all the nutrients, vitamins, trace elements explode and bring a hundred times higher quantity of macronutrients vitamins [1]. Additionally, as mentioned previously, germination in itself marks a moment of intense molecular processing activity in the seed. In fact, it is a chemical reaction for the seeds to release enzyme inhibitors transforming the starch into simple sugar; by splitting amino acids in proteins and lipids into fatty acids directly assimilated at the small intestine, even those which protect the cardiovascular system. Moreover, it promotes an increasing of nutritional value of the seed.

Some vitamins, absent from the dry seed, may even appear, such as vitamin C in wheat germ. Dry seeds have nutritional properties definitely lower than germinated seeds [4]. In fact, the germinated seeds are rich in vitamins, minerals, enzymes and especially out of proteins of excellent quality. Furthermore, these seeds contain lipids which appear to be unsaturated fatty acids, even those that protect the cardiovascular system. The germinated seeds are rich in fibers and have a lower glycolic index. They reduce fat storage, muscle wasting and are very satisfying while bringing few calories. Vitamins are essential nutrients which must be supplied to the body in small amounts on a regular basis to perform various chemical and physiological functions in the human body [5]. They are widely distributed in natural food sources and can be easily introduced into the diets to meet daily needs. Although the vitamins are a group of organic compounds which have structural and chemical properties, they can be conveniently classified into two groups according to their solubility: water soluble vitamins and fat soluble vitamins. Water soluble vitamins comprises vitamins A [6] [7], D

(Eiff, Monakhova, and Diehl, 2015), E [8] [5], and K [8] and other carotenoids with varying degrees of vitamin A activity [6]. Fat soluble vitamins comprises vitamin C (Silva *et al*, 2013) and vitamin B [9], namely thiamine (vitamin B1) [10], riboflavin (vitamin B2) [7], niacin (vitamin B3), pyridoxine (vitamin B6) [11], pantothenic acid (vitamin B5), biotin (vitamin B8), folic acid (vitamin B9) and cyanocobalamin (vitamin B12) [12]. Vitamins solubility not only decides their distribution in the different food groups, but is also an important factor to consider for their analysis and quantification. Vitamins have a variety of uses in food products as colorants, antioxidants, and nutritional additives, in particular.

Fenugreek, "*Trigonella-foenum-graecum*", is a legume of the family of Fabaceae, under family of Faboideae, Tribe of Trifolieae, genre of Trigonella. It is a small annual herbaceous plant from 20 to 50 cm tall with leaves composed of three leaflets and yellowish or white flowers of triangular form (hence the name of trigonelle) giving rise to fruit pods that contain ten to twenty seeds with strong characteristic odor. Fenugreek seeds are very hard, oblong, and angular and of color brown clearly. It is used mainly as medicinal herb and condiment.

Fenugreek is very widespread around the Mediterranean basin of which it is cultivated in Tunisia in great quantity, which justifies our choice within the framework of the valorization of this species.

In addition to their culinary properties, recent scientific studies have confirmed the medicinal value of fenugreek seeds [13]: a diuretic, hypertensive on the heart, cholesterol lowering (cholesterol and triglycerides reducer), galactagogue, stimulating the pancreas, anti-inflammatory, an appetite opener used in cases of anorexia and loss of appetite, emollient (its ongoing mucilage provides an emollient effect which moistens the respiratory mucosa and quenches thirst), antidiabetic agents, retarding the evolution of certain cancers.

According to the Food and Drug Administration, we can consider fenugreek seeds as a product "generally to safe" for human health [13].

The carob tree, known under the scientific name *Ceratonia siliqua*, belongs to the genus *Ceratonia* of the subfamily of Caesalpinioideae, of family Fabaceae (Leguminous plants), part of the order Fabales (Rosales) Subclass Rosidae, of class Magnoliopsida, of subphylum angiosperms and of Tracheobionta branch. The carob tree is native of the Mediterranean Region. It is found mainly naturally in Spain, Morocco, Algeria, Tunisia, Egypt, Greece, Italy, Turkey, Portugal, and Cyprus [14].

Recent studies seek to develop all components of carob seed which represents 10% of the total weight of the carob pod (30% - 33% seed coat, 42% - 46% endosperm and 23% - 25% cotyledon) [15] and show their important roles in food industries as a stabilizing agent, and pharmaceutical industries [16].

Scientific researchers showed that carob seed are rich in phenolic compounds [17], sugar, fatty acids [18], tannins, proteins [19] and minerals. Some vitamins

are identified and quantified in the pod (pulp and seeds) and in the pulp but not in the seed. For this reason and given the interest of the valuation of seed.

In this study we will follow the evolution of vitamins in those fenugreek and carob seed, optimize processing conditions by Instant Controlled Pressure Drop Process "DIC" (Allaf, 2014) and to assess the impact of this technology on the bioavailability and ensure of vitamins seeds: the seeds will be treated before germination, then the vitamins are extracted before and after DIC treatment and analyzed by reverse-phase liquid chromatography (RP-HPLC). The final objective of this study is to increase the bioavailability and ensure preservation of vitamins by DIC process, subsequently transform the seeds into powder in order to introduce them as a food supplement.

2. Materials and Methods

2.1. Vegetable Materials

The carob seed (*Ceratonia siliqua*) are harvested in the month of September 2014 from INRGRF (National Institute for Research in Rural Engineering, Water and Forestry), while fenugreek seeds (*Trigonella foenum-graecum*) are bought from a sales center of seed at *BouSalem* North-West city of Tunisia country 36°36'40"N, 8°58'11"E.

2.2. Methods

Carob seed and fenugreek seeds were stored in the dark for 4 days and 15 days respectively at ambient temperature (25°C). Carob seed did not germinate without pretreatment in particular once it was dried, it became very hard and did not thus absorb water thereby, preventing the seed to germinate which was not the case with fenugreek seeds [15].

In fact, a good seed yield is achieved in the case of acid treatment. We should then follow this acid treatment protocol that involves soaking the carob seed for 20 min in concentrated sulfuric acid (H₂SO₄) and then 20 minutes in soapy water with a few drops of Tween 20 as wetting agent, then 20 minutes in the solution of Benlate used as fungicide agent [15].

The treated carob seed and fenugreek seeds washed with distilled water were germinated after 15 days and 4 days respectively in a room of culture in conditions controlled at a temperature of 25°C ± 2°C. In the laboratory, various samples were crushed and stored away from light in glass vials for further analysis.

2.2.1. DIC Treatment

Instant Controlled pressure Drop Process (shown in **Figure 1**) was introduced in 1988 by the team of Professor Allaf [20]. Applied to food products, the DIC provides a best way to couple perfectly controlled thermal and mechanical treatment.

Given the interest of this technique, germinated fenugreek and germinated carob were divided and treated with DIC treatment. For both species studied an experimental of two parameters, shown in **Table 1**.

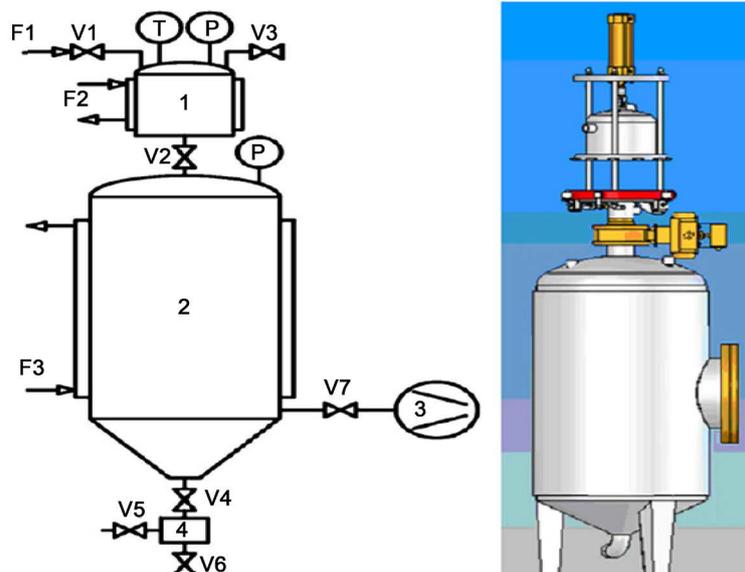


Figure 1. Photo and schematic diagram of the pilot apparatus instant controlled pressure drop DIC: (1) autoclave with heating jacket; (V2) rapid valve; (2) vacuum tank with cooling Water jacket; (3) vacuum pump; (4) extract container; F1&F2steam flow; F3 cooling water flow.

Table 1. Independent variables used in developing experimental data and experimental design used in DIC treatment.

Independent variables		$-\alpha$	-1	0	$+1$	$+\alpha$
Saturated steam pressure P (kPa)		200	300	400	500	600
Processing time (s)		10	15.9	30	44.1	50
Trial N°.	1 2 3 4 5 6 7 8 9 10 11 12 13					
Pressure (kPa)	400 600 400 400 540 540 400 260 260 400 200 400 400					
Time (s)	30 30 50 30 44.1 15.9 30 15.9 44.1 30 30 10 30					

α is axial distance, $\alpha = \sqrt[3]{N}$. N is the number of experiments of orthogonal design; $N = 2^n$. n is the number of parameters; $n = 2$ In this case, $n = 2$ and $\alpha = 1.4142$. Exp: number of the experiment.

Treatment with DIC performed on fenugreek seeds and carob seed are carried out according to an design of experiment DoE using 2 operating parameters: the saturated steam pressure P ranged from 200 to 600 kPa and the treatment time t ranged from 10 to 50s. The operating conditions shown in **Table 1**.

2.2.2. Vitamins A and E

Carob and fenugreek seeds were processed to determine the concentrations of vitamin A and E, based on the methods of analysis relating to control of authorized additives suitable foods [21] [22]. The samples were saponified with potassium hydroxide solution and the vitamins extracted into hexane which will be removed by evaporation. The residue obtained was dissolved in methanol. Then the concentrations of vitamin A and E of the methanol extract were determined by reverse phase liquid chromatography RP-HPLC with reference to a calibration curve of retinol for Vitamin A and alpha-tocopherol to vitamin E [23].

2.2.3. Vitamins B

The preparation of the samples was performed based on the decree of January 1998 of Official assays vitamins A, B, D and E in foods (consolidated to July 22, 2015, Department of State, Ministry of economy, Finance and Privatization in Tunisia) and methods developed in research studies [5] [9]. This method consisted initially of an acid attack followed by further enzymatic hydrolysis.

2.2.3.1) Acid hydrolysis

A 0.5 g of sample, well homogenized, was mixed with 6.5 ml of a hydrochloric acid solution (0.1 N). The resulting mixture was placed in a water bath at 60°C for 30 minutes. And thereafter one proceeds immediately cooling.

2.2.3.2) Enzymatic hydrolysis

After pH adjustment (according to experience) to 4.2 by the addition of sodium acetate solution (2.5N), 0.5 g of Taka-diestase enzyme is added, and the mixture is put in an oven at 37°C for 12 h. After incubation of 12°C to 37°C, samples treated were undergoing to quenching and quantitatively the mixture is decanted into a 100 mL volumetric flask. Then, it adjusted with distilled water. Finally, filter the supernatant chromatographic analysis for high pressure liquid HPLC.

2.2.3.3) Reversed-phase high performance liquid chromatography (RP-HPLC)

the HPLC system (Agilent) was equipped with a pump type technology Agilent 1200 series, a vacuum degassing unit model G1322A, a UV-VIS spectrometer to 8 wavelengths, a fluorescence detector (G1321 Agilent 1200 Series), an analytical C 18 column (Agilent) (4.6 mm × 250 mm, 5 µm). The mobile phase of the HPLC system was comprised of pure methanol and sodium dihydrogen phosphate NaH₂PO₄ (10:90 v/v). During the analysis the column was equilibrated at 30°C. The chromatographic peaks were recorded and elaborated automatically by employing a computerized program "Agilent ChemStation". The analysis was performed by gradient elution of wavelength at room temperature, at a flow rate of 1 mL/min. The total execution time required is less than 20 minutes.

Table 2. Program of wavelength changes during elution time for five vitamins water-soluble determinations in fenugreek and carob seed.

Vitamins	Time (min)	Wavelengths (nm)
Biotin (B8)	0.0 - 2.5	204
Nicotinamide (B3)	2.6 - 3.8	261
Thiamine (B1)	3.9 - 4.5	234
Pyridoxine (B6)	4.6 - 5.0	275
Pantothenic acid (B5)	5.1 - 7.0	210

The program of wavelength changes during elution time for vitamins B determination shown in **Table 2**.

2.2.4. The Membrane Integrity

The effect of treatment with DIC on membrane integrity is determined for seeds germinating according to the method of Singh [24] [25]. This parameter has been included in order to have more information about the stability of the membrane and the relative ion content. The seeds were washed with distilled water and were placed in sealed bottles containing 100 ml of distilled water and incubated at 25°C for 2 hours. The electrical conductivity of the solution C_1 was measured using an electrical conductivity type WTW Wissenschaftlich-Technische Werkstätten LF521. This measurement is used to detect ion leakage caused by the treatment of DIC in relation to total ions normally present in the sample. The samples were autoclaved at 120°C for 20 minutes and the electric conductivity C_2 was obtained after stabilization at 25°C. This measure reveals the maximum ion leakage.

The leakage of electrolytes is determined by the following formula:

$$\text{The membrane integrity (\%)} = \frac{C_1}{C_2} * 100$$

2.2.5. Statistical Analysis

The different experiment results are obtained following the average of three separate determinations. It was mentioned that the statistical significance of the results found was analyzed using ANOVA-EXCEL.

3. Results and Discussion

3.1. Vitamin B

Treated samples were analyzed by HPLC according to the chromatographic conditions shown in 2.2.2.3 paragraph. The compounds were conveyed under pressure by the mobile phase which is in this case composed of a solution of sodium dehydrogenate phosphate and Methanol (80% NaH_2PO_4 and 20% methanol).

Correlation coefficients show the linearity of the calibration curve, which allows using them for the determination of the concentration of each vitamin

present in each sample expressed in mg/L. Six working solutions were prepared for each analyte whose range is between 1 and 30 mg/L for B1, B3, B5, B6 and seven solutions between 1 and 30 mg/L for B8. The analysis was performed in triplicate to determine the linearity of the assay. The regression lines were calculated by the method of least squares of the areas of the peaks relative to the analyte. The equations corresponding to the five regression analytes were

$$\text{B1: } y = 25.82754x + 6.26753$$

$$\text{B3: } y = 33.21959x - 1.42661$$

$$\text{B5: } y = 7.15590x + 5.776e^{-1}$$

$$\text{B6: } y = 13.70389x + 9.16444e^{-1}$$

$$\text{B8: } y = 11.89793x + 2.37982$$

x: Amount et y: Area

They were consistently linear in the already mentioned range for all compounds. The linearity was checked by analysis of variance of the regression (Table 3). A value of r above 0.9949 for all vitamins, ($P < 0.001$) except for thiamine with $r = 0.9781$. The coefficient of determination (r^2) is more than 95.66% for thiamine and 99.66% higher than for others. Six determinations of the same sample were performed to assess the accuracy of the method. Six determinations of the same sample were performed to assess the accuracy of the method. Table 4 illustrates the accuracy of the method for the determination of vitamins B1, B3, B5, B6, and B8.

As we have reported our study intend to identify and to quantify thiamine (B1), niacin (B3), pantothenic acid (B5), pyridoxine (B6) and biotin (B8) in the non germinated and germinated seeds treated or not by the DIC process.

The content of vitamins B in different samples is determined in 100 g of dry seeds DS. The data outlined in Table 5 represent the mean values of vitamins content in fenugreek and carob samples.

The content of thiamine, niacin, pantothenic acid, pyridoxine and biotin available in dry and germinated seeds for both species studied (FB, FGNT, CB, CGNT) varied. Thiamin content increased almost 5 times during germination for carob species. On the contrary, for the fenugreek, a disappearance of vitamin

Table 3. Linearity of standard curves of vitamins B₁, B₃, B₅, B₆, and B₈.

Vitamins	r	r ²	F ^a _{exp}	DF ^b	P
Biotin (B ₈)	0.9949	99.88	21,247.5	1.5	($P < 0.001$)
Nicotinamide(B ₃)	0.9998	99.96	165,492.10	1.5	($P < 0.001$)
Thiamine (B ₁)	0.9781	95.66	100,026.5	1.5	($P < 0.001$)
Pyridoxine (B ₆)	0.9998	99.96	27,417.53	1.5	($P < 0.001$)
Pantothénic acid (B ₅)	0.9999	99.98	7686.97	1.5	($P < 0.001$)

F^a (1.5; 0.001) = 6.61. F tab and Fexp are tabulated and experimental Snedecor's F values, respectively in ANOVA analysis, DF^b, degrees of freedom.

Table 4. Peak area range and concentration, correlation data of the calibration curves and quantification limit of determined vitamins in fenugreek and carob seed.

Vitamins	Concentration range (mg/L)	Surface broad peak	Correlation	Detection limits (mg/L)	Quantification limits (mg/L)
Biotin (B ₈)	1 - 30	59.83426 - 348.10776	0.9949	0.006	0.022
Nicotinamide	1 - 30	31.45451 - 1000.048	0.9998	0.008	0.028
Thiamine (B ₁)	1 - 30	18.14213 - 714.6253	0.9781	0.012	0.042
Pyridoxine (B ₆)	1 - 30	14.31208 - 409.0012	0.9998	0.002	0.007
Pantotenic	1 - 30	8.05888 - 214.6708	0.9999	0.001	0.0035

Table 5. Mass concentration of vitamins B group for carob (CB) and fenugreek species (FG) (mg/100g dry seeds (DS)).

Vit	CB ¹	CGNT ²	CGμDIC ¹ ³	CGμDIC ² ³	CGμDIC ³ ³	CGμDIC ⁵ ³	CGμDIC ⁶ ³
B1	0.354 ± 0.011	1.792 ± 0.013	1.023 ± 0.002	0.762 ± 0.005	1.089 ± 0.009	0.686 ± 0.007	
B3	1.076 ± 0.006	0.972 ± 0.001	0.628 ± 0.001	3.238 ± 0.001	0.634 ± 0.009	0.589 ± 0.036	
B5	2.339 ± 0.050	2.109 ± 0.023	1.764 ± 0.009	1.701 ± 0.016	3.899 ± 0.005	1.714 ± 0.042	
B6	7.907 ± 0.027	0	0	0	0	0	
B8	0.491 ± 0.001	1.167 ± 9.2051E-06	0.801 ± 0.001	0.327 ± 0.001	0.711 ± 2.3639E-05	0.611 ± 0.009	
Vit	FB ¹	FGNT ²	FGμDIC ¹ ³	FGμDIC ² ³	FGμDIC ³ ³	FGμDIC ⁵ ³	FGμDIC ⁶ ³
B1	32.6 ± 0.005	0	174 ± 0.001	299.4 ± 0.001	126.7 ± 0.001	144.2 ± 0.001	120.6 ± 0.001
B3	3.098 ± 0.001	3.082 ± 0.002	4.333 ± 0.001	4.434 ± 0.003	0	3.917 ± 0.001	0
B5	0	0	0	0	0	0	0
B6	0	0	0	0	0	0	0
B8	1.275 ± 0.001	0.526 ± 7.8224E-06	3.605 ± 0.001	3.498 ± 1.2016E-08	3.971 ± 0.001	3.943 ± 0.001	3.397 ± 0.001

1) CB/FB: Ungerminated Carob seed/Ungerminated fenugreek seed. 2) FG/CG NT: Fenugreek or Carob/seed germinated not treated by the DIC pilot apparatus. 3) FG/CG μDIC 1, 2, 3, 5, 6: Fenugreek or Carob/seed germinated treated by the DIC pilot apparatus according to the experiments conditions shown in **Table 2**. Vit: vitamins.

B1 is observed when the seeds were germinated. Niacin content decreased by 9% for the carob and 0.5% for fenugreek after germination. Pantothenic acid increased by 9.8% during the germination of seeds of carob tree. Dry fenugreek seeds, however, were free of pantothenic acid even after germination content has not changed.

Germination of carob promoted the disappearance of the pyridoxine while it is away for ungerminated and germinated fenugreek.

When carob seed were germinated, the biotin content has increased by 2.4 times, while for the fenugreek content decreased by 59%.

From the results, we can conclude that the behavior of each legume lag phase and during germination is very specific and depends on the species and type of legume. The carob seed contain the various groups of vitamins B and the contents also depend on applied conservation treatment, with small losses in their vitamin content.

We can explain this variation of Vitamin B in untreated germinated seeds and

those treated by the DIC by the sensitivity and the chemical instability of vitamins B during the drying process at 45°C [26].

The carob C is the most affected as fenugreek, actually fenugreek F seeds showed the greatest changes: an increase thiamine content, niacin and biotin for sprouts treated with DIC. As a result, the considerable loss of the content of thiamin, niacin and biotin for untreated germinated seeds and a significant increase for those treated with DIC. This confirms the effect of the DIC in vitamins conservation purposes.

As we reported in the experimental section which followed a plan of experience in both parameters (processing time and pressure) for the treatment of DIC samples, we selected few points for HPLC analysis. The choice adopted is based on the following selection of points from the center point, the maximum and minimum points. It was noted that the repetition of analysis for each sample is of the order of three to verify the repeatability and reproducibility of results.

For the fenugreek, it is clear that the treatment for 30 s at 60 mPa corresponding to the point central is the most effective treatment from point of yield perspective. This finding is founded on the increase in the contents of vitamins B. This confirms the interest of DIC in the cell expansion promoting the increased availability of vitamins [27]. Moreover, this has been proven for phenolic compounds and oligosaccharides [28].

The contents of vitamins B1, B3, B5, B6 and B8 are variable for the samples of carob treated by the DIC. In fact, we choose, as for the fenugreek, the central point ($P = 0.4$ mPa; $t = 30$ s) while basing itself on the results found and shown in the **Table 5**. Our key objective was to choose the maximum condition from contents point of yield and apparition of the different vitamins.

To recapitulate: The two species studied are rich thiamine (Vitamin B1), niacin (vitamin B3) and biotin (vitamin B8) which have nutraceutical and pharmaceutical benefits. The role of quoted vitamins is proven in several research studies [10]).

It is known that vitamins do not exist in a simple form. In fact, thiamin may be present in seeds in various forms such as, mono-, di- and tri-phosphates thiamine. Similarly, niacin, nicotinic acid, nicotinamide, nicotinamide dinucleotide (NAD) and nicotinamide-adenine dinucleotide phosphate (NADP) are the predominant forms relating to the niacin [26].

The losses of vitamins can be explained by the multiple effects of several factors. First of all, it is necessary to consider the removal or reduction of the vitamin with the process of drying.

The rapid hydration of legumes can favor the mass transfer from the moist seeds. Our finding showed that higher vitamin losses obtained in fenugreek corresponds to their rate of faster hydration.

3.2. Vitamin A

The fat-soluble vitamin, vitamin A, exists in the biological matrix in different

forms: retinol, retinal, retinoic acid, retinyl palmitate and retinyl phosphate. These molecules are altered by oxygen in the air and deteriorations accelerated by the light and heat.

Germination makes it possible to enrich the seeds caroubier in vitamins A. On the other hand, it promotes a slight rating decrease for the fenugreek (FB, FGNT). Indeed, we found that the content of vitamins A are about 24.33 g and 26.747 g for 100 g of dry matter or dry seeds DS respectively for ungerminated CB and germinated CGNT carob seed. Germinated Carob seed are not consumed despite their high nutritional value.

The ungerminated and germinated seeds of two studied species are rich in vitamin A. It is possible to insulate it and develop it in nutraceutical and pharmaceutical sight considering vitamin A is known for these roles important in the vision, the growth of the bones, the cell multiplication, with the synthesis of certain proteins, with the reproduction, absorption of the iron and with the regulation of the immune system [6] [7].

Table 6 shows that it will have a reduction in the content of vitamin A for the fenugreek and carob samples treated by the DIC. As we have previously reported that this technique is a technique of conservation and not the generation of the chemical elements, it can be exploited it as a means of decontamination removing the problems of mold generation during germination.

3.3. Vitamin E

Alpha-tocopherol is not found for the two species studied. This may be explained by the higher rate of decomposition of vitamin E due to their chemical instability. Vitamin E is detected in crude samples and germinated for carob and fenugreek. Given the sensitivity of vitamin E can be explained as alpha tocopherol undergoes oxidation reactions. A number of phenomena can affect the quality: loss of the nutritional value (vitamins oxidation).

3.4. Membrane Integrity

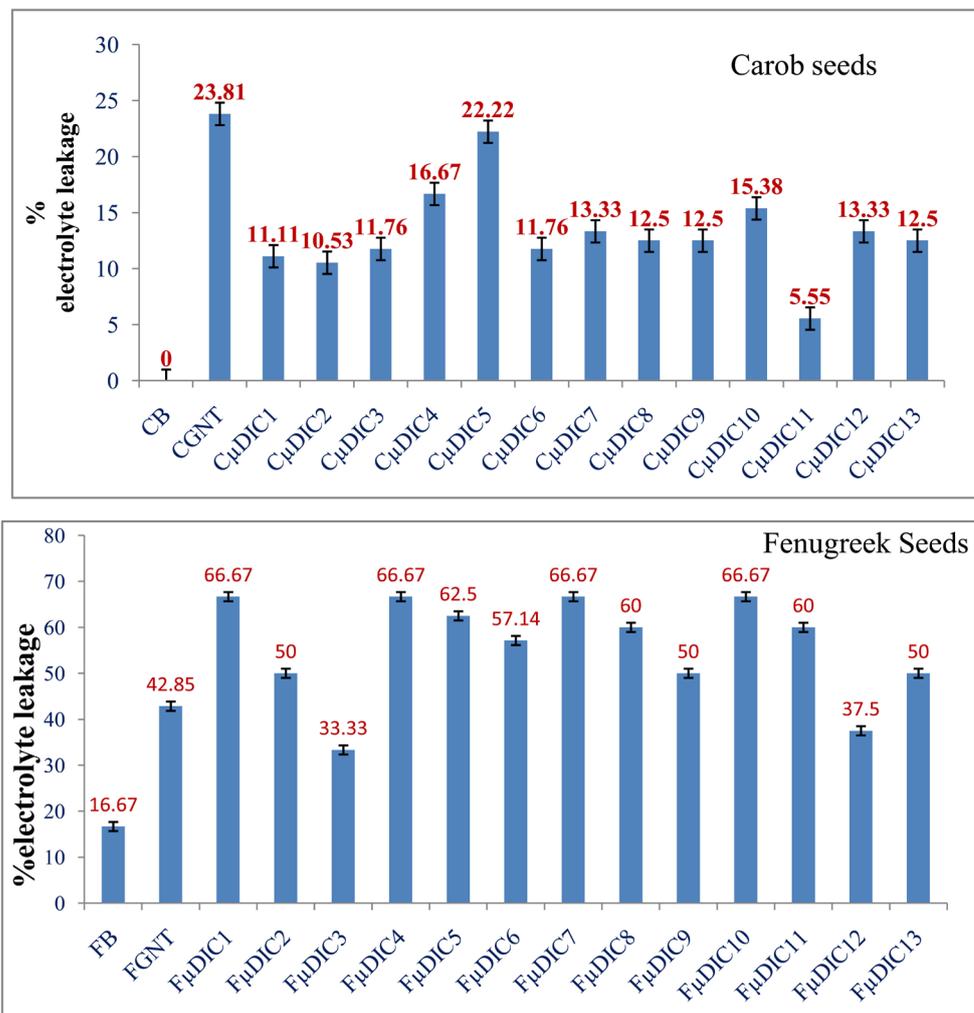
The following interpretation is based on the analysis of **Figure 2**:

The electrolyte percent are about 16.66% and 0% respectively for ungerminated

Table 6. Mass concentration of vitamin A for carob and fenugreek species (μg [Retinol]/100g DS).

CB ¹	CGNT ²	CG μDIC^3									
		1	2	3	4	5	6	7	8	11	12
24.330 \pm 0.002	26.747 \pm 0.204	48.826 \pm 0.002	0	50.626 \pm 0.008	48.909 \pm 0.002	0	0	48.775 \pm 0.001	0	0	0
FB ¹	FGNT ²	FG μDIC^3									
		1	2	3	4	5	6	8	11	12	
55.404 \pm 0.012	54.468 \pm 0.009	48.449 \pm 0	0	0	47.562 \pm 0.015	0	0	4.593 \pm 0.009	0	0	

1) CB/FB: Ungerminated Carob seed/Ungerminated fenugreek seed. 2) FG/CG NT: Fenugreek Carob/seed germinated not treated by the DIC pilot apparatus. 3) FG/CG μDIC 1, 2, 3, 4, 5, 6, 7, 8, 11, 12: Fenugreek or Carob/seed germinated treated by the DIC pilot apparatus according to the experiments conditions shown in **Table 2**.



CB/FB: Ungerminated Carob seed/Ungerminated fenugreek seed; FG/CG μ DIC 1, 2, 3, 4, 5, 6: Fenugreek or Carob/seeds treated by the DIC pilot apparatus according to the six experiment conditions in **Table 2**; FG/CG NT: Fenugreek or Carob/seeds not treated by the DIC pilot apparatus.

Figure 2. Percentage of electrolyte leakage in Carob(C) and fenugreek (F) seeds.

fenugreek seeds and ungerminated carob (control samples). Germination and treatment DIC promotes increased electrolyte leakage. This reflects the alteration of cellular structures in both species [29].

An alteration of the integrity of the cell membrane is expressed by a high percentage of electrolyte leakage in the treated samples than in the control samples [25]. These data could reflect an adaptation to stress or treatment after a brief membrane disintegration [30].

Measuring the intracellular ion leakage to the outside environment, through the cell membrane, allows us to determine the membrane integrity of the cell [31]. In fact, the increase in conductivity of the medium reflects the percentage increase in electrolyte leakage [30]. The cell walls are structures to see rigidity and expansion of plant cells [32].

The integrity of cell membranes is essential for the survival and structural variation of the plant cell. However, these membranes constantly undergo ger-

mination changes before and after DIC treatment, which confirms and proves our choice of DIC treatment conditions for vitamin A, B1 (thiamine), B3 (niacin), B8 (biotin) content.

3.5. Comparative Study for the Presence of Vitamins in Fenugreek

Table 7 shows the content of vitamins sought in the fenugreek ungerminated and germinated. The results evaluated in our study, on the one hand has elucidated that fenugreek is an excellent source of vitamins A, B1, B3 and B8, and on the other hand in agreement with previous studies [33] [34].

Meanwhile, fenugreek samples used in this study did not contain vitamins B5 and B6 similar to the data published in USDA and in the sales data sheets fenugreek seeds. It is mentioned that samples of fenugreek studied respectively from Kingdom of Saudi Arabia, India and America [34]. This can be explained by various factors such as the origin of crop seeds, soil, climate and culture variety.

4. Conclusions

This study has confirmed that germinated seeds for the two species are the important source for vitamin A and B. For the two species studied, the transition from latency to germination allows the generation of vitamins A, groups B and E. Because of the sensitivity of vitamin E, chromatograms show their degradation which may be the objective of another study.

Overall DIC improves transport and bioavailability within tissues. In fact, this technique makes it possible to open and explode the cellular strong boxes and subsequently it increases the levels of water-soluble and fat-soluble vitamins presenting in the germinated and non-germinated seeds for the two studied species: carob and fenugreek.

In fact, DIC process concerns numerous transformations of instantaneity thermodynamics and involves large operations of hydro-thermo-mechanical treatments (HTM). D.I.C. is distinguished by the possibility of a perfect control of process parameters (treatment time, levels of pressure, vacuum level, number of cycles, moisture content of the product, etc.). Treatments are many and varied, and therefore can be adapted to a very large number of applications of extraction of vitamin and protein.

Our perspective (work in progress and apart from the vitamins of the present study) is to increase the bioavailability and ensure preservation of Heat Shock Protein (HSP) by DIC process, therefore the seeds rich in vitamins and HSP (precisely the Small HSP) will be transformed into powder and consumed as nutritional supplements.

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Table 7. Mass concentration of vitamins in non-germinated and germinated fenugreek seeds.

Vitamins	Seeds	Units	Value/100g DS	References
			18 - 30	[35]
			3	[36]
Vitamin A	Ungerminated	µg	96	[34]
			3	[37]
			55.40	Present work
			36	[37]
	Germinated	µg	54.46	Present work
			12 - 43	[35]
Vitamin C	Ungerminated	mg	3	[37]
			3	[38]
			43	[34]
			0.41	[35]
			0.34	[34]
Vitamin B1	Ungerminated	mg	0.32	[37]
			0.32	[38]
			0.326	Present work
	Germinated	mg	1.74	Present work
			0.36	[35]
Vitamin B2	Ungerminated	mg	0.37	[37]
			0.37	[38]
			0.290	[34]
			6	[35]
			1.64	[37]
Vitamin B3	Ungerminated	mg	1.1	[34]
			1.64	[38]
			3.098	Present work
	Germinated	µg	4.333	Present work
Vitamin B5	Ungerminated	mg	0	Present work
			0	Present work
			0.6	[36]
Vitamin B6	Ungerminated	mg	0.6	[37] [38]
			0	Present work
	Germinated	mg	0	Present work
Vitamin B8	Ungerminated	µg	1.275	Present work
			3.605	Present work
Vitamin B9	Ungerminated	µg	84	[34]
			57	[37]

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