

Original Research Article

Evaluation of Bioaccessibility of Vitamin C from Four Different Commercial Amla Products Using a Modified *In Vitro* Digestion Model

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Abstract: The present study aims to evaluate the bioaccessibility of vitamin C from 4 different Amla-based products compared with the Raw Amla using a modified dynamic customized in vitro digestion model. We chose 4 different amla products including amla juice, honey amla, dry amla, and amla powder for our study for the estimation of vitamin C. The results indicated that before in vitro digestion, the quantity of vitamin C is higher (45 mg/g) compared with other formulations. This may be due to the degradation of the amla matrix during powder making. Then, we carried out a dynamic in vitro digestion process sequentially from the mouth phase into the intestinal phase. Briefly, equal volumes of amla product and salivary enzymes were mixed thoroughly for 5 minutes, transferred to the stomach phase by mixing with porcine gastric juice, and made a pH of 3 for 2 hours. Finally, the digested foodstuffs were transferred and mixed with porcine intestinal juice for 2 hours. Once the intestinal digestion is finished, the overall digest is filtered using 0.3-micron filter paper for collecting micellar phase from the supernatant for vitamin C quantification. The results indicated that the amount of vitamin C entered the micellar is higher in the case of amla powder compared with other formulations (25 mg/g). Therefore, among the different amla products, dry amla (57.8 %) and amla juice (56.8%) exhibited better sources of vitamin C to facilitate bioactivity and also ameliorate vitamin C deficiency disorders.

Keyword: Amla (*Phyllanthus emblica*), ascorbic acid (vitamin C), *in vitro* digestibility, bioaccessibility, food matrix.

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INTRODUCTION

Bioavailability and bioaccessibility measurements are the key components in food and nutraceutical industries to elucidate the number of essential compounds like carbohydrates, lipids, proteins, vitamins, and minerals that enter into circulation after digestion is over. In most cases, the available number of essential components in the foodstuffs is not equal to the number of components entering into circulation. This will make us seriously concerned to address the recommended daily allowances of all the essential nutrients for the betterment of cellular activity. Moreover, there may be a reason for the development of different nutritional formulations as well as supplements to meet the daily need for minerals and vitamins in the market. Among the different factors, the food matrix is playing an important role in regulating the bioavailability

and bioaccessibility of essential nutrients. Due to various technical difficulties, recently the researchers carried out various techniques to find the true availability of essential nutrients in the blood as well as the target tissue and cells for better biological activities that include the INFOGEST model and TIM model. Nevertheless, depending on the study field employed and techniques adopted, the bioavailability of the tested components may vary due to the difficulties in mimicking the functional gastrointestinal systems in the in vitro conditions. Recently, the researchers modified the INFOGEST into dynamic mode as well as introduce beneficial microbes and also use another model specifically elucidate the digestibility of the small intestine by keeping the food directly in the porcine intestine and closing the intestine for 2 hours then measuring the bioavailability of essential nutrients in

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circulation. Before drawing any conclusions about a possible health effect on an essential nutrient, it is crucial to examine how the digestive process affects bioactive chemicals and their stability and the amount of a substance that is liberated from its matrix and made accessible for absorption in the gastrointestinal system is known as bioaccessibility (e.g., enters the bloodstream). This phrase refers to the digestive processes that turn food into assimilable form, as well as the absorption of nutrients into intestinal epithelial cells and systemic, intestinal, and hepatic metabolism. Definitions based on absorption, however, exclude the positive effects of unabsorbed nutrients, such as calcium binding of bile salts in the tract. *In vitro* digesting techniques that typically mimic stomach and small intestine digestion, occasionally followed by Caco-2 cell absorption, are used to evaluate bioaccessibility [1].

Among the different food supplements, is Indian gooseberry also known as *Emblica officinalis Gaertn. / Phyllanthus emblica Linn. (Euphorbiaceae)*. Amla berries are processed into powder for capsules and juiced for extracts due to their high antioxidant content, vitamin C. The quantity of vitamin C in Indian gooseberries is 20 times more than that in an orange. It has been demonstrated in several studies that amla has anti-diabetic, hypolipidemic, anti-microbial, anti-inflammatory, antioxidant, hepatoprotective, and antiemetic properties. The ascorbic acid level in amla fruit is the second highest of all commercially grown fruits, making them a rich source of vitamin C. The recommended daily intake of vitamin C for adults is 90 mg for men and 75 mg for women [2]. Amla fruit contains several bioactive phytochemicals, the bulk of which are polyphenols (ellagic acid, chebulinic acid, gallic acid, chebulagic acid, apigenin, quercetin, corilagin, luteolin, etc.), as well as a significant amount of vitamin C (ascorbic acid) [3].

In India, it is consumed with salt and red chili powder, cooked into a pickle, or turned into a sweet treat. In addition to these other forms, amla is also offered as a powder, liquid, oil, tablet, and spice. Many people also consume amla tea and dried amla because of its nutritional advantages [4]. The USDA National Nutrient Database reports that Indian gooseberries are low in calories, with 100 grams of the fruit having only 44 calories [4-6].

There is a need to refine and validate the method with suitable *in vitro* assays to ascertain its limitations and applications. This is because some *in vivo* digestion events cannot be replicated, in addition to the fact that there are numerous *in vitro* digestion models reported in previous literature [7, 8].

Additional factors have also been extensively discussed, including the presence of phospholipids, the distinction between individual digestive enzymes like pancreatin and bile salts and gastric lipase and their

mixtures, and the ratio of food bolus to digestive fluids. In the current consensus paper, within the cost INFOGEST network, we suggest a general, standardized, and useful static digestion method based on physiologically relevant conditions that can be applied for a variety of endpoints and may be modified to accommodate additional specific requirements. Concerning the *in vitro* data and enzymes that are currently available, a frameset of parameters, including oral, gastric, and small intestinal digestion, are outlined and their relevance is discussed [7, 8]. At this juncture, the present work is aimed to evaluate the bioavailability and bioaccessibility of vitamin C in 5 different amla-based products (Amla Fruit, Amla Powder, Amla Juice, Dry Amla, and Honey Amla) using modified *in vitro* digestion models.

MATERIALS AND METHODS

Materials

Amla (*Phyllanthus emblica*), Amla Juice (Hebridean), Amla Powder (Indus Valley), Dry Amla, and Honey Amla (Saaral) were used as the source of vitamin C. Potassium Iodide (Merck, India), Iodine Crystals (Merck, India), Soluble Starch (Merck, India), Potassium Chloride (Himedia, India), Hydrochloric Acid (Merck, India), Sodium Hydroxide (Merck, India) and Glycine (Merck, India) were used for the measurement of vitamin C.

Methods

Vitamin C Quantification by Redox Titration

It was performed using the Redox titration method reported earlier in the literature. Vitamin C is assayed by the Iodometric titration method using iodine as the standard solution. Ascorbic acid in the different amla products before and after *in vitro* digestion, oxidized to form dehydro-ascorbic acid and iodine which forms a blue-black color as an endpoint and calculated the vitamin C concentration using the formula reported previously in the literature [9].

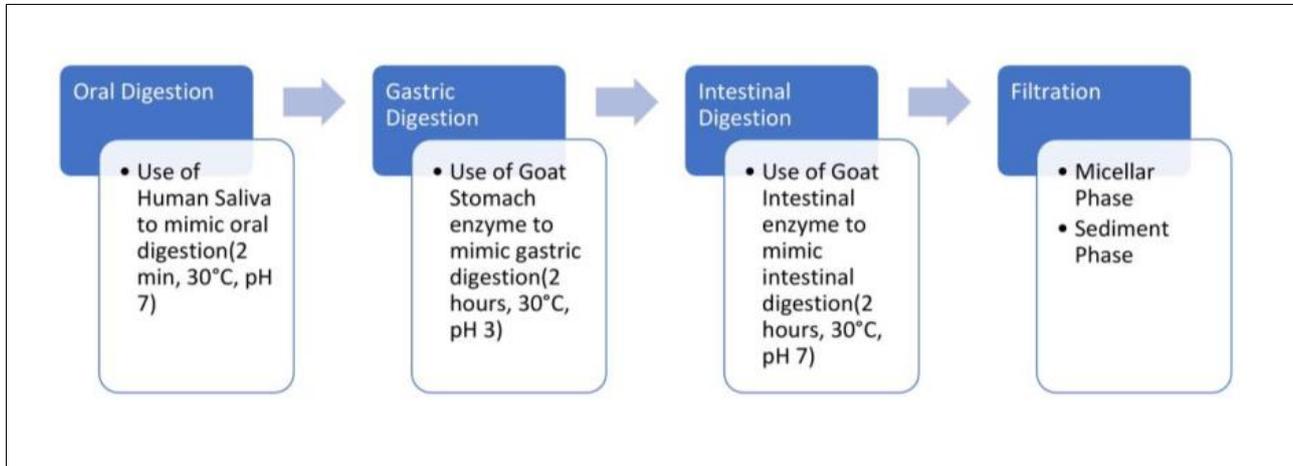
Invitro Digestibility of Amla Products

In vitro, digestibility of different amla products was performed using the modified protocol reported in the literature [10]. Briefly, for Mouth digestion: Saliva was extracted from healthy human volunteers and diluted in a 1:1 ratio and used as a medium for mouth digestion [11]. For Stomach Environment: The Stomach Peritoneal Wall of *Capra hircus* was scrapped to extract 10 ml of enzyme which was diluted to 50 ml using distilled H₂O [12]. For Intestinal Environment: The Intestinal Peritoneal Wall of *Capra Circus* was scrapped to extract 10 mL of enzyme and diluted to 50 mL for the process.

To 2 ml of samples 2 ml of freshly extracted human saliva was added and using a mortar and pestle a light mixing was done to ensure the process of chewing for 2 minutes. Then, 2 ml of the sample was kept aside for further analysis. The remaining sample was in a conical flask to which 4 ml of the gastric enzyme extract

and 10 ml of acidic buffer (pH 3) [10], were added the mixture and placed in a shaker for 2 hrs at 120 rpm at normal room temperature. After 2 hours, 2 ml of the sample was kept aside for further analysis, the remaining volume of the stomach digest product was taken and mixed with 4 ml of intestinal enzyme extract and 10 ml

of basic buffer (pH 7.4) was added and kept in a shaker of 2 hrs at 75 rpm in room temperature. The intestinal raw digestion was ultracentrifuged to 10,000 rpm at 25 °C for 10-15 min, the received supernatant and pellet were separated, and each phase was analyzed for vitamin C using redox titration protocol [9-12] (Scheme 1).



Scheme 1: A schematic flowchart of in vitro simulated digestion

Further, the bioaccessibility of digested vitamin C was calculated using the formula expressed below.

$$\text{Bioaccessibility} = \frac{C_{\text{Micelle phase}}}{C_{\text{Digesta}}} \times 100,$$

Where C micelle (Supernatant) and C digest (Pellets) are nutraceutical concentrations in the micelle phase and the intestinal raw digest [13], and the bioavailability of digested vitamin C is expressed by the following equation [13].

RESULTS AND DISCUSSION

The bioavailability of dietary vitamin C plays an important role to meet the RDA and also to overcome vitamin C-based complications. Vitamin C is consumed in various forms that include tablets and food supplements like amla powder, honey amla, etc. The major concern is about the bioavailability of vitamin C to favor biological functions. Various in vitro digestion models were proposed to evaluate the bioavailability and bioaccessibility of essential nutrients. Bioaccessibility and bioavailability of essential nutrients are mainly depending upon the architectural pattern of the food matrix that holds the biological components as well as the role of the digestive tract to release these components

in the intestine thereby facilitating the absorption of molecules and entering into the circulation. At this juncture, the present work aims to evaluate the bioavailability and bioaccessibility of vitamin C from five different amla products. The available vitamin C for the selected amla products and crushed raw amla was estimated using iodometric titration methods. The results indicated that the powder form of amla has the maximum amount of vitamin C compared to other amla products even compared with crushed raw amla. This may be attributed to the complexity of the food matrix holding vitamin C unavailable for the estimation. Moreover, there is no significant difference in the quantity of available vitamin C in amla juice and honey amla compared with amla powder which can be due to the combination of solvents that may block the release of vitamin C for quantification. These observations are also concurrent with reports available in the literature for comparing available essential nutrients in different food supplements (Figure 1) [14].

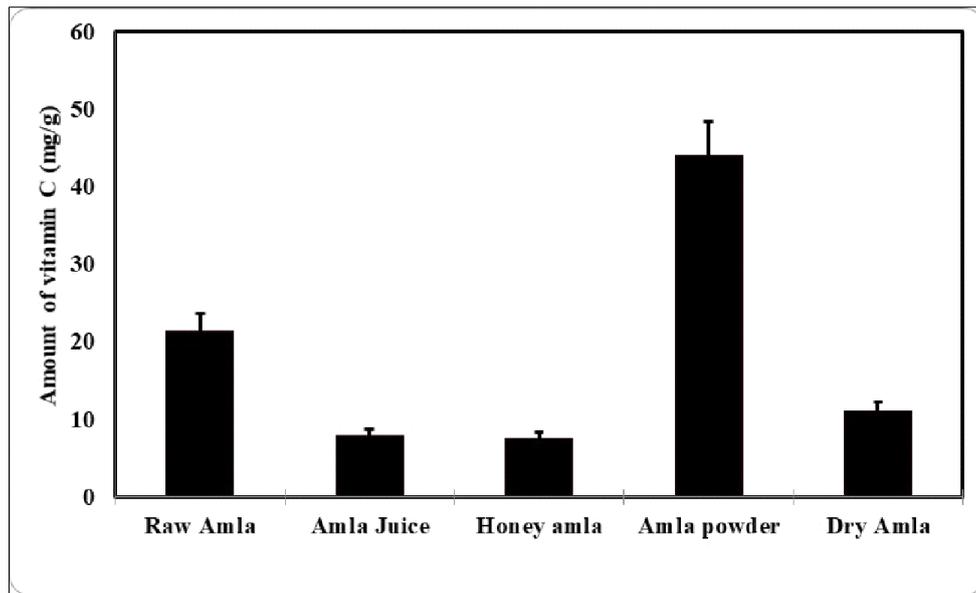


Figure 1: Amount of Vitamin C in Amla and its Products in the raw condition measured in our laboratory using titrimetric methods. Values are expressed as mean±S.D. n=5.

The bioavailability of vitamin C influences various biological activities and also regulates various metabolic reactions. In addition, vitamin C reduces oxidative stress due to its potent antioxidant activity. In this study, the *in vitro* digestibility of different commercially available amla products was carried out using a modified *In vitro* digestion model reported earlier in the literature. It indicates that the amount of vitamin C

released after mouth digestion is equal to the crushed amla (Raw Amla) and other products. This may be due to the lesser time contact in the mouth close to one or two minutes and also the salivary enzymes are not capable of degrading the food matrix extensively (2.8%) to release vitamin C from different food forms rich in ascorbic acid (Figure 2).

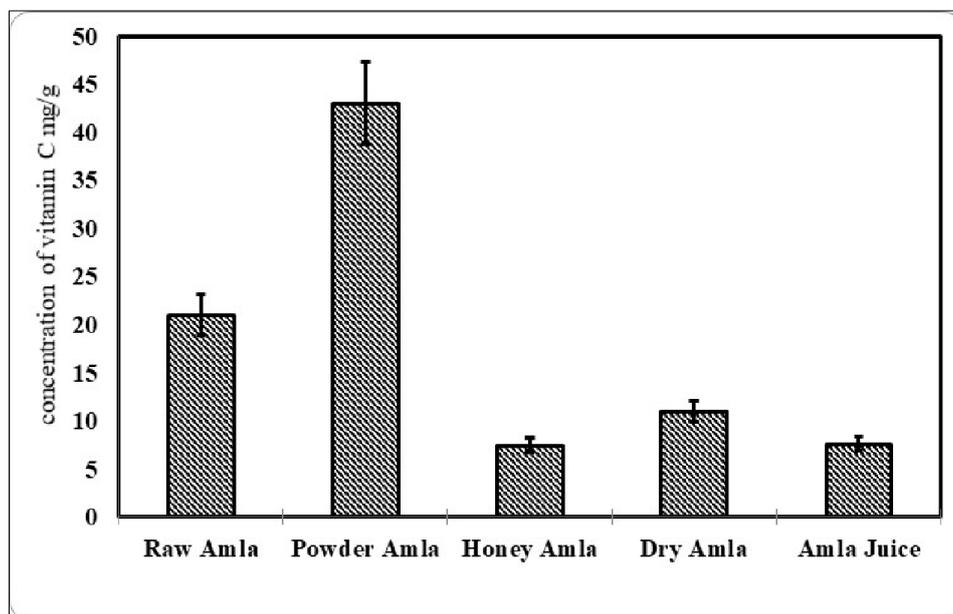


Figure 2: Release of Vitamin C in Raw Amla and its Products after mouth digestion. Values are expressed as mean±S.D. n=5.

In continuation, the digested sample from the mouth was then transferred to gastric juice and kept for 2 hours to form chyme, and further, the digested amla products were transferred to intestinal juice that contains intestinal enzymes and the pH is 8. After 2 hours, the

digested samples were separated into pellets and supernatant by filtration. The filtrate is considered as micellar phase whereas the pellet is considered as sediment phase (Figure 3) [13].

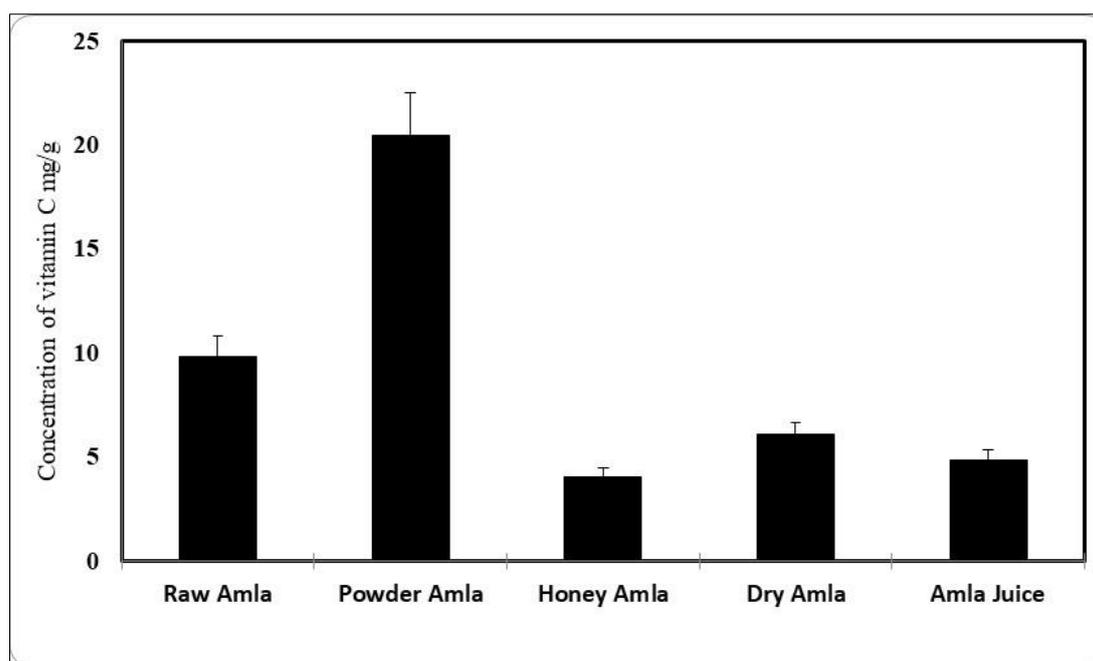


Figure 3: Release of Vitamin C in Raw Amla and its Products after intestinal digestion in the micellar phase. Values are expressed as mean± S.D. n=5.

After intestinal digestion, relatively 50 % of the Amla is in the micellar phase in that among all the products in powder amla close to 60 % in the micellar phase compared with all others. This may be attributed to the difference in the matrix that encapsulated/entrapped vitamin C thereby blocking its release into the micellar phase for better availability [15]. Further, the bioaccessibility of vitamin C was quantified using the formula mentioned in the methods. The results indicated that the bioaccessibility of vitamin C in raw amla, dry amla, powder amla, amla juice, and honey amla is 50.6 %, 57.8 %, 51.2 %, 56.8 %, and 52.3 % respectively. From that, the maximum amount of vitamin C is released and entered into circulation from dry amla compared to other amla products.

CONCLUSION

We completed the Quantification of Vitamin C in various commercial sources of Amla. We also successfully mimicked the digestion pathway by the usage of animal-extracted crude enzymes. Amla in the form of powder provided to be the highest source of vitamin C ranging from 44.03 mg/g in native states to 39.79 mg/g after intestinal digestion, whereas Honey Amla provided to be the least source of vitamin C ranging from 7.57 mg/g in native states till 7.74 mg/g after intestinal digestion, therefore for a common man who has access to all commercial amla products like Amla fruit, Amla Juice, Amla Powder, Dry Amla, Honey Amla, Etc. when he/she consumes Amla Powder or Amla fruit daily can thus meet the required dietary supplement of Vitamin C and needn't consume synthetic drugs in a longer run

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Consent for Publication: Not applicable.

Availability of Data and Materials: Not applicable.

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Conflict of Interest: The authors declare no conflicts of interest, financial or otherwise.

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REFERENCES

- Baliga, M. S., & Dsouza, J. J. (2011). Amla (*Emblca officinalis* Gaertn), a wonder berry in the treatment and prevention of cancer. *European Journal of Cancer Prevention*, 20(3), 225-239.
- Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds. Washington (DC): National Academies Press (US); 2000.
- Variya, B. C., Bakrania, A. K., & Patel, S. S. (2016). *Emblca officinalis* (Amla): A review for its phytochemistry, ethnomedicinal uses and medicinal potentials with respect to molecular mechanisms. *Pharmacological research*, 111, 180-200.

4. Tewari, R., Kumar, V., & Sharma, H. K. (2019). Physical and chemical characteristics of different cultivars of Indian gooseberry (*Emblica officinalis*). *Journal of food science and technology*, *56*, 1641-1648.
5. Ruangchakpet, A., & Sajjaanantakul, T. (2007). Effect of browning on total phenolic, flavonoid content and antioxidant activity in Indian gooseberry (*Phyllanthus emblica* Linn.). *Agriculture and Natural Resources*, *41*(5), 331-337.
6. Muzaffar, K., Sofi, S. A., Makroo, H. A., Majid, D., & Dar, B. N. (2022). Insight about the biochemical composition, postharvest processing, therapeutic potential of Indian gooseberry (amla), and its utilization in development of functional foods—A comprehensive review. *Journal of Food Biochemistry*, *46*(11), e14132.
7. Lucas-González, R., Viuda-Martos, M., Pérez-Alvarez, J. A., & Fernández-López, J. (2018). In vitro digestion models suitable for foods: Opportunities for new fields of application and challenges. *Food Research International*, *107*, 423-436.
8. Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T. O. R. S. T. E. N., Bourlieu, C., ... & Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food—an international consensus. *Food & function*, *5*(6), 1113-1124.
9. Satpathy, L., Pradhan, N., Dash, D., Baral, P. P., & Parida, S. P. (2021). Quantitative determination of vitamin C concentration of common edible food sources by redox titration using iodine solution. *Letters in Applied NanoBioScience*, *10*(3), 2361-2369.
10. Venkatasubramanian, P., Koul, I. B., Varghese, R. K., Koyyala, S., & Shivakumar, A. (2014). Amla (*Phyllanthus emblica* L.) enhances iron dialysability and uptake in in vitro models. *Current science*, 1859-1866.
11. Ariza, M. T., Forbes-Hernandez, T. Y., Giampieri, F., Gasparri, M., Soria, C., Martínez-Ferri, E., & Battino, M. (2017). Effects of in vitro gastrointestinal digestion on strawberry polyphenols stability. *Acta Horti*, *1156*, 389-396. DOI: 10.17660/Acta.Hort.2017; 1156.58.
12. Pérez-Vicente, A., Gil-Izquierdo, A., & García-Viguera, C. (2002). In vitro gastrointestinal digestion study of pomegranate juice phenolic compounds, anthocyanins, and vitamin C. *Journal of agricultural and food chemistry*, *50*(8), 2308-2312.
13. Dima, C., Assadpour, E., Dima, S., & Jafari, S. M. (2020). Bioavailability and bioaccessibility of food bioactive compounds; overview and assessment by in vitro methods. *Comprehensive Reviews in Food Science and Food Safety*, *19*, 1541-4337.
14. Rein, M. J., Renouf, M., Cruz-Hernandez, C., Actis-Goretta, L., Thakkar, S. K., & da Silva Pinto, M. (2013). Bioavailability of bioactive food compounds: A challenging journey to bioefficacy. *British journal of clinical pharmacology*, *75*(3), 588-602.
15. Winuprasith, T., Khomein, P., Mitbumrung, W., Suphantharika, M., Nitithamyong, A., & McClements, D. J. (2018). Encapsulation of vitamin D3 in pickering emulsions stabilized by nanofibrillated mangosteen cellulose: Impact on in vitro digestion and bioaccessibility. *Food hydrocolloids*, *83*, 153-164.

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