



## Germination on the nutritional properties of seeds of four melon varieties

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**ABSTRACT:** Fruit seeds, in terms of nutrition, are as wholesome as the grains cultivated exclusively for human consumption. The fruit pulp-processing agroindustry, more often than not, discards these seeds as unprofitable waste. The inclusion of seeds as a consumer product, equal in value to the pulp itself, can be made possible by adopting processes that increase their worth, and ideally at minimal costs. The controlled germination process is suitable for such an endeavor. The strategy presented here, is low in cost and permits the modulation of the constituents of the seeds by breaking the continuity of the process at the precise time, thus enabling the formation of a new product, which possesses bioactive properties. This research was performed with the focus on exploring the ways that the germination time influences the constituents of the seeds in four melon varieties. The Cantaloupe, Gália, Pele-de-Sapo and Orange melon seeds were studied and the water content, ash, proteins, lipids, total and reducing sugars, total phenolic compounds, tannins, flavonoids and anthocyanins were determined in the samples. This was done *in natura* and post germination specifically at 24, 48, 72 and 96 hours. Germination altered all the parameters, except the ash content. In Pele-de-Sapo, the protein content dropped, while in the others it showed an increase. Germination caused a rise in the values of the total and reducing sugars, as well as the total phenolic compounds, tannins, flavonoids and anthocyanins, showing variations during the intermediate times, based on the variety. Mostly, higher values were achieved at 96 h of the germination process.

**Key words:** *Cucumis melo* L., bioactive compounds, phenolic compounds, agricultural residues, sustainability, environment.

### Efeito da germinação nas propriedades nutricionais de sementes de quatro variedades de melão

**RESUMO:** Sementes de frutas são materiais tão nutritivos quanto grãos produzidos especificamente para o consumo, no entanto, comumente essas sementes são desprezadas pela agroindústria de processamento de polpas de frutas como resíduo sem valor. A inserção desse tipo de material como produto de consumo tão nobre quanto à própria polpa pode ser viabilizada pela utilização de processos que lhe agreguem valor, preferencialmente com os menores custos. O processo de germinação controlada pode ser utilizado com esse fim. Trata-se de uma estratégia de baixo custo, que permite modular os constituintes das sementes pela interrupção do processo no tempo adequado, permitindo a criação de um novo produto, com destaque para as propriedades bioativas. O trabalho foi realizado com o objetivo de se estudar o efeito do tempo de germinação sobre os constituintes das sementes de quatro variedades de melão. Foram utilizadas sementes de melões das variedades Cantaloupe, Gália, Pele-de-Sapo e Orange, determinando-se o teor de água, cinzas, proteínas, lipídios, açúcares totais e redutores, compostos fenólicos totais, taninos, flavonoides e antocianinas nas amostras *in natura* e após germinação em tempos de 24, 48, 72 e 96 h. Com exceção do teor de cinzas, todos os parâmetros foram alterados pela germinação. Na Pele-de-Sapo o teor de proteínas foi reduzido, nas demais, foi aumentado. Açúcares totais e redutores, compostos fenólicos totais, taninos, flavonoides e antocianinas tiveram teores elevados com a germinação, variando em tempos intermediários conforme a variedade, em sua maioria com valores mais altos atingidos em 96 h de processo germinativo.

**Palavras-chave:** *Cucumis melo* L., compostos bioativos, compostos fenólicos, resíduos agrícolas, sustentabilidade, meio ambiente.

### INTRODUCTION

Brazil, ranks globally as the third largest fruit producer, cultivating a total of 37 million tons, covering 19 species. China, which supplies 265 million tons, and India, raising 93 million tons (FAO, 2018) are the only two countries ahead of Brazil. Of all the fruit trees raised in Brazil, the melonis remarkable, having an annual turnover of 614,000 tons, cultivated in more than 24,000 hectares. The Northeast region is

the biggest contributor, particularly the states of Rio Grande do Norte, Ceará, Pernambuco (São Francisco Valley) and Piauí (IBGE, 2020).

Melon (*Cucumis melo* L.) is a tropical fruit, having great commercial worth, enjoyed and popular across the globe, for its highly appealing properties and sensory attraction. However, a large part of the fruit is composed of husk and seeds.

The utilization of such residues is a wise way of minimizing the wastage of nutritious

agricultural raw materials, by increasing their value, and creating new food products. This will raise the income of the producers, as well as reduce the negative effects on the environment by lowering the waste, effectively using the discarded material and making full use of the cultivation.

Germination, a biochemical process that causes complex cellular changes, triggers the metabolic activity of the seed embryo in a coordinated and sequential way. Germination involves the mechanisms of hormone regulation and protein hydration, besides the transportation of reserves, synthesis of macromolecules, heightened respiration and cell elongation (RAJJOU et al., 2012; BEWLEY et al., 2013). During the germination process, the reserve materials namely, carbohydrates, lipids and proteins require conversion into soluble compounds, which must later be transported to the growth regions to make the necessary nutrients available until the seedlings acquire autotrophic capacity (ATAÍDE et al., 2017). Germination involves wide-ranging phytochemical changes which result from the dynamic and complex flow of nutrients, in which remobilization, degradation and accumulation (NELSON et al., 2013) take place. Apart from these, germination contributes towards the decrease in enzyme inhibitors, which are one of the antinutritional factors (AGUILERA et al., 2013).

The germination process is seen to raise the nutritional value of the seeds by initiating biochemical alterations, based on the plant type, genetics and conditions under which it occurs (MARTINEZ et al., 2011). According to LEITE et al. (2016), in their assessment of the functional properties of the sorghum seeds (*Sorghum bicolor* L. Moench) *in natura* and germinated, the process of germination caused a boost in the nutritional value, particularly in the protein content, while it lowered the percentage of tannins. From the studies of CANTELLI et al. (2017), it is evident that the biochemical changes caused by the germination process in some soybean genetic lines induced a rise in the protein and isoflavone contents, and a drop in the contents of the trypsin inhibitor and phytic acid (phytates), recognized as the anti-nutritional factors.

In light of the observations cited above, the present study was performed to confirm the effect exerted by the different times of germination on the composition of the melon seeds belonging to the Cantaloupe, Gália, Pele-de-Sapo and Orange varieties.

## MATERIAL AND METHODS

The seeds of four varieties of melon were studied: Cantaloupe and Gália (*Cucumis melo* L. var.

reticulatus), Pele-de-Sapo and Orange (*Cucumis melo* L. var. inodorus).

Purchased in the fruit trade, fruits at the ripe maturation stage alone were selected. After sanitizing them with 1% sodium hypochlorite solution, they were cut lengthwise and the seeds were extracted by hand and placed a sieve. They were then washed under running water, rubbing lightly to remove the aril. Next, the surface water was evaporated at 26 °C ( $\pm 1$ ) ambient temperature and 80 % average relative humidity for 24 hours. Finally, they were transferred to airtight glass bottles and stored for later use.

The germination process of the seeds was performed in a BOD-type germination chamber, under controlled conditions, where the temperature was kept steady at 25 °C ( $\pm 1$ ) and relative humidity was at 99%. Adopting the methodology of BRASIL (2009), 100 seeds of each melon variety were taken and sown on Germitest paper sheets, moistened with distilled water at 2.5 times their mass and wrapped as rolls. These were labeled and put inside a germination chamber for a 96-hour time period. This time was selected using preliminary tests, when the primary root and the cotyledon were observed to be completely protruded. This stage was considered the one where the seeds were fully germinated. In this study, 20 rolls of each melon variety were used.

In the course of the germination process, at each 24-hour cycle (0, 24, 48, 72 and 96 h) seed samples were drawn to assess the degree of alteration in the composition. Analysis of the seeds was performed in triplicate, following the official analytical methods (AOAC, 2016): the water content was evaluated using the gravimetric method in an oven maintained at 105 °C for 24 h; the ash content was assessed with muffle incineration at 550 °C, with the results given as percentage (%) (d.b.); the protein concentration was quantified following the Kjeldahl method, and the total nitrogen content was ascertained, with the crude protein content calculated by multiplying the total nitrogen content by a factor of 6.25; the lipid value was determined by the BLIGH & DYER (1959) methodology, employing chloroform, methanol and water.

Using the methodology adopted by YEMM & WILLIS (1954), the total sugars were evaluated based on the Anthrone method. Here, 0.5 g of the sample was diluted in 50 mL of distilled water, and these extracts were diluted through filtration; the absorbance was noted using a spectrophotometer set at 620 nm. The reducing sugars were ascertained by the technique adopted by MILLER (1959), in line with the Dinitrosalicylic Acid Method (DNS), where

the extracts were first drawn from diluting 1 g of the sample in 25 mL of distilled water. These were then filtered and the absorbance reading was done using a spectrophotometer set at 540 nm.

Applying the methodology of WATERHOUSE (2006), the total phenolic compounds were evaluated using Folin-Ciocalteu reagent. First, the extracts were composed by diluting 1 g of the sample in 50 mL of distilled water; then following a 30-minute period of rest the extracts were filtered. Next, the absorbance reading was taken in a spectrophotometer at 765 nm. A standard solution of gallic acid was employed to acquire the analytical curve, and the concentrations of the total phenolic compounds in the samples were given in mg of gallic acid equivalent to per hundred grams of dry base (mg EAG/100 g d.b.).

To calculate the tannins, the Folin-Ciocalteu methodology (GOLDSTEIN & SWAIN, 1963) was adopted; the same extract from the analysis of total phenolic compounds was used. The analytical curve was obtained by using a standard solution of tannic acid. The tannin concentrations present in the samples were cited as mg of tannic acid equivalent per hundred grams of dry base (mg EAT/100 g d.b.).

Using spectrophotometry, the flavonoids and anthocyanins were calculated, based on the methodology followed by FRANCIS (1982). After the extracts were obtained using ethanol-HCl 85:15 (v/v) solution, by macerating 1 g of the sample in 10 mL of the solution, they were left at 5 °C/24h, ensuring they were kept away from light; next, the extract was filtered through cotton and the volume was completed to 10 mL. The absorbance reading was taken at 374 nm for the flavonoids and 535 for the anthocyanins.

All the analytical determinations were repeated in quadruplicate. The experimental data drawn from the study of the samples were submitted to analysis of variance (ANOVA), adopting a wholly randomized design. The means compared using Tukey 5% probability test ( $P \leq 0.05$ ), employing

the Assistet<sup>®</sup> software (Version 7.7) (SILVA & AZEVEDO, 2016).

## RESULTS AND DISCUSSION

A significant rise was observed in the level of the water content ( $P \leq 0.05$ ), over the germination period, in the seeds of all four melon varieties assessed (Table 1). The initial water content of the seeds, prior to the start of the germination process, which stagnated around 5% (d.b.), showed a substantial rise exceeding 70% at the end of 96 h; this was due the hydration of the seeds, crucial for the occurrence of the biochemical transformations, which lead to plant growth (WARLE et al., 2015).

A similar finding of the increased water content was revealed in linseed seeds by WANG et al. (2016). In their study, they reported that during germination the water content in the *in natura* seeds was 4.14 %, while in the sprouts it rose to a high of 94.66 %, post 8 days of germination. According to the study of SHREEJA et al. (2021), germination affected the nutritional composition of the buckwheat seeds, recording 15.78 % escalation in the water content after 48 h of germination.

In their studies, MARTINEZ et al. (2011), determined the chemical alterations noticed in soybean prior to and post the germination process. They too reported that the water content (in percentage) shot up from 6.71 to 63.72 % in the germinated seeds. In fact, BERNI et al. (2011), in their assessment of the influence exerted by germination on the centesimal make-up reported that the composition of the wheat grains *in natura* had 9.7 % water, which rose to 67.8 % at 104 h of germination. Greater values of the water content were reported in Pele-de-Sapo and Gália seeds after 96 h of germination.

From the data given in table 2; although, significant statistical variations are evident in the ash content of the samples, no specific variety revealed

Table 1 - Effect of germination time on water content of four melon seed varieties.

Varieties	-----Germination time/Water content (% d.b.)-----				
	<i>In natura</i>	24 h	48 h	72 h	96 h
Pele-de-Sapo	4.93±0.01 bE	42.99±0.04 bD	58.59±0.03 aC	73.86±0.04 aB	85.84±0.06 aA
Gália	5.30±0.02 abE	34.38±0.24 cD	46.08±0.29 cC	70.61±0.32 bB	85.52±0.08 aA
Cantaloupe	4.47±0.07 cE	33.85±0.12 cD	39.69±0.02 dC	65.36±0.68 cB	78.30±0.17 cA
Orange	5.49±0.03 aE	47.03±0.17 aD	52.77±0.09 bC	63.60±0.04 cB	83.57±0.18 bA

Mean ± standard deviation followed by the same lowercase letters in columns and uppercase letters in rows do not differ statistically, according to Tukey's test at 5% probability.

Table 2 - Effect of germination time on ash levels of four melon seed varieties.

Varieties	Germination time / Ashes (% d.b.)				
	<i>In natura</i>	24 h	48 h	72 h	96 h
Pele-de-Sapo	3.55±0.03 abA	3.06±0.05 bB	3.43±0.14 aA	3.50±0.07 aA	3.18±0.09 cB
Gália	3.16±0.10 cC	3.12±0.07 bC	3.51±0.02 aB	3.49±0.09 aB	3.72±0.07 aA
Cantaloupe	3.41±0.12 bB	3.48±0.07 aB	3.37±0.02 aB	3.33±0.06 bB	3.80±0.04 aA
Orange	3.68±0.01 aA	3.51±0.09 aB	3.46±0.06 aB	3.50±0.02 aB	3.56±0.02 bAB

Mean ± standard deviation followed by the same lowercase letters in columns and uppercase letters in rows do not differ statistically, according to Tukey's test at 5% probability.

superiority. Similarly, as the germination time progressed, no alterations could be attributed to it. According to CHAVAN et al. (1989), the reduced ash content might have been caused by the loss in dry matter, when soaking with water occurs during germination.

In the study done by THAKUR et al. (2021), regarding the influence exerted by germination on the nutritional and antinutritional properties, as well as on the bioactive compounds of amaranth, wheat and quinoa seeds, a non-significant drop ( $P \leq 0.05$ ) in the ash content was observed, post the germination process. From the studies done by SILVA et al. (2020), in terms of the ways germination time affected the physical characteristics of soybean, a non-significant decline in the ash content of the seeds (6.59%) was reported, when compared to that of the sprouts (5.45%).

When CANTELLI et al. (2017), assessed the physicochemical properties of certain genetic lines in the germinated seeds of soybean, they did not observe any significant differences ( $P \leq 0.05$ ) among the ash contents. However, TATSADJIEU et al. (2004), reported that the reduction in the ash content was indicative of mineral loss induced by the water absorbed by the seed and the radicle being developed during the germination process. It appears

that the highest ash levels (mineral content) of the seeds germinated after 96 h were from the Gália and Cantaloupe varieties.

From table 3, the protein content appears to show significant fluctuations ( $P \leq 0.05$ ) in all the four seed varieties during the germination period, with reductions at the times of 24 and 48 h; this is followed by the rising values from this time onwards, until the higher values are achieved at the final time of 96 h. This emphasizes that at this germination time the Orange variety seeds, which possess higher protein content, concur with the seeds *in natura*. These oscillations showed increases of 5.74, 4.88 and 6.11% in the protein content of the seeds of the melon varieties of Gália, Cantaloupe and Orange, respectively, in comparison with the ones *in natura*.

Many researchers also noted alterations in the percentage of the proteins present in the seeds and grains during germination. In fact, UKPONG et al. (2021), in a comparative study of rice grains from the Farro 57 cultivar, in terms of the nutritional composition, recorded higher values for the protein content in germinated rice (14.54-15.01%), followed by brown rice (10.99 %), but lower values for parboiled rice (10.16 %); however, THAKUR et al. (2021), assessed the influence exerted by germination

Table 3 - Effect of germination time on protein levels of four melon seed varieties.

Varieties	Germination time / Proteins (g/100 g d.b.)				
	<i>In natura</i>	24 h	48 h	72 h	96 h
Pele-de-Sapo	37.16±0.03 bB	33.81±0.20 aD	30.63±0.06 aE	35.58±0.03 aC	38.60±0.14 dA
Gália	34.48±0.06 cB	33.60±0.03 bC	27.62±0.03 bD	33.53±0.03 bC	40.22±0.06 bA
Cantaloupe	34.35±0.18 cB	31.96±0.03 dC	23.45±0.02 dD	31.58±0.02 cC	39.23±0.06 cA
Orange	38.72±0.06 aB	32.90±0.02 cC	26.55±0.02 cD	33.05±0.03 bC	42.83±0.09 aA

Mean ± standard deviation followed by the same lowercase letters in columns and uppercase letters in rows do not differ statistically, according to Tukey's test at 5% probability.

on the nutritional characteristics of the amaranth seeds and reported 7.01 % increase in the protein content post germination for 72 h; but BENIWAL et al. (2019), recorded 4.81% rise in the protein content in the amaranth seed after 48 hours of germination.

It was CANTELLI et al. (2017), who investigated the ways that germination affects the seeds of the BRS 216 cultivar and three soybean lines (BRM09-10505, BRM10-60599 and PF133002). They reported increases of 12.03, 3.89, 7.77 and 8.84%, respectively, post day four of germination. The rise in the protein content is possibly partly a result of new amino acids being synthesized biologically, as well as to a reduction in the dry matter, carbohydrates in particular, when the processes of respiration during germination, seed transformation and protein synthesis are taking place (PARNSAKHORN et al., 2018; JAN et al., 2017; ZHANG et al., 2015).

As germination progressed, the lipid content increased significantly in the seeds of the Pele-de-Sapo, Cantaloupe and Orange varieties ( $P \leq 0.05$ ) when compared with the seeds *in natura* (Table 4). For the samples of the Pele-de-Sapo and Gália, the highest values were observed at 48 h; however, in Cantaloupe these values were achieved at 24 and 96 hours, while in the Orange they were reported at 24, 48 and 96 h. It was ATAÍDE et al. (2017), who reported that the lipid content escalation at 96 h of germination was probably due to their synthesis to supply energy to the root starting to develop because, at this point in time, the primary root protrusion had already taken place. The seeds of the Cantaloupe variety were confirmed to have the highest lipid content both in the *in natura* seeds and in those which germinated after 96 hours.

In all the varieties, 72 h germination time was the one at which the lowest values were observed among the samples of the germinated seeds. In fact, THAKUR et al. (2021), reported similar reductions in the germination of quinoa and buckwheat, where

the authors noted a decline from 6.39 to 5.15 % and from 1.40 to 1.15 % in the lipid content, respectively, after 72 hours of germination; however, SHREEJA et al. (2021), in buckwheat, indicated a reduction from 3.13 g/100g to 2.05 g/100g for the lipid content, post the germination time of 48 hours.

A significant decrease was observed in the lipid content of the two *Chenopodium* cultivars (*Chenopodium album*) after 48 h of germination by JAN et al. (2017). However, SKUPIEŃ et al. (2017), attributed the lowered lipid content in the initial hours of the germination process to the process of triacylglycerol degradation during seed imbibition, which supplied the carbon and energy for growth, post-germination.

Right through the germination period the total sugars and reducing sugars revealed a remarkable rise among the four seed varieties assessed (Tables 5 and 6), with the Pele-de-Sapo variety, recording the highest values after 96 h. When compared to the seeds *in natura*, both sugars displayed an initial rise in the samples that were germinated for 24 h; however, a reduction was noted at the end of 48 h when compared to the earlier time, followed by an increase once again in the remaining times, achieving the maximum value at 96 h for all the four varieties.

In their study, ZHU et al. (2017), reported that the elevated sugar content in the germinated seeds may be linked to the enzymatic activities that induce complex carbohydrates to be hydrolyzed to simple sugars. Studies by WARLE et al. (2015), in their evaluation of the nutritional quality of germinated soybeans, reported a significant spike in the total sugar content from 3.55 to 5.60 % just after the germination process. The authors attributed this increase to the  $\alpha$ -amylase and  $\beta$ -amylase enzyme activity during germination.

The escalation in the sugar content, while germination progresses, particularly the rise in cellulosic glucose, results from a metabolic reaction

Table 4 - Effect of germination time on lipid levels of four melon seed varieties.

Varieties	-----Germination time / Lipids (g/100 g d.b.)-----				
	<i>In natura</i>	24 h	48 h	72 h	96 h
Pele-de-Sapo	33.43±0.70 cD	40.47±0.17 bC	54.15±1.32 aA	32.99±0.04 bD	49.26±0.26 bB
Gália	34.65±0.45 bC	38.39±0.21 cB	44.83±0.19 cA	31.85±0.27 cD	39.76±0.24 cB
Cantaloupe	37.68±0.43 aD	50.98±0.86 aA	47.19±0.17 bB	39.21±0.15 aC	51.22±0.25 aA
Orange	33.69±0.26 bcB	37.21±0.31 dA	36.63±0.77 dA	28.75±0.12 dC	37.45±0.28 dA

Mean ± standard deviation followed by the same lowercase letters in columns and uppercase letters in rows do not differ statistically, according to Tukey's test at 5% probability.

Table 5 - Effect of germination time on total sugar levels of four melon seed varieties.

Varieties	Germination time / Total sugars (g/100 g d.b.)				
	<i>In natura</i>	24 h	48 h	72 h	96 h
Pele-de-Sapo	1.39±0.01 dE	3.14±0.00 aC	2.86±0.01 aD	4.52±0.01 aB	7.73±0.01 aA
Gália	1.76±0.00 cD	2.78±0.01 cB	2.10±0.01 bC	2.79±0.02 bB	6.62±0.02 bA
Cantaloupe	1.80±0.00 bD	2.30±0.00 dB	1.95±0.01 cC	2.20±0.00 cB	4.35±0.01 dA
Orange	1.88±0.02 aD	2.94±0.01 bB	1.93±0.01 dD	2.09±0.00 dC	5.52±0.04 cA

Mean ± standard deviation followed by the same lowercase letters in columns and uppercase letters in rows do not differ statistically, according to Tukey's test at 5% probability.

(LI et al., 2014). In fact, UKPONG et al. (2021), determined the influence exerted by germination time on the nutritional composition of the Farro 57 cultivar rice grain, as well as noted the oscillation in the content of the reducing sugars (5.14 % for 12 h; 11.23% for 24 h; and 8.88% for 36 h of germination). It was AMADEU et al. (2021), who determined the physicochemical properties of pumpkin seeds, prior to and post germination, and recorded a steady rise in the levels of the reducing sugars in the seeds, post 48 h of germination.

At 96 hours, the total phenolic compounds showed increased levels exceeding 100 % when the fresh and germinated seeds were compared (Table 7). During the intermediate germination times (24, 48 and 72 h), oscillations of statistical significance were confirmed; however, no clear behavior of these oscillations could be observed among the varieties evaluated. The total phenolic compounds showed their maximum levels in the germinated Gália melon seeds at 96 h ( $1399.80 \pm 57.22$  mg EAG/100g d.b.), indicating a rise of nearly 2.3 times when compared with their initial concentration, in the seeds *in natura*.

From the paper of JAN et al. (2017), it is evident that during the germination process, the seed

cell wall degradation enzymes are activated, which facilitates the release of the phenolic compounds which are bound to it, freeing them, and thus inducing the rise in the total phenolic content, as the germination time increases.

In their study, on the effect of germination on broad beans (*Vicia faba* L.), lupine seeds (*Lupinus albus*), chickpea seeds (*Cicer arietinum* L.), lentil seeds (*Lens culinaris*), fenugreek seeds (*Trigonella foenum-graecum* L.) and the common bean (*Phaseolus vulgaris*) SALEH et al. (2017), as well as in the research of KIM et al. (2016), who assessed the ways germination, at 25 °C for 6 days, affected the functional compounds of soybean (*Glycine max* L.), reported that such a rise was induced by the synthesis of the phenolic compounds during the process of germination.

An approximately 82.36 % increase was evident in the total phenolic content of the flaxseed sprouts on day ten of germination, in comparison to the flaxseed prior to the germination. Sprouting enhances the accumulation of the total phenolic compounds, which may be understood as a good source of phenolics in the foods linked to health benefits (WANG et al., 2016). Other studies like those of PAUCAR-MENACHO et al. (2018) and CARCIOCHI et al. (2014), also reported an almost

Table 6 - Effect of germination time on reducing sugar levels of four melon seed varieties.

Varieties	Germination time / Reducing sugars (g/100 g d.b.)				
	<i>In natura</i>	24 h	48 h	72 h	96 h
Pele-de-Sapo	0.88±0.02 bE	2.27±0.02 aC	2.12±0.02 aD	3.96±0.02 aB	6.51±0.02 aA
Gália	1.02±0.03 aC	0.67±0.00 cD	0.56±0.01 cE	1.89±0.01 bB	5.53±0.02 bA
Cantaloupe	0.31±0.01 dD	0.57±0.02 dC	0.54±0.00 cC	0.66±0.00 dB	2.87±0.01 dA
Orange	0.39±0.01 cE	1.03±0.01 bB	0.66±0.00 bD	0.80±0.00 cC	4.19±0.03 cA

Mean ± standard deviation followed by the same lowercase letters in columns and uppercase letters in rows do not differ statistically, according to Tukey's test at 5% probability.

Table 7 - Effect of germination time on the levels of total phenolic compounds of four melon seed varieties.

Varieties	-----Germination time / Total phenolics (mg EAG/ 100 g d.b.)-----				
	<i>In natura</i>	24 h	48 h	72 h	96 h
Pele-de-Sapo	594.25±10.86 aC	563.56±6.17aC	1014.72±21.39 aB	995.41±26.46 aB	1218.74±49.93 bA
Gália	604.65±15.60 aC	489.14±19.33 bD	486.33±6.74dD	796.89±16.61 bB	1399.80±57.22 aA
Cantaloupe	493.90±11.35 bD	435.02±8.36 cE	641.72±7.78 cB	560.79±7.21dC	1223.81±34.80 bA
Orange	524.53±12.50 bD	513.92±34.35 bD	911.43±9.44 bB	746.42±13.97 cC	1156.32±53.14 cA

Mean ± standard deviation followed by the same lowercase letters in columns and uppercase letters in rows do not differ statistically, according to Tukey's test at 5% probability.

doubling of the phenolic compounds in the quinoa sprouts from 42 h of germination, when compared with the quinoa seeds.

The germinated melon seeds revealed a remarkable rise in the tannin content when compared to the *in natura* samples (Table 8), although variations were observed at different times, based on the variety. At 72 hours, a decrease was observed in the total phenolics, showing lower values than in the *in natura* samples, for all four varieties. At 96 hours, the levels once again were elevated, significantly exceeding those achieved at 48 hours for all the varieties; however, the highest levels were observed in the Cantaloupe, Pele-de-Sapo and Orange varieties.

Findings that were quite different were reported by PADMASHREE et al. (2019) and THAKUR et al. (2021), in which the first assessed the ways germination affected the anti-nutritional characteristics of two quinoa varieties and recorded a significant decrease in the tannin content of 8.98 % in the white quinoa and 12.86 % in red quinoa, post 48 h of germination; the second study confirmed the influence exerted by germination on the antinutritional characteristics of quinoa for the 72 h period and reported a 27.08 % drop in the tannin content of the sprouts in comparison to that of the seeds.

From the research of MODGIL & SOOD (2017), it is clear that during germination the seed coat with its tannin constituents breaks, causing the tannin losses. Also, new complex components are produced. Some amount of the losses occur because of the tannins leaching into the water during the soaking process.

In table 9, the flavonoid contents of the melon seeds *in natura* are listed, with the germination times up to 96 h. The germination process resulted in significant rises of the flavonoid content in all the four varieties, and a progressive increase was evident between each time interval in the Pele-de-Sapo, Gália and Orange varieties. The Cantaloupe variety; however, showed an increase at 24 h, after which very slight variations were seen in the times that followed. But there was another increase again, by about 40 mg/100 g, between 72 and 96 h, but the levels; however, were lower by more than 25% in comparison to the other varieties. After 96 h of germination, the highest flavonoids levels were evident in the Gália and Pele-de-Sapo varieties.

During the germination process the increase in the flavonoid levels could have been caused by the enzymes involved in the flavonoid biosynthesis, or from the polyphenols such as tannins, having high

Table 8 - Effect of germination time on total tannins levels of four melon seed varieties.

Varieties	-----Germination time / Tannins (mg EAT/ 100 g d.b.)-----				
	<i>In natura</i>	24 h	48 h	72 h	96 h
Pele-de-Sapo	510.32±8.00 cD	568.16±14.19 dC	775.57±9.15 cB	457.36±8.02 bE	956.26±37.94 aA
Gália	578.19±10.04 aD	671.79±9.40 cC	769.73±23.04 cB	445.60±16.38 cE	834.97±32.47 bA
Cantaloupe	530.02±4.23 bD	744.31±10.72 aC	841.77±11.16 bB	485.26±10.23 aE	961.97±42.53 aA
Orange	590.42±10.05 aD	725.04±9.04 bC	892.89±17.99 aB	479.32±4.80 aE	956.24±32.36 aA

Mean ± standard deviation followed by the same lowercase letters in columns and uppercase letters in rows do not differ statistically, according to Tukey's test at 5% probability.

Table 9 - Effect of germination time on flavonoid levels of four melon seed varieties.

Varieties	-----Germination time / Flavonoids (mg/100 g d.b.)-----				
	<i>In natura</i>	24 h	48 h	72 h	96 h
Pele-de-Sapo	9.76±0.19 bE	20.18±0.03 bD	26.26±0.06 bC	40.75±0.10 aB	83.22±0.63 aA
Gália	12.78±0.52 aE	18.08±0.88 cD	21.70±0.02 cC	33.08±0.11 bB	83.31±1.47 aA
Cantaloupe	12.57±0.44 aD	17.25±0.07 cC	18.95±0.03 dB	17.79±0.04 dC	57.00±0.48 cA
Orange	12.73±0.39 aE	21.65±0.03 aD	28.14±0.06 aC	31.21±0.03 cB	77.20±1.72 bA

Mean ± standard deviation followed by the same lowercase letters in columns and uppercase letters in rows do not differ statistically, according to Tukey's test at 5% probability.

molecular weight (DREWNOWSKI & GOMEZ-CARNEROS, 2000). After 96 h of germination, the highest flavonoid levels were observed in the Gália and Pele-de-Sapo varieties.

From the study of WANG et al. (2016), performed on germinated and non-germinated flaxseeds, the total flavonoid content was observed to rise significantly over the germination times, peaking on day ten ( $1133.27 \pm 37.86$  mg/100 g). The increase was about 55 times when compared to  $20.61 \pm 4.55$  mg/100g, the initial concentration in the non-germinated seeds. It was KUMARI et al. (2015), who investigated the ways the duration of germination impacted the nutritional constituents of two soybean varieties (*Glycinemax* L.); they reported a strong spike in the total flavonoid content post 72 h of germination, with 21 % for the black soybean and 11 % for the yellow soybean, in comparison to the samples of the control.

In table 10, the anthocyanin values are listed for both the *in natura* and germinated seed samples, for up to 96 h and of 48 h of germination; statistical superiority is observed at 96 h, in all the four varieties, concurring with the behavior of the flavonoids. The Orange variety showed the highest anthocyanin content in the germinated seeds (at 96 h).

The remarkable rise throughout the germination period of the melon seeds indicated the positive effect this process exerts on the synthesis of bioactive compounds, producing samples abundant in the total phenolic compounds, flavonoids and anthocyanins.

## CONCLUSION

Barring the ash content, the germination process was observed to alter all the constituents assessed in this study, in all four varieties of the melon seeds. The germination process caused the values of the constituents to vary during the times of the intermediate assessments, but showed either stabilization or decline between 24 and 72 hours of the process, and an increase again in most of the determinations and samples by the culmination of 96 hours.

The germination process effectively altered the melon seeds in terms of the nutritional composition, enabling the control of the nutrient concentration, as well as the synthesis of bioactive compounds, in line with the germination time and fruit variety.

Table 10 - Effect of germination time on anthocyanin levels of four melon seed varieties.

Varieties	-----Germination time / Anthocyanins (mg/100 g d.b.)-----				
	<i>In natura</i>	24 h	48 h	72 h	96 h
Pele-de-Sapo	8.80±0.30 cD	12.92±0.09 bC	14.25±0.12 cC	24.93±0.08 bB	47.52±0.55 cA
Gália	10.01±0.31 aD	14.69±0.70 aC	15.62±0.06 bC	28.03±0.07 aB	52.62±0.56 bA
Cantaloupe	9.94±0.36 aD	12.11±0.05 bC	13.89±0.04 cC	16.58±0.02 cB	41.87±0.18 dA
Orange	9.31±0.38 bE	12.80±0.07 bD	17.47±0.09 aC	24.69±0.01 bB	61.67±0.01 aA

Mean ± standard deviation followed by the same lowercase letters in columns and uppercase letters in rows do not differ statistically, according to Tukey's test at 5% probability.

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## DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

## AUTHOR'S CONTRIBUTIONS

All the authors have made equal contributions to the manuscript with respect to design and writing. All the authors have critically reviewed the manuscript and approved the final version.

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