



FUNCTIONAL PROPERTIES, AND *IN VITRO* MINERAL BIOAVAILABILITY OF DEFATTED *CUCUMIS MELO* AND *CITRULLUS VULGARIS* SEED FLOURS

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Abstract

Citrullus vulgaris and *Cucumis melo* seeds are the most underutilized byproducts with excellent nutritional and therapeutic values. Therefore, in this study, functional properties, mineral content, and *in vitro* bioavailability of selected minerals of *Cucumis melo* and *Citrullus vulgaris* seeds were assessed. *Citrullus vulgaris* seeds flour showed better functional properties than that of *Cucumis melo*. Also, defatted seed flours of both the samples showed significantly ($p < 0.05$) improved color, water, and oil binding properties, emulsifying and foaming properties, however, defatted seeds showed significantly ($p < 0.05$) lower bulk density as compared to whole seed flours. Moreover, *Cucumis melo* seeds showed significantly ($p < 0.05$) higher content of calcium, zinc, iron, sodium and potassium in comparison with *Citrullus vulgaris* seed, whereas non-significant ($p > 0.05$) difference was observed in the copper content of both seed flours. *Cucumis melo* seed showed significantly ($p < 0.05$) higher bioavailability of the zinc and calcium in comparison with *Citrullus vulgaris* seeds, however, the non-significant difference was observed in mineral bioavailability of iron.

Keywords: Functional properties, *Cucumis melo*, *Citrullus vulgaris*, Minerals content, Bioavailability.

Introduction

Over the past years, waste utilization of fruits and vegetables as a source of functional ingredients in different food formulations showed promising interests to the scientists (Meethal *et al.*, 2017; Chhikara *et al.*, 2018; Chawla *et al.*, 2019; Suri *et al.*, 2019; Kaur *et al.*, 2019; Dhiman *et al.*, 2020; Paul *et al.*, 2020; Bassi *et al.*, 2020). More innovations have been revealed on the utilization of fruits and vegetable by-products as natural and highly nutritive food ingredients (Sadh *et al.*, 2018a; Singh *et al.*, 2018; Banga *et al.*, 2019; Chaudhary *et al.*, 2020; Iid *et al.*, 2020). Watermelon (*Citrullus vulgaris*) and cantaloupe (*Cucumis melo*) seeds are among the most underutilized by-products contain an excellent amount of proteins and essential fatty acids (Lakshmi and Kaul 2011). In Ayurveda and Chinese folk medicines, *Cucumis melo* seeds are used as an antitussive, hepatoprotective, febrifuge, and vermifuge constituent, besides, melon seeds extract can be used as an antidiabetic and anti-chronic eczema component (Marino *et al.*, 2009). As well, in various countries, watermelon and cantaloupe seeds are directly consumed by humans as snacks after salting and roasting (Lakshmi and Kaul, 2011). Furthermore, both seeds also consist of an excellent amount of trace minerals such as iron, zinc, and calcium, however, the presence of certain antinutritional factors limits the mineral bioavailability of these seeds (Sadh *et al.*, 2018b). Also, it is well known that majorly antinutritional factors are the polyphenolic constituents that are present in the hydrophobic parts of the seeds, therefore defatting of the seeds can also remove the antinutritional components from the seeds (Lakshmi and Kaul, 2011; Ananthanarayan *et al.*, 2018; Kumar *et al.*, 2018a; Kumar *et al.*, 2018b). As well, micronutrient malnutrition is a severe menace to the health and efficiency of billions of people worldwide, even though it is largely preventable (Chawla *et al.*, 2017; Gupta *et al.*, 2019; Kumar *et al.*, 2020; Mishra *et al.*, 2020). Among all the micronutrients, deficiency of iron, zinc, and calcium is a primary concern not only due to the severe health

consequences they may cause, however every age group of the world population is affected in both developing and developed countries (Gupta *et al.*, 2015). Furthermore, the evaluation of the functional properties of seed flour is challenging due to the presence of several components and factors (Joshi *et al.*, 2015). Moreover, only a few reports have been published to access the functional properties and *in vitro* mineral bioavailability of micronutrients from *Citrullus vulgaris* and *Cucumis melo* seeds. Since, there has been improved interest in non-conventional natural food rich in nutraceuticals, the seed of watermelon and cantaloupe could be used as functional food additives. Therefore, the present study was carried out to assess the functional properties and *in vitro* mineral bioavailability of iron, zinc, and calcium of watermelon and cantaloupe seed flours.

Materials and Methods

Chemicals and reagent

Petroleum ether, Tween 80, nitric acid, sulphuric acid, and perchloric acid were procured from SD Fine India Pvt., Ltd., (Mumbai, India). Digestive enzymes for gastrointestinal assay such as human pancreatic lipase, phospholipase A2, α -amylase, colipase, cholesterol esterase, mucin, bovine serum albumin, pepsin, pancreatin, and taurocholate salts were procured from Sigma Chemical Co (Madrid, Spain). Cellulose dialysis membrane with 16 mm internal diameter and 14000 Da molecular weight cut-off was obtained from Himedia Laboratories Pvt., Ltd. (Mumbai, India). Soybean oil was purchased from the local market of Sirsa, Haryana, India. All chemicals used in the present investigation were of 'Analytical Reagent' grade. Triple distilled water and acid-washed glassware were used throughout the experiments.

Collection and defatting of seeds

Fresh uncoated watermelon and cantaloupe seeds were obtained from Amritash Herbocare Ellenabad (Haryana), India. Seeds were washed three times to remove dust and foreign particles using triple distilled water. Washed seeds were then grounded using a high-speed blender (Braun AG

Frankfurt A.M. Mx 32, Germany) to obtain the seed flour. The flour was then defatted for 8h using a Soxhlet apparatus (Jain Scientific works, Ambala) using petroleum ether (boiling point 40-60 °C, flour to solvent ratio of 1:10 w/v) as fat extraction solvent. Defatted seed flours were then air-dried in a hot air oven at room temperature (30°C) and grounded again to fine seed flour using a high-speed blender. Both whole seed and defatted seed flour samples were stored in sterilized airtight glass containers at refrigerated temperature (4-7 °C) until further analysis.

Functional properties of seed flour

Bulk density

The bulk density of both the flour samples was determined by the method proposed by Sadh *et al.* (2018b). Briefly, whole seed and defatted seed flour samples were carefully filled in a 5.0 ml graduated measuring cylinder contains a minimum count of 0.5 ml. Measuring cylinder (bottom part) was gently and carefully tapped ten times till further attenuation of the seed flour samples after complete filling up to the 5.0 ml mark. Bulk density of all the seed flour samples was calculated as the weight of the seed flour sample occupied in per unit volume of the sample and it was expressed as g/cm³.

Color measurements of the seed flour

To evaluate the change in color after defatting of the seed flour, Hunter lab color flex colorimeter (Hunter associates laboratory Inc., USA) was used. Herein, L* = whiteness a* = redness to greenness and b* = yellowness to blueness were the color directs of the colorimeter.

Water and oil binding properties of seed flour

Water and oil binding efficacy of whole and defatted seed flour were determined by following the process proposed by Chawla *et al.*, (2017). To determine these properties of seed flour samples, pre-weighed 15 ml centrifuge tubes were used. Briefly, a 1g sample of all the seed flour samples was mixed gently in 10 ml triple distilled water and soyabean oil and poured in the centrifuge tubes. Samples were kept undisturbed for 30 min and tubes were then centrifuged at 5000 ×g (27 °C) for 5 min. The supernatant of both oil and water was discarded and the weight of tubes with pallets was taken again. Both water and oil absorption capacity were expressed as g of water and g of oil reserved per g of the seed flour samples.

Emulsifying efficacy of seed flour

Emulsifying activity and stability were determined by following the method proposed by Shilpashree *et al.* (2015). Briefly, a 20 ml soybean oil was sonicated with 1% seed flour solutions using probe ultra sonicator (Sonics and Materials Inc. New Town, USA) with 5s pulse rate for 10 min. The prepared emulsion samples were centrifuged at 1500×g for 5 min and the height of the emulsified sample was then measured for the calculation of emulsifying activity.

$$EA \% = \frac{\text{Emulsified layer height}}{\text{Weight of the total content}} \times 100$$

Also, for the calculation of emulsion stability, heating of emulsion was carried out at 80°C for 30 min before centrifuging at 1500×g for 5 min and calculated as follows:

$$ES\% = \frac{\text{Emulsified layer after heating}}{\text{Emulsified layer before heating}} \times 100$$

Foaming capacity of seed flour

Foam capacity (FC) and foam stability (FS) were measured for the whole and defatted flour samples. The method proposed by Chawla *et al.* (2017) was followed for the evaluation of foam capacity and foam stability. Briefly, a 2% (w/v) seed flour solution was prepared using a magnetic stirrer (500rpm, 30 min). Samples were then whipped in an auto-mix blender (230V AC Sujata Mixer, Ambala, Haryana, India) at its maximum speed for exactly 15 min. The sample was then immediately shifted to a 100 ml graduated measuring cylinder. The volume was measured before and after stirring. Foam stability was determined by change in the foam volume in the measuring cylinder after 60 min (30°C).

$$FC (\%) = \frac{\text{Whipped volume (ml)} - \text{the volume before whipping (ml)}}{\text{the volume before whipping (ml)}} \times 100$$

$$FS (\%) = \frac{\text{Volume after resting (ml)} - \text{The volume before whipping (ml)}}{\text{the volume before whipping (ml)}} \times 100$$

Mineral content of seeds

Method purposed by AOAC (2005) was followed to determine mineral content (copper, zinc, iron, and calcium) of seed flour. In the case of potassium and sodium, atomic emission mode was used, whereas, absorption mode was used for copper, zinc, iron, and calcium using AAS (AA-7000, Shimadzu, Japan). Ashing of all seed flour samples was done (at 550°C for 8 h) and obtained ash was solubilized in tri acid mixture (Nitric acid, sulphuric acid, and perchloric acid) and heated for complete dissolution.

In-vitro bioavailability of minerals of seed flour

In-vitro mineral bioavailability of iron, zinc, and calcium was determined by the simulated gastro-intestinal model as proposed by Chawla *et al.* (2017) with slight modification (i.e. 5 g seed flour sample was taken). Different components and concentrations of both inorganic and organic solutions, digestive solutions such as saliva, gastric juice, duodenal juices, and bile constituents were carefully prepared and used as described by (Granado-Lorencio *et al.*, 2007).

Statistical analysis

Microsoft Excel, 2016 (Microsoft Corp., Redmond, WA, USA) was used for the calculation of means and standard error mean). A significant difference between values was calculated and verified by one-way analysis of variance (ANOVA) and the assessment between means was calculated by critical difference value (Kaushik *et al.*, 2018).

Results and Discussion

Color estimation and Bulk density

Typical color parameters of seed flour samples are presented in Figure 1a. It is evident from the results that the extraction of fat from both seed flours significantly affected (p<0.05) all the color parameters. Color parameters of flour samples of *Citrullus vulgaris* showed a significant difference (p<0.05) in comparison with both full fat and defatted seed flour of *Cucumis melo*, respectively. Defatted samples were whiter in comparison to their full-fat samples as lipid-soluble pigments were removed during defatting (Joshi *et al.*, 2015). Fat extraction also reduced the redness and yellowness of both the seed flours. Joshi *et al.* (2015) and Turan *et al.* (2015) reported similar changes in color after defatting almond, Brazil nut, hazelnut, peanut, and soybean flour. The bulk density of both seed flours is presented in Figure 1b. Bulk density of all the seed flour samples is ranged from 0.34

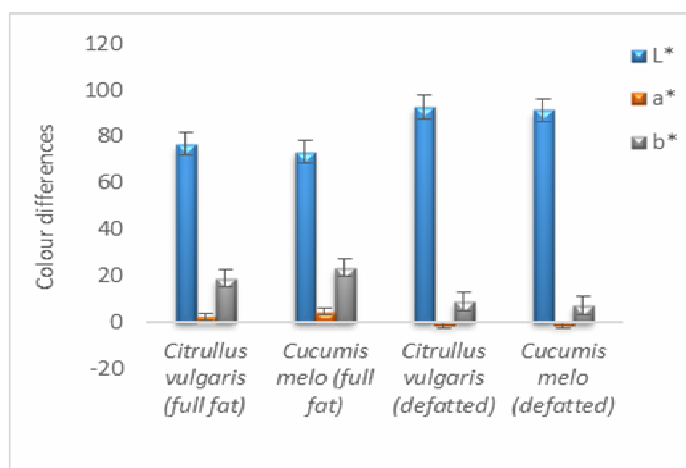
to 0.62 g/cm^3 . Results from the figure revealed a significant difference in the bulk density of all seed flour samples. Defatting of seeds led to a significant decrease in the bulk density of both seed samples. Significantly ($p < 0.05$) higher bulk density of *Citrullus vulgaris* than *Cucumis melo* seed flours was observed. Our results were in accordance with the findings Joshi *et al.* (2015) and Turan *et al.* (2015) who reported a significant difference between defatted and full-fat flours of almond, Brazil nut, hazelnut, peanut, and soybean seed in terms of bulk density.

Water and oil binding capacity

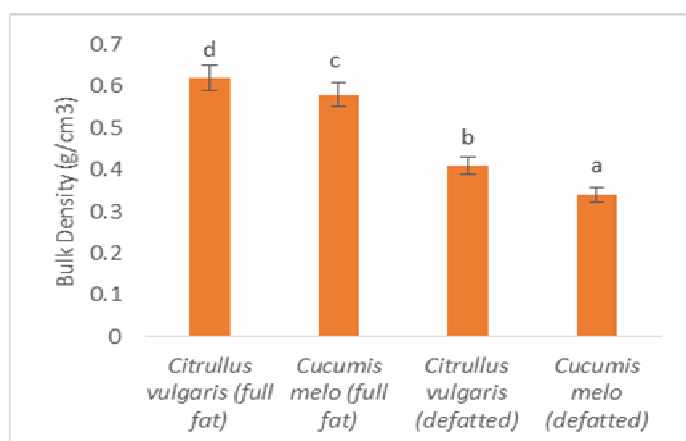
Results of water and oil binding efficacy of all the seed flour is represented in (Figure 2a). Water and oil binding capacity of all seed flour samples were ranged from 0.76-2.15 and 0.81-2.39 g/g, respectively. All the seed flour samples showed significant ($p < 0.05$) difference in terms of water and oil binding capacity, however *Cucumis melo* (full fat) seed flour showed significantly ($p < 0.05$) lower water and oil binding capacity than that of other full fat and defatted seed flour samples. Defatting opens both the hydrophobic and hydrophilic domains of the seed flours, therefore, increased water and oil binding capacity was observed in defatted seed samples. However, defatted *Citrullus vulgaris* seeds showed significantly ($p < 0.05$) higher water and oil binding efficacy. A similar trend in water-binding capacity was reported in watermelon and pumpkin seeds by Lakshmi and Kaul (2011). Joshi *et al.*, (2015) reported similar oil binding capacity of defatted flour samples of almond, Brazil nut, hazelnut, peanut and soybean seeds.

Emulsifying activity, stability, and foaming properties

Emulsifying activity and stability of all the samples are represented in (Figure 2b). Results from the figure clearly revealed a significant ($p < 0.05$) difference in terms of the emulsifying activity and stability of all the seed flour samples. Emulsifying properties and stability of all the samples were ranged from 43.32-51.34% and 38.34-46.78%, respectively. The extraction of fat from seed flours led to a significant increase ($p < 0.05$) in the emulsifying activity of the seed flours. The defatting of seed flours also significantly improved emulsion stability. Seed flours (full fat and defatted) of *Citrullus vulgaris* showed significantly ($p < 0.05$) higher emulsifying activity and stability in comparison with *Cucumis melo*. The increase in emulsifying activity and stability of *Citrullus vulgaris* seed flours might be due to their higher protein content and surface charge. As well, the defatting of both the seed flour significantly ($p < 0.05$) improved the foaming properties of both the seed flours as shown in (Figure 2c). Herein, seed flour of *Citrullus vulgaris* showed significantly ($p < 0.05$) improved foaming properties in comparison with seed flours of *Cucumis melo*. Results were in line with the findings of Lakshmi and Kaul (2011); Joshi *et al.* (2015) who reported significantly increased emulsifying and foaming capacity of the defatted flour samples of almond, Brazil nut, hazelnut, peanut and soybean seeds.



(a)

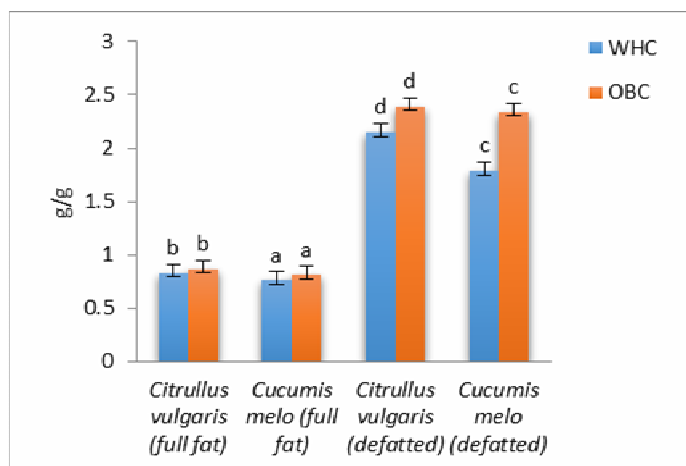


(b)

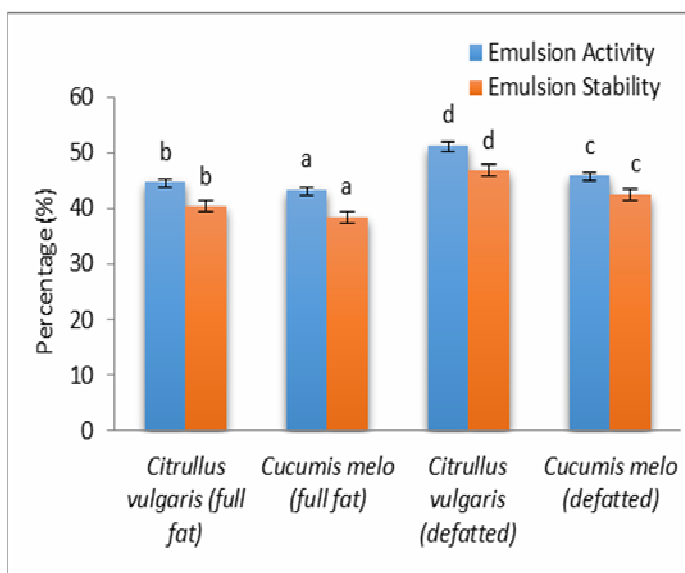
Fig. 1: (a) Colour differences for different color parameters of full fat and defatted seed flour samples of *Citrullus vulgaris* and *Cucumis melo*. The color coordinates L* = whiteness; a* = redness to greenness and b* = yellowness to blueness (b) Bulk density of full fat and defatted seed flour samples of *Citrullus vulgaris* and *Cucumis melo*

Data are presented as means \pm SEM (n=3).

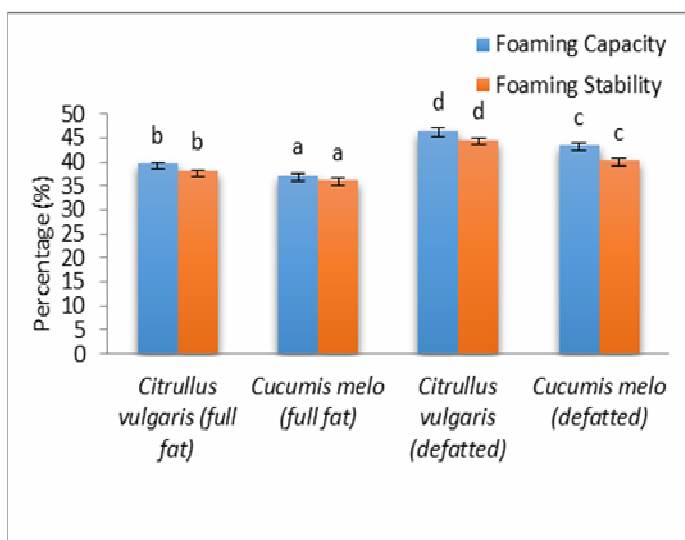
^{a-d}Means within the row with different lowercase superscript are significantly different ($p < 0.05$) from each other



(a)



(b)



(c)

Fig. 2: Functional properties of full fat and defatted seed flours of *Citrullus vulgaris* and *Cucumis melo* (a) Water holding capacity and Oil binding capacity (b) Emulsion activity and Emulsion stability (c) Foaming capacity and Foaming stability

Data are presented as means±SEM (n=3).

^{a-d}Means within the row with different lowercase superscript are significantly different (p<0.05) from each other

Mineral content and *in-vitro* bioavailability of iron, zinc, and calcium

Minerals are important ingredients as they play vital physiological processes in our body. Zinc, iron, copper, calcium, sodium and potassium content of seeds of cantaloupe and watermelon were determined using an Atomic absorption spectrophotometer and data is presented in Table 1. Cantaloupe showed significantly higher (p<0.05) zinc, iron, calcium, sodium, and potassium content in comparison to watermelon whereas no difference (p<0.05) was observed in copper content. Bioavailability (iron, zinc, and calcium) of seeds was examined using an *in vitro* simulated gastrointestinal digestion system. It can be inferred from the results that cantaloupe seeds showed significantly increased the bioavailability of the zinc and calcium in comparison with *Citrullus vulgaris* seeds, however, non-significant (p<0.05) difference was observed in the mineral bioavailability of iron (Figure 3).

Table 1: Minerals content in seeds of *Cucumis melo* and *Citrullus vulgaris*

Minerals	<i>Cucumis melo</i> seeds (ppm)	<i>Citrullus vulgaris</i> seeds (ppm)
Zinc	6747.04±52.48 ^b	3364.29±7.47 ^a
Iron	112.12±0.22 ^b	105.34±0.61 ^a
Copper	39.67±1.06 ^a	38.77±0.36 ^a
Calcium	1282.08±2.38 ^b	468.90±1.55 ^a
Sodium	24062.28±51.91 ^b	12484.83±18.63 ^a
Potassium	19614.77±35.55 ^b	14376.37±10.99 ^a

Data are presented as means±SEM (n=3).

^{ab}Means within rows with different lowercase superscript are significantly different (p<0.05) from each other

Conclusion

Defatted seed flours of both the sample showed significantly improved color parameters, bulk density, water, and oil binding properties, emulsifying and foaming properties, respectively. *Citrullus vulgaris* seed flours showed significantly higher functional properties than seeds of *Cucumis melo*. *Citrullus vulgaris* showed significantly (p<0.05) lower mineral content in comparison with *Cucumis melo*. *Cucumis melo* seed showed significantly (p<0.05) increased the bioavailability of the zinc and calcium in comparison with *Citrullus vulgaris* seeds, however, the non-significant difference was observed in the bioavailability of iron.

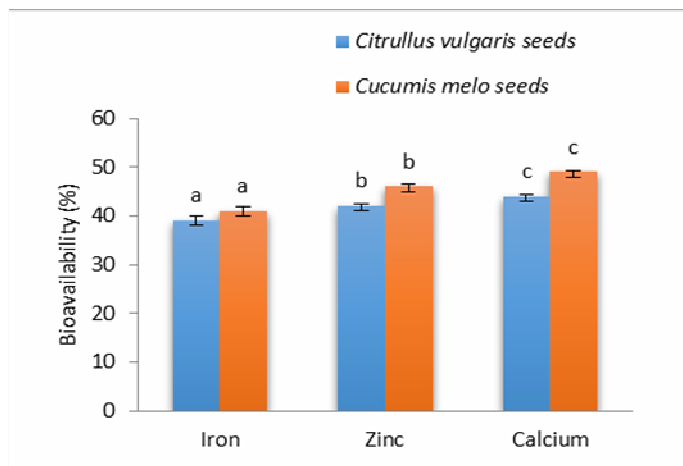


Fig. 3 : *In vitro* bioavailability of iron, zinc, and calcium of *Citrullus vulgaris* and *Cucumis melo* seeds

Data are presented as means±SEM (n=3).

^{a-b}Means within the row with different lowercase superscript are significantly different (p<0.05) from each other.

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Abbreviations

WBC Water binding capacity, **OBC** Oil binding capacity, **AAS**, Atomic absorption spectrophotometer

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