



Original article

Isoflavones from black chickpea (*Cicer arietinum* L) sprouts with antioxidant and antiproliferative activityDomínguez-Arispuro Dulce-María^a, Canizalez-Román Adrián^{a,b}, Reyes-Moreno Cuauhtémoc^a, Milán-Noris Ada-Keila^a, Milán-Carrillo Jorge^a, Acosta-Smith Erika^b, Cuevas-Rodríguez Edith-Oliva^{a,*}^a Programa Regional de Posgrado en Biotecnología, Programa de Posgrado en Ciencia y Tecnología de Alimentos, Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa, Mexico^b CIASaP, Programa de Posgrado en Ciencias en Biomedicina Molecular, Facultad de Medicina, Universidad Autónoma de Sinaloa, 80246 Culiacán Sinaloa, Mexico

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ABSTRACT

Black chickpea is a good source of bioactive compounds, particularly isoflavones. Sprouting improves nutraceutical value in chickpea seeds. This study aimed to explore the role of sprouting of black chickpea seeds on the synthesis of isoflavones and evaluate the impact of the soluble isoflavone on cellular antioxidant activity (CAA) and antiproliferative activity in breast cancer cells. Isoflavones were identified and quantified by HPLC-UV-MS. The CAA and antiproliferative activity were determined in HepG2 cells and MDA-MB-231 cancer cells, correspondingly. In sprouted black chickpea, six isoflavones (formononetin, biochanin-A, and its glycosides) were identified and the total isoflavones content increased (0.31 to 35.72 $\mu\text{gBA}/\text{mg}$ of extract). The CAA was increased five times from 137.2 to 788.2 $\mu\text{MEQ}/100$ g of sample. The bioactive compounds in sprouted chickpea decreased the proliferation of MDA-MB-231 cell line. Also caused morphological changes such as cell shrinkage, rounding and nuclear fragmentation. The results herein suggest that bioactive compounds, as isoflavones, in sprouted black chickpea showed a potential antioxidant and antiproliferative activity. Therefore, it may be considered as a value-added product or ingredient for produce functional foods.

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1. Introduction

Desi chickpea is a small grain with pigmented seed coat (red, green, brown, and black) and scarcely use for human consumption (Heiras-Palazuelos et al., 2013; Domínguez-Arispuro et al., 2018; Milán-Noris et al., 2018). Chickpea is a good source of bioactive compounds, as isoflavones. The main isoflavones present in chickpea are biochanin-A and formononetin. Also, genistein and daidzein are reported in small amounts (Wu et al., 2012). Isoflavones are considered as estrogenic polyphenols (structurally similar to estrogens) with potent anticarcinogenic properties, they mimic the main mammalian estrogen (estradiol) that allows them to bind

to the estrogen receptor (Velentzis et al., 2008; He and Chen, 2013). However, previous reports have shown that, isoflavones presented cancer prevention due to non-hormonal mechanisms (Sánchez-Chino et al., 2015), these compounds suppress angiogenesis, induces apoptosis, inhibits DNA topoisomerases and cancer cell differentiation (Garrido et al., 2003; Messina, 1999; Nagaraju et al., 2013).

Moreover, the profile and content of isoflavones in plants are influenced by several factors as genotype, agriculture practices, weather and processing conditions (Gao et al., 2015; Pérez-Martín et al., 2017; Milán-Noris et al., 2018). Sprouting of seeds is considered as an affordable and natural bioprocess that improve the nutritional and nutraceutical characteristic of chickpea seeds (Sharma et al., 2018). In previous studies, it has been reported that sprouting increases significantly the content of isoflavones in desi chickpea (Milán-Noris et al., 2018). Moreover, black chickpea sprouting was optimized to enhance antioxidant capacity (Domínguez-Arispuro et al., 2018). This study aimed to explore the effect of sprouting of black chickpea seed on the synthesis of isoflavones, cellular antioxidant and antiproliferative activity in breast cancer cells which lack hormone receptor (MDA-MB-231).

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2. Materials and methods

2.1. Plant materials

Black chickpea seeds were grown (December-2017 to April-2018) at Culiacan Experimental Station, National Research Institute for Forestry, Agriculture and Livestock (INIFAP), Sinaloa, México. Black chickpea seeds were collected, cleaned, and preserved at 4 °C until analysis.

2.2. Production of sprouted chickpea flour

Sprouted chickpea flours were processed in compliance with the methodology developed by Domínguez-Arispuro et al. (2018). Black chickpea seeds were washed with purified water during 30 s. After that, the seeds were washed-out and rinsed with distilled water. Then, the soaked seeds were placed in sprouted containers. Containers were introduced in a sprouting chamber (33 °C/171 h), controlling the relative humidity at 80–90%. The sprouts obtained were immediately stored at –80 °C, freeze-dried, and milled to obtain sprouted chickpea flours (SCF). On the other hand, whole raw black chickpea seeds were ground to produce unsprouted flour (UCF). It was used as a control. Both flours were preserved at 4 °C in tightly sealed containers.

2.3. Extraction of soluble phenolic compounds

The soluble phenolics from the unsprouted and sprouted chickpea flours were extracted as previously reported (Heiras-Palazuelos et al., 2013). Briefly, one gram of sample (UCF or SCF) were mixed with 10 mL of ethanol: water (80:20, v/v) and stirred in a rotator tube at 50 rpm by 10 min. Then, the samples were centrifugated (3000g/25 °C) by 10 min. The supernatant was evaporated under reduced pressure to dryness in a rotavapor and stored at –20 until analysis. For biological test, the extracts were reconstituted in serum free culture medium.

2.4. Identification and quantification of isoflavones

Soluble phytochemical extracts were reconstituted in 80% aqueous methanol and filtered with a 0.2 µm membrane filter. The identification of isoflavones in chickpea extract was performed in an HPLC coupled to an ion trap (IT) mass detector (1100 series, Agilent Technologies, Santa Clara, CA). Ionization was accomplished using an electrospray source (ESI) at 300 °C, capillary temperature at 4 kV, voltage nebulizer pressure at 50 psi and nitrogen gas flow rate at 10 mL/min. The mass scan range was from 150 to 2000 (*m/z*) and the data was captured in positive mode. The separation was adapted for chickpea samples and achieved using an Eclipse XDB C18 column with 3 mm × 150 mm and 3.5 µm of dimension (Agilent Technologies, Santa Clara, CA) with a flow rate of 0.4 mL/min and a column temperature of 30 °C. The solvents used as mobile phase, were (A) water with formic acid (0.1%) and (B) acetonitrile. The elution was: the first 8 min increased from 0 to 10% B, 8 to 16 min increased to 35% B, 16 to 26 min increased to 90% B and 26 to 36 min increased to 100% B.

The quantification of isoflavones were performed in a HPLC-UV. The separation was as described for HPLC-IT-MS. The injection volume was 2 µL of chickpea extracts and detection was recorded at 260 nm. The compounds were confirmed by UV spectra and retention time (Milán-Noris et al., 2018). The isoflavones in black chickpea were reported as Biochanin A equivalents (µg BA eq/mg of extract)

2.5. Cell studies

2.5.1. Cell culture

Liver cancer cells (Hep-G2) were used for the evaluation of the cellular antioxidant activity. Hep-G2 was grown WME medium, supplemented with FBS (5%), HEPES (10 mM), L-glutamine (2 mM), insulin (5 µg/mL), hydrocortisone (0.05 µg/mL), penicillin (50 units/mL), streptomycin (50 µg/mL), and gentamycin (100 µg/mL) (Wolfe et al., 2008). MDA-MB-231, triple-negative breast cancer (TNBC) cells, characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor (HER)-2 expression. Murine fibroblast cells (NIH-3T3) were grown in DMEM (Dulbecco's Modified Eagles) medium, supplemented with 10% FBS (heat-inactivated) (Life, USA) and 1% antibiotic solution (100 IU/mL penicillin: 100 µg/mL streptomycin). Cell culture was preserved (37 °C, 5% CO₂) in a humidified atmosphere.

2.5.2. Cytotoxicity assay

The cytotoxicity of chickpea extracts on HepG2 cells was performed using the methylene blue test (Felice et al., 2009). HepG2 cells were placed (4×10^4 cells per well) in 96-well plate with 100 µL of growth culture medium and incubated for 24 h under controlled conditions (37 °C; 5% CO₂). 100 µL of soluble extracts were added to each well at different concentrations. The microplate was placed on a tabletop shaker for 20 min to facilitate uniform elution. Cytotoxicity was calculated by a 10% reduction of absorbance (570 nm) reading for each concentration compared to untreated cells.

2.5.3. Cellular antioxidant activity (CAA)

HepG2 cells were placed (6×10^4 /well) on a 96 wells microplate. After 24 h of incubation (37 °C, 5% CO₂), cells were tested with 100 µL of soluble extracts from UCF, SCF or quercetin, (at different treatments) and 25 µM of DCFH-DA by 60 min. The effectiveness of phytochemicals, on cell absorption or cell membrane binding, was determined applying two protocols (with PBS wash and without PBS wash). Then 100 µL of HBSS (600 µM ABAP) was added to the cells, and the plate was immediately placed in the preheated microplate reader (Victor™ X3 Multi-Label, Perkin Elmer, Inc., MA, USA) and maintained at 37 °C. The emission (535 nm) and excitation (485 nm) reading were recorded each 5 min for 60 min. The CAA of each sample was calculated as follows: dividing the EC₅₀ of quercetin standard (positive control) by the EC₅₀ of each sample. The data was reported as µmol of quercetin equivalents/100 g sample (Wolfe et al., 2008).

2.5.4. Morphological changes on breast cancer cell

The evaluation of the effect of chickpea extracts in the cell morphology (MDA-MB-231) was tested in 8 wells plates (1×10^4 cells per well). The samples were tested at 10 mg/mL concentration for 24 h with a double fluorescent staining (Dapi/Phalloidin). After that cells were fixed with formaldehyde (4%) for 30 min. Then, the cells were permeabilized with a triton (0.2%). The cells morphological changes were observed in a fluorescent microscope at excitation/emission of 353/460 nm (Dapi) and 535/585 nm (phalloidin). The cells exposed to 40 µM/mL cisplatin (antineoplastic drug) were used as a positive control and untreated cells as a negative control.

2.5.5. Antiproliferative activity

Antiproliferative activity in MDA-MB-231 and NIH-3T3 was determined by the MTT assay (Xu and Chang, 2009) with some modifications. Cancer cells were grown with DMEM (37 °C, 5% CO₂) for 24 h. The 96-wells plates were prepared with 100 µL of a suspension (MDA-MB-231 or NIH-3T3) containing 1×10^4

cells/mL and permitted to grow for 24 h. The chickpea extracts were suspended in DMEM medium. The samples were tested at different concentrations (0, 0.3, 0.5, 1, 5, 10, 15 mg/mL) for 12 h. Following, 20 μ L of MTT stock solution was added to each well and incubated at 37 °C for 4 h. The supernatant was removed, and after that, 100 μ L of dimethyl sulfoxide was added in each well to solubilize purple formazan crystals. The plates were read at 570 nm in a scanning multi-well spectrophotometer (Synergy HT, Bio-Tek, Winooski, VM). The cell viability and IC₅₀ in MDA-MB-231 and NIH-3T3 cells were calculated using controls (untreated cells) as 100% viable. Positive (40 μ M cisplatin) and negative (untreated cells) controls were used for comparison purposes.

2.6. Statistical analysis

All statistical analyses were executed in the Minitab statistical software version 16. The measurements, in this study, were carried out in triplicate. Data were depicted as mean \pm standard deviation (SD) and analyzed by analysis of variance followed by Duncan tests to identify differences among treatments. A *p* value \leq 0.05 was considered significance. IC₅₀ and EC₅₀ were calculated with Graph-Pad Prism Software version 5.0.

3. Results

3.1. Identification and quantification of isoflavones

The isoflavone content in black chickpea samples (unsprouted and sprouted) are depicted in Table 1. In Fig. 1 are showed the representative chromatograph of isoflavone profile of black chickpea sprouts. Six principal isoflavones were identified: Isoformononetin glycoside ([M+H]⁺ = 431; 259 nm), formononetin malonyl glycoside ([M+H]⁺ = 531; 249 nm), biochanin-A glycoside ([M+H]⁺ = 447; 261 nm), biochanin-A malonyl glycoside ([M+H]⁺ = 533; 260 nm), formononetin ([M+H]⁺ = 269; 249 nm) and biochanin-A ([M+H]⁺ = 285; 261 nm). In UCF samples, biochanin-A and its derivatives were observed, and biochanin-A was detected as the major isoflavone with 0.14 μ gBAEq/mg of extract. Interestingly, in SCF sample, the profile and content of isoflavones increased during the sprouting process. Formononetin and its derivatives were found only in SCF sample. The total isoflavones content (TIC) in the sprouted black chickpea (171 h/ 33 °C) increased from 0.31 to 35.72 μ g BAEq/mg of extract, close to 115-fold. The biochanin-A malonyl glycoside was the main isoflavone (7.91 μ gBAEq/mg) in SCF sample, representing 22% of TIC.

3.2. Cellular antioxidant activities of black chickpea extracts

Fig. S1 shows the cytotoxic effect of UCF and SCF on HepG2 cell line. The cellular antioxidant activity and EC₅₀ values for black chickpea extracts with and without PBS wash protocols are shown

Table 1
Isoflavones content in unsprouted (UCF) and sprouted (SCF) black chickpea extracts.

Compound	UCF (μ g BA eq/mg e)	SCF (μ g BA eq/mg e)
Isoformononetin glycoside	ND	6.18 \pm 0.05
Formononetin malonyl glycoside	ND	6.82 \pm 0.44
Biochanin-A glycoside	0.06 \pm 0.00 ^b	6.35 \pm 0.14 ^a
Biochanin-A malonyl glycoside	0.10 \pm 0.01 ^b	7.91 \pm 0.09 ^a
Formononetin	ND	4.07 \pm 0.39
Biochanin-A	0.14 \pm 0.00 ^b	4.36 \pm 0.49 ^a
Total	0.31 \pm 0.00^b	35.72 \pm 0.72^a

Mean \pm standard deviation; ND: not detected; μ g BA eq/mg e: micrograms of Biochanin-A equivalents/milligram of extract.

in Table 2. In the protocol with PBS wash, the CCA were of 41.2 μ mol QE/100 g sample (UCF) and 222.2 μ mol QE/100 g sample (SCF). The sprouting process of black chickpea seeds improves CAA values significantly, increasing nearly five times compared with its unsprouted counterpart. Besides, as expected, EC₅₀ values were reduced from 28 (UCF) to 5.1 (SCF) mg/mL by sprouting. Regarding the protocol without washing (sterile PBS), similar tendency was obtained as in the protocol with washing. That is, the sprouting increased the antioxidant activity in SCF close to 470% approximately, the values improved from 137.2 to 788.2 μ M EQ/100 g of sample, and the EC₅₀ values decreased after the bioprocess (4.91 to 0.88 mg/mL).

3.3. Morphological changes induced on MDA-MB-231 cells by UCF and SCF extracts

The cytotoxic damage of the UCF or SCF extracts was confirmed and compared with positive (cisplatin 40 μ M) and negative controls (untreated) (Fig. 2) using double staining (Dapi and Phalloidin) protocol. The bioactive compounds of both extracts caused contraction of the plasma membrane and damage in the nuclear membrane. Although, the cell line decreased the size and suffered a rounding concerning to the original structure. The bioactive compounds of the SCF extracts significantly affected membranes, clearly observing a fragmented nucleus and undefined membrane. These results suggest that both raw and sprouted of black chickpea treatments were cytotoxic for cells but did not determine whether it promote programmed cell death or apoptosis.

3.4. Effect of chickpea extracts on the proliferation of MDA-MB-231

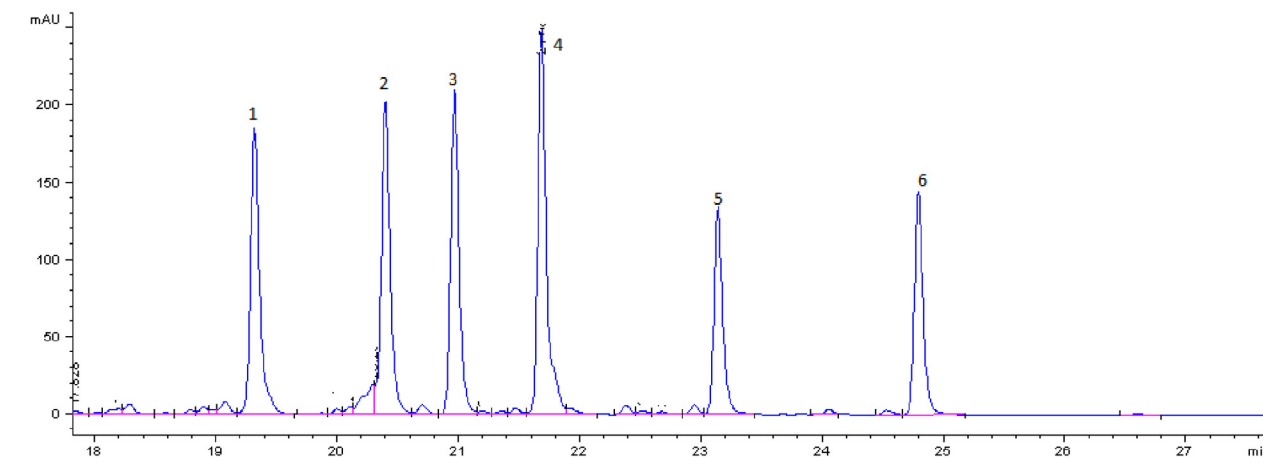
The effect of black chickpea extracts in the viability of breast cancer cell are shown in Table 3. The UCF extract had low effect in cancer cells viability at 0.3 to 0.5 mg/mL concentrations. However, at higher doses (1 to 15 mg/mL) stronger reductions in cell viability were observed. The SCF extract reduced cell proliferation in TNBC cells in dose-dependent effect. The IC₅₀ of SCF was 11 mg/mL.

The effect of the chickpea extracts was analyzed in non-transformed cells (Table 4)NIH-3T3 cell line (murine fibroblast cell) to study the specificity of the extract towards the proliferation of cancer cells. The results indicate that the NIH-3T3 cell line was affected by exposure to both soluble chickpea extracts, with statistically significant differences (*p* < 0.05) between unsprouted and sprouted extracts. The IC₅₀ value for SCF extract was 14 mg/mL. The higher dose tested of UCF extract (15 mg/mL) in both cell lines were not enough to determinate IC₅₀.

4. Discussion

In this work, the sprouting effect of black chickpea in the synthesis of isoflavones was assayed. Furthermore, the cellular antioxidant (HepG2) and antiproliferative activity (MDA-MB-231) of its bioactive compounds were explored.

The six isoflavones were identify in sprouted black chickpea: formononetin, biochanin-A and its derivatives. Those compounds showed the *m/z* and UV max according to previously reports (Guardado-Félix et al., 2017; Milán-Noris et al., 2018). During the sprouting process, the plant reactivates the secondary metabolism, promoting the synthesis of bioactive compounds such as phenolic compounds, which are boost when they are compared with the raw seed (Dominguez-Arispuro et al., 2018). Previous studies have shown that sprouting process modify the content and profile of isoflavonoid in Kabuli chickpea seeds (Wu et al., 2012;



Peak	UV _{max} (nm)	Ions [M+H] ⁺	Compound
1	259	431	Isoformononetin-glycoside
2	249	531	Formononetin malonyl-glycoside
3	261	447	Biochanin-A glycoside
4	260,288	533	Biochanin-A malonyl-glycoside
5	249	269	Formononetin
6	261	285	Biochanin-A

Fig. 1. Representative chromatogram at 260 nm, showing the principal isoflavones profile of sprouted black chickpea.

Table 2
Cellular antioxidant activity and EC₅₀ of unsprouted (UCF) and sprouted (SCF) black chickpea extracts.

		UCF	SCF
*EC ₅₀	PBS	28 ± 4.40 ^a	5.19 ± 0.12 ^b
	Without PBS	4.91 ± 0.73 ^a	0.88 ± 0.10 ^b
**CAA	PBS	41.21 ± 6.02 ^b	222.2 ± 5.60 ^a
	Without PBS	137.2 ± 18 ^b	778.3 ± 77.80 ^a

Mean ± standard deviation. Letters within a column indicate significant differences between UCF and SCF (p < 0.05) extracts.

* EC₅₀ (mg/mL): Median Effective Concentration;

** μmol EQ/100 g: micromole equivalents of quercetin.

Guardado-Félix et al., 2017; Milán-Noris et al., 2018). Sprouting, activates the central phenylpropanoid pathway and isoflavonoid branch pathways in legumes, by increasing the phenylalanine ammonia-lyase (PAL) activity. This enzyme is known as the initiator of isoflavones synthesis (Luczkiewicz and Glod 2003; Wu et al., 2012; Guardado-Félix et al., 2017).

In the analysis of the cellular antioxidant activity with both protocols in each sample, significant differences were observed, which depend on the degree of absorption and association with the cell membrane of the bioactive compounds present in the soluble extract. When the cells were washing with PBS, many of the components of the medium were removed. Washing is important since it allows the removal of the component from the medium that may cause interference. After wash the cells, only the compounds that cross the cell membrane barrier or those that are firmly bound to the cell membrane, provide antioxidant activity. The effectiveness

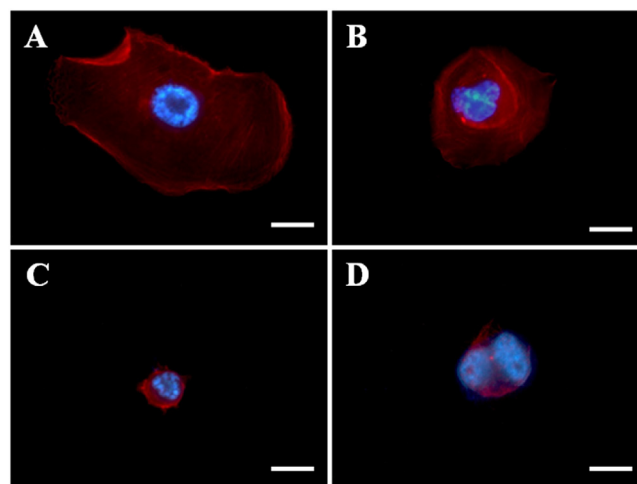


Fig. 2. Morphological changes of MDA-MB231 cells. (A) Untreated, (B) Cisplatin 40 μm, (C) 10 mg/mL of UCF(unsprouted black chickpea extracts), (D) 10 mg/mL of SCF (sprouted black chickpea extracts). Note: Cell shrinkage, rounding, DNA condensation and nuclear fragmentation in B, C, and D panels. Scale bars 20 μm.

of cell absorption or membrane binding combined with its ability to sequester free radicals dictates the efficacy of the phytochemicals in the samples. So, the bioactive compounds present in both extracts have two ways to exert antioxidant activity. Those compounds could act at the level of the cell membrane and break the chain reaction of free radicals at the level of the cell surface, or they

Table 3

Effect of unsprouted (UCF) and sprouted (SCF) black chickpea extracts on the viability of MDA-MB-231 breast cancer cells.

Concentration (mg/mL)	% Viability	
	UCF	SCF
0	100	100
0.3	96.40 ± 1.31 ^{ab,A}	90.53 ± 2.75 ^{a,B}
0.5	96.16 ± 0.68 ^{a,A}	83.46 ± 6.00 ^{a,B}
1	94.63 ± 0.68 ^{b,A}	82.33 ± 6.04 ^{a,B}
5	92.90 ± 0.85 ^{c,A}	58.90 ± 5.58 ^{b,B}
10	79.25 ± 0.80 ^{d,A}	51.95 ± 5.75 ^{b,B}
15	72.00 ± 1.71 ^{e,A}	46.68 ± 4.53 ^{c,B}
*IC ₅₀	>15 mg/mL	11.11 ± 0.12 mg/mL

Mean ± standard deviation; Lowercase letters within a column indicate significant differences between concentration ($p < 0.05$); Uppercase letters within a row indicate significant differences between UCF and SCF ($p < 0.05$).

* IC₅₀ (mg/mL): median inhibitory concentration.

Table 4

Effect of unsprouted (UCF) and sprouted (SCF) black chickpea extracts on the viability of NIH-3 T3 cell line.

Concentration (mg/mL)	% Viability	
	UCF	SCF
0	100	100
0.3	99.15 ± 0.21 ^{ab,A}	97.27 ± 0.71 ^{a,B}
0.5	92.27 ± 2.41 ^{b,A}	91.21 ± 1.76 ^{b,A}
1	86.14 ± 0.73 ^{c,A}	87.08 ± 1.97 ^{b,A}
5	86.39 ± 2.41 ^{c,A}	71.10 ± 5.85 ^{c,B}
10	62.01 ± 3.94 ^{d,A}	56.77 ± 5.56 ^{d,A}
15	56.39 ± 2.74 ^{e,A}	49.39 ± 3.65 ^{e,B}
*IC ₅₀	>15 mg/mL	14.35 ± 0.6 mg/mL

Mean ± standard deviation; Lowercase letters within a column indicate significant differences between concentration ($p < 0.05$); Uppercase letters within a row indicate significant differences between UCF and SCF ($p < 0.05$).

* IC₅₀ (mg/mL): median inhibitory concentration.

can cross the membrane and react with intracellular ROS (López-Alarcón and Denicola, 2013).

Furthermore, in the SCF sample, biochanin-A malonyl glycoside was the main isoflavone, so antioxidant mechanism may be related to this compound. Biochanin-A reduces oxidative stress through the decrease of the malondialdehyde levels in diabetic rats, increased the levels of endogenous antioxidant enzymes (catalase, superoxide dismutase), thus modifying the total antioxidant status (de Camargo et al., 2019). Besides, phenolic compounds acting as antioxidants such as glycosylated isoflavones, bind the HepG2 cell membrane strongly and act at the level of the cell surface. This behavior has been explained due to their molecular size, polarity and solubility and they cannot easily enter into the cell (Wolf and Liu et al., 2008).

Additionally, the isoflavones in chickpea extracts showed antiproliferative effect in MDA-MB-231 cancer cell line. Those compounds may affect the membrane of MDA-MB-231. It was observed that the chickpea treatments caused morphological changes suggesting cell damage and possible apoptosis. The characteristic morphology of cells undergoing apoptosis includes cell membrane modifications as loss of asymmetry, membrane fixation, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation (Hengartner 2000).

According to literature, isoflavones inhibits cell growth and induces apoptosis in MDA-MB-231 cells. It modulates the antioxidant system of cancer cells causing the accumulation of reactive oxygen species and cell death (Ullah et al., 2011). NFκB signaling pathway is involved in the regulation of critical biological processes such proliferation and apoptosis, a common active pathway in TNBC. Soy bean isoflavones inhibited cell proliferation and trig-

gers apoptosis in TNBC, causing a dose-dependent response, decreasing NFκB/p65 protein levels and DNA-binding activity of NFκB, downregulation of Bcl-2, up-regulation of Bax, and apoptotic protein (Li et al., 2008; Pan et al., 2012; Zhu et al., 2013; He and Chen 2013).

5. Conclusions

The sprouting process is an efficient alternative to boost the content and profile of isoflavones in black chickpea, increasing cellular antioxidant activity, and decreasing the proliferation of MDA-MB-231 breast cancer cells. The results herein suggest that bioactive compounds in sprouted black chickpea showed a potential antioxidant and antiproliferative activity. Thus, SCF seems to be a good candidate as ingredient for the development of functional foods and to valorize black chickpea for direct human consumption.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.sjbs.2020.11.048>.

References

- de Camargo, A.C., Favero, B.T., Morzelle, M.C., Franchin, M., Alvarez-Parrilla, E., de la Rosa, L.A., Geraldí, M.V., Maróstica Júnior, M.R., Shahidi, F., Schwember, A.R., 2019. Is chickpea a potential substitute for soybean? Phenolic bioactives and potential health benefits. *Int. J. Mol. Sci.* 20, 2644. <https://doi.org/10.3390/ijms20112644>.
- Dominguez-Arispuro, D.M., Cuevas-Rodríguez, E.O., Milán-Carrillo, J., León-López, L., Gutiérrez-Dorado, R., Reyes-Moreno, C., 2018. Optimal germination condition impacts on the antioxidant activity and phenolic acids profile in pigmented desi chickpea (*Cicer arietinum* L.) seeds. *J. Food Sci. Technol.* 55 (2), 638–647. <https://doi.org/10.1007/s13197-017-2973-1>.
- Felice, D., Sun, J., Liu, R., 2009. A modified methylene blue assay for accurate cell counting. *J. Funct. Foods* 1, 109–118. <https://doi.org/10.1016/j.jff.2008.09.014>.
- Gao, Y., Yao, Y., Zhu, Y., Ren, G., 2015. Isoflavone content and composition in chickpea (*Cicer arietinum* L.) sprouts germinated under different conditions. *J. Agric. Food Chem.* 63, 2701–2707. <https://doi.org/10.1021/jf5057524>.
- Garrido, G.A., De La Maza C, M.P., Valladares, B.L., 2003. Potential health benefits of phytoestrogens. Fitoestrogenos dietarios y sus potenciales beneficios en la salud del adulto humano. *Rev. Med. Chile* 131, 1321–1328. <http://doi.org/10.4067/S0034-98872003001100015>.
- Guardado-Félix, D., Serna-Saldívar, S.O., Cuevas-Rodríguez, E.O., Jacobo-Velázquez, D.A., Gutiérrez-Urbe, J.A., 2017. Effect of sodium selenite on isoflavonoid contents and antioxidant capacity of chickpea (*Cicer arietinum* L.) sprouts. *Food Chem.* 226, 69–74. <https://doi.org/10.1016/j.foodchem.2017.01.046>.
- He, F.J., Chen, J.Q., 2013. Isoflavones and breast cancer incidence: differences between Chinese women and women in Western countries and possible mechanisms. *Food Sci. Hum. Well.* 2, 146–161. <https://doi.org/10.1016/j.fshw.2013.08.002>.
- Heiras-Palazuelos, M.J., Ochoa-Lugo, M., Gutiérrez-Dorado, R., López-Valenzuela, J. A., Mora-Rochín, S., Milán-Carrillo, J., Garzón-Tiznado, J.A., Reyes-Moreno, C., 2013. Technological properties, antioxidant activity and total phenolic and flavonoid content of pigmented chickpea (*Cicer arietinum* L.) cultivars. *Int. J. Food Sci. Nutr.* 64, 69–76. <https://doi.org/10.3109/09637486.2012.694854>.
- Hengartner, M.O., 2000. Insight review articles: the biochemistry of apoptosis. *Nature* 207, 770–776. <https://doi.org/10.1038/35037710>.
- Li, Z., Li, J., Mo, B., Hu, C., Liu, H., Qi, H., Wang, X., Xu, J., 2008. Genistein induces cell apoptosis in MDA-MB-231 breast cancer cells via the mitogen-activated protein

- kinase pathway. *Toxicol. in vitro* 22, 1749–1753. <https://doi.org/10.1016/j.tiv.2008.08.001>.
- López-Alarcón, C., Denicola, A., 2013. Evaluating the antioxidant capacity of natural products: a review on chemical and cellular-based assays. *Anal. Chim. Acta* 763, 1–10. <https://doi.org/10.1016/j.aca.2012.11.051>.
- Luczkiewicz, M., Glod, D., 2003. Callus cultures of 'Genista' plants—in vitro material producing high amounts of isoflavones of phytoestrogenic activity. *J. Plant Sci.* 165, 1101–1108. [https://doi.org/10.1016/S0168-9452\(03\)00305-4](https://doi.org/10.1016/S0168-9452(03)00305-4).
- Messina, M.J., 1999. Legumes and soybeans: Overview of their nutritional profiles and health effects. *Am. J. Clin. Nutr.* 70, 439S–450S. <https://doi.org/10.1093/ajcn/70.3.439s>.
- Milán-Noris, A.K., Gutiérrez-Urbe, J.A., Santacruz, A., Serna-Saldívar, S.O., Martínez-Villaluenga, C., 2018. Peptides and isoflavones in gastrointestinal digests contribute to the anti-inflammatory potential of cooked or germinated desi and kabuli chickpea (*Cicer arietinum* L.). *Food Chem.* 268, 66–76. <https://doi.org/10.1016/j.foodchem.2018.06.068>.
- Nagaraju, G.P., Zafar, S.F., El-Rayes, B.F., 2013. Pleiotropic effects of genistein in metabolic, inflammatory, and malignant diseases. *Nutr. Rev.* 71, 562–572. <https://doi.org/10.1111/nure.12044>.
- Pan, H., Zhou, W., He, W., Liu, X., Ding, Q., Ling, L., Zha, X., Wang, S., 2012. Genistein inhibits MDA-MB-231 triple-negative breast cancer cell growth by inhibiting NF- κ B activity via the Notch-1 pathway. *Int. J. Mol. Med.* 30, 337–343. <https://doi.org/10.3892/ijmm.2012.990>.
- Pérez-Martín, L., Bustamante-Rangel, M., Delgado-Zamarreño, M.M., 2017. Classification of lentils, chickpeas and beans based on their isoflavone content. *Food Anal. Methods* 10, 1191–1201. <https://doi.org/10.1007/s12161-016-0675-3>.
- Sánchez-Chino, X., Jiménez-Martínez, C., Dávila-Ortiz, G., Álvarez-González, I., Madrigal-Bujaidar, E., 2015. Nutrient and nonnutrient components of legumes, and its chemopreventive activity: a Review. *Nutr. Cancer* 67, 401–410. <https://doi.org/10.1080/01635581.2015.1004729>.
- Sharma, S., Singh, A., Singh, B., 2018. Characterization of in vitro antioxidant activity, bioactive components, and nutrient digestibility in pigeon pea (*Cajanus cajan*) as influenced by germination time and temperature. *J. Food Biochem.* 43, 12706–12718. <https://doi.org/10.1111/jfbc.12706>.
- Ullah, M.F., Ahmad, A., Zubair, H., Khan, H.Y., Wang, Z., Sarkar, F.H., Hadi, S.M., 2011. Soy isoflavone genistein induces cell death in breast cancer cells through mobilization of endogenous copper ions and generation of reactive oxygen species. *Mol. Nutr. Food Res.* 55, 553–559. <https://doi.org/10.1002/mnfr.201000329>.
- Velentzis, L.S., Woodside, J.V., Cantwell, M.M., Leatham, A.J., Keshtgar, M.R., 2008. Do phytoestrogens reduce the risk of breast cancer and breast cancer recurrence? What clinicians need to know. *Eur. J. Cancer* 44, 1799–1806. <https://doi.org/10.1016/j.ejca.2008.05.019>.
- Wolfe, K.L., Kang, X., He, X., Dong, M., Zhang, Q., Liu, R.H., 2008. Cellular antioxidant activity of common fruits. *J. Agric. Food Chem.* 56 (18), 8418–8426. <https://doi.org/10.1021/jf801381y>.
- Wu, A., Song, L., Feng, S., Liu, Y., He, G., Yioe, Y., Liu, S., Huang, D., 2012. Germination dramatically increases isoflavonoid content and diversity in chickpea (*Cicer arietinum* L.) seeds. *J. Agric. Food Chem.* 61, 8120–8125. <https://doi.org/10.1021/jf3021514>.
- Xu, B., Chang, S.K.S., 2009. Phytochemical profiles and health-promoting effects of cool-season food legumes as influenced by thermal processing. *J. Agric. Food Chem.* 57, 10718–10731. <https://doi.org/10.1021/jf902594m>.
- Zhu, H., Bhajjee, F., Ishaq, N., Pepper, D.J., Backus, K., Brown, A.S., Zhou, X., Miele, L., 2013. Correlation of Notch1, pAKT and nuclear NF- κ B expression in triple negative breast cancer. *Am. J. Cancer Res.* 3, 230–239.