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Bioactive Compounds Bioavailability of Microencapsulated Foshou Fruit Effervescent Tablets: in Vitro Simulated Gastrointestinal

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Abstract **– This study aimed to evaluate the extractability factor and bioavailability of phenolic compounds and antioxidant activity of effervescent tablets of microencapsulated Foshou fruit extract by in vitro simulated gastrointestinal model. Total phenolic content and antioxidant capacity of the effervescent tablets were estimated in chemical extract (CE), buffer extract (BE), the extract after simulated digestion(GE), and the extract after simulated absorption(AE). The effervescent tablets of microencapsulated Foshou fruit extract by different carrier agents formulations were used in this study, the carrier agents formulations were Gum arabic/ Maltodextrin/ Modified starch/ Whey protein (GMSW); Gum arabic/ Maltodextrin/ Modified starch (GMS); Gum arabic/ Maltodextrin/ Whey protein (GMW); Gum arabic/ Modified starch/ Whey protein (GSW); Maltodextrin/ Modified starch/ Whey protein (MSW). The GE had the highest total phenolic content (15.43 mg GAE/g tablet) and antioxidant activity (IC_{50} 7.40 mg tablet). The GMS tablets showed the highest total phenolic content and antioxidant activity among all extracts. GMS tablets showed the highest extractability factor for phenolic compounds in BE (1.81), GE (2.82), and AE (2.43), moreover in terms of extractability factor for antioxidant activity in BE (1.28), GE (2.44), and AE (1.69). The highest bioavailability index was in MSW tablets (0.90) for phenolic compounds, and in GSW tablets (0.73) for antioxidant activity. The results of this study indicate the possibility of using effervescent tablets as a method of improving the bioavailability of phenolic compounds and antioxidants in microencapsulated Foshou fruit extract.**

Keywords **– Bioavailability, Extractability Factor, Simulated Gastrointestinal, Foshou Fruit, Effervescent Tablets, Microencapsulation.**

I. INTRODUCTION

The food holding components with supposed beneficial effects on human health has become a subject of growing interest throughout the last decades. An epidemiological confirmation has extensively linked phenolic-rich foods with the prevention of many chronic pathologies, including diabetes, cardiovascular diseases, and neurodegenerative diseases, in addition to some cancer types, in this context, consumer demand for foods that exert beneficial effects on human health, drives to the proffer of newfangled products wealthy in phenolic compounds (Castello et al., 2018; Chen, Gnanaraj, Arulselvan, El-Seedi, & Teng, 2019). The useful effects of phenolic compounds can be really effective only if they reach the relevant tissues and function in sufficient concentration to generate a biological effect (Barros & Junior, 2019). Improvement of bioavailability of phenolic compounds is critical to increase their therapeutic possibility against chronic ailments at targeted tissues (Spigoni et al., 2017).

The bioavailability of bioactive compounds after human digestion decides their biological action in the body.

In vitro gastrointestinal digestion is being used to make the first screening of bioactive compounds behavior after human digestion. The in vitro gastrointestinal digestion simulates the physical processes (Temperature, Agitation, and pH) and chemical processes (Salinity and Enzymatic) that take place during gastrointestinal digestion and give information about the changes that happen in bioactive compounds (Lucas-Gonzalez, Viuda-Martos, Alvarez, & Fernandez-Lopez, 2018).

The digestion and absorption of biologically active compounds and their delivery systems are often assessed through laboratory simulated gastrointestinal models, which provide an alternative without the need for human or animal experiments. Laboratory gastrointestinal models have been widely applied in research on functional foods and drugs due to their time-saving ability and low cost along with high replication potential (Bouayed, Hoffmann, & Bohn, 2011). Simulated digestion methods typically include oral, gastric and small intestinal phases. The simulated digestion methods try to simulate physiological conditions in vivo, taking into account the digestion time, presence of digestive enzymes and their concentrations, pH, and salt concentrations (Minekus et al., 2014).

Encapsulation technology is being broadly considered recently as a potential technique to increase the bioavailability and absorption of phenolic compounds (Martins, Poncelet, Rodrigues, & Renard, 2017). Microencapsulation of phenolic compounds to increase their bio-availabilities for therapeutic potentials is commonly accomplished using several chosen methods like spray-drying, where it is the most available method for pharmaceutical and food industries since phenolic compounds and their bioactivity can easily be protected through this method (Calderon-Oliver, Pedroza-Islas, Escalona-Buendia, Pedraza-Chaverri, & Ponce-Alquicira, 2017; Kalusevic et al., 2017). Based on our previous study Mahdi et al. (2019) we found that the aqueous extract of Foshou fruit has a good content of phenolic compounds with high antioxidant activity. Depending on that, we did a study about microencapsulation of the Foshou fruit extract with different encapsulation carrier agents by spray-drying (not published yet), and the production of effervescent tablets from the Foshou fruit extract microcapsules as an oral dose (not published yet).

Under the light of these studies, this study was prepared to investigate the bioavailability of phenolic compounds and the antioxidant activity of effervescent tablets of the Foshou fruit extract. An in vitro digestion method simulating the mouth, stomach and small Intestine was employed for this aim. Furthermore, a dialysis step for simulation of absorption was included in the model.

II. Materials and Methods

2.1. *Material and Chemicals*

The encapsulation carrier agents were food grade, all chemicals and solvents used were of analytical grade and stored in ideal conditions according to the manufacturers specifications. Dialysis bags (molecular weight cut-off of 10,000 Da, a flat width of 25 mm) and clips with a width of 4 mm were obtained from Viskase Co. (Lombard, Illinois, USA).

2.2. *Foshou Fruit Extract Microcapsules Mixtures*

Four carrier agents were mixed by different ratio to make 5 formulations for microencapsulation the Foshou fruit extract. The carrier agents formulations were GMSW (25% Gum arabic, 25% Maltodextrin, 25% Modified starch, 25% Whey protein); GMS (33.33% Gum arabic, 33.33% Maltodextrin, 33.33% Modified starch); GMW

(33.33% Gum arabic, 33.33% Maltodextrin, 33.33% Whey protein); GSW (33.33% Gum arabic, 33.33% Modified starch, 33.33% Whey protein); MSW (33.33% Maltodextrin, 33.33% Modified starch, 33.33% Whey protein).

2.3. *Formulation the Tablets*

Each tablet contains Citric Acid (20%), Sodium bicarbonate (30%), Stevia (10%) and Foshou fruit extract microcapsules mixtures (40%).

2.4. *Methods*

2.4.1. *In Vitro Digestion and Absorption*

2.4.1.1. *Preparation the Simulated Digestive Juices*

Table 1 shows the simulated digestive fluids composition according to the method described by Flores, Singh, Kerr, Pegg, and Kong (2014). The simulated digestive fluids were prepared on the day of the experiment and the pH was adjusted with HCl (1 M) or NaOH (1 M).

Table 1. The simulated formula of the saliva, gastric, duodenal, and bile fluids.

	Artificial saliva	Gastric juice	Duodenal juice	Bile juice
Distilled water	500 ml	500 ml	500 ml	500 ml
NaCl	58.5 mg	2.752 g	7.012 g	5.259 g
KCl	74.5 mg	0.824 g	0.564 g	0.376 g
Urea	0.2 g	0.085 g	0.1 g	0.25 g
$NaHCO_3$	1.05 g	-	3.388 g	5.785 g
Concentrated HCl	-	6.5 ml	0.180 ml	0.150 ml
NaH_2PO_4	-	0.266 g	-	-
$CaCl_2.2H_2O$	-	0.399 g	-	-
NH_4Cl	-	0.306 g	-	-
KH_2PO_4	-	-	80.0 mg	-
$MgCl_2$	-	-	50.0 mg	-
Mucin	0.5 g	3.0 g	-	-
α-Amylase	1.0 g	-	-	-
Pepsin	-	2.5 g	-	-
Pancreatin	-	-	9.0 g	-
Lipase	-	-	1.5 g	-
Bile salts	-	-	-	30 g
pH	6.8 ± 0.2	1.30 ± 0.02	8.1 ± 0.2	8.2 ± 0.2

2.4.1.2. *Simulation the Digestion*

In order to simulate the digestion in mouth, stomach, and intestines the method described by Kuck, Wesolowski, and Noreña (2017) was followed with minor modification. In the beginning, 1 g tablet was dissolved

in 4 mL distilled water into Erlenmeyer flasks (125 mL) and incubated in shaking incubator for 10 min at 37 °C. To simulate the digestion in mouth, 6 ml of artificial saliva was added, and then the mixture was shaken (150 rpm) for 5 min at 37 °C (pH 6.8). For simulating the digestion in the stomach, 12 ml of gastric juice was added, and then the mixture was shaken (150 rpm) for 120 min at 37 °C (pH 2-3). Finally, for simulating the digestion in the small intestine, 12 ml of duodenal juice and 6 ml of bile juice were added, and the mixture was shaken (150 rpm) for 120 min at 37 °C (pH 6.5-7). The thermal inactivation of the enzymes at 70 °C for 10 min was used for stopping the enzymatic hydrolysis. At the end of the in vitro digestion process, the mixtures were centrifuged for 5 min at 5000 rpm.

2.4.1.3. *Simulation of the Absorption*

The dialysis method was used for simulation the absorption after simulated digestion according to (Bouayed et al., 2011; Silva et al., 2018) with some modification. The dialysis bag was cut to a length of 14 cm, washed (outer and inner surface) with 0.9% NaCl solution and then sealed with clip from one side. About 10 mL of sample was added into the dialysis bag (bubble-free) then sealed with clip and weighed. The sealed dialysis bag was placed into Erlenmeyer flasks (125 mL) with 50 mL of PBS buffer (Phosphate buffered saline, pH 7.4) the flasks were placed in the shaking incubator (150 rpm) for 120 min at 37 °C. The PBS buffer was prepared by dissolving 8 g NaCl, 200 mg KCl, 1.44 g $Na2HPO_4$, and 240 mg $KH2PO_4$ in 1000 mL deionized water. The PBS buffer with the compounds that passed through the dialysis bag was used to estimate the bioavailability of microencapsulated bioactive compounds in terms of its total phenolic compound, and antioxidant activity.

2.4.2. *Chemical Extracts (CE)*

Chemical extracts were obtained by dissolving 1 g tablet in 40 mL of methanol 80% into Erlenmeyer flasks (125 mL) and then shaken in shaking incubator (150 rpm) for 4 h at 37 °C.

2.4.3. *Buffer Extracts (BE)*

Buffer extracts were obtained by dissolving 1 g tablet by 40 mL of PBS buffer (phosphate-buffered saline, pH 7.4) into Erlenmeyer flasks (125 mL) and then shaken in shaking incubator (150 rpm) for 4 h at 37 °C.

2.4.4. *Determination of Total Phenolic Compound (TPC)*

The TPC contents was determined using the Folin–Ciocalteu assay according to Mohammed et al. (2019). Briefly, 100 µL of the dissolved tablets solution was added to 5 mL (0.2 N) of Folin phenol reagent. After 5 min, 4 mL of 7.5% sodium carbonate solution was added. After incubation at 25°C in the dark for 105 min, the optical density at 765 nm was measured using a UV spectrophotometer. The results were expressed as mg of gallic acid equivalents per gram tablet (mg GAE/g tablet).

2.4.5. *Determination of Antioxidant Activity by DPPH$^{\bullet}$ - SA Assay*

The scavenging activity against DPPH$^{\bullet}$ was estimated according to AL-Ansi et al. (2019). Briefly, DPPH$^{\bullet}$ solution (3.5 mL) was added to 100 µL of the samples. After incubation for 40 min in the dark at 25 °C, absorbance was recorded at 517 nm. The IC_{50} was expressed as mg tablet.

$$\text{DPPH}^{\bullet}\text{-SA} = \frac{\text{Absorbance of the control} - \text{Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

2.4.6. *Extractability Factor*

Extractability factor was determined to investigate the efficiency of extraction in different extracts according to Gawlik-Dziki et al. (2015):

2.4.6.1. *Extractability Factor for Phenolic Compounds*

$$\text{Extractability factor of buffer extract} = \frac{\text{Phenolics content in buffer extract}}{\text{Phenolics content in chemical extract}}$$

$$\text{Extractability factor of simulated digestion} = \frac{\text{Phenolics content after simulated digestion}}{\text{Phenolics content in chemical extract}}$$

$$\text{Extractability factor of simulated absorption} = \frac{\text{Phenolics content after simulated absorption}}{\text{Phenolics content in chemical extract}}$$

2.4.6.2. *Extractability Factor for Antioxidant Activity*

$$\text{Extractability factor of buffer extract} = \frac{\text{Antioxidant activity in chemical extract}}{\text{Antioxidant activity in buffer extract}}$$

$$\text{Extractability factor of simulated digestion} = \frac{\text{Antioxidant activity in chemical extract}}{\text{Antioxidant activity after simulated digestion}}$$

$$\text{Extractability factor of simulated absorption} = \frac{\text{Antioxidant activity in chemical extract}}{\text{Antioxidant activity after simulated absorption}}$$

2.4.7. *Bioavailability Index*

Bioavailability index was determined to investigate the bioavailability of phenolic compounds and antioxidant according to Swieca, Gawlik-Dziki, Dziki, and Baraniak (2017):

$$\text{Phenolic compounds bioavailability index} = \frac{\text{Phenolics content after simulated absorption}}{\text{Phenolics content after simulated digestion}}$$

$$\text{Antioxidant bioavailability index} = \frac{\text{Antioxidant activity after simulated absorption}}{\text{Antioxidant activity after simulated digestion}}$$

2.5. *Statistical Analysis*

Total phenolic contents and antioxidant activity by DPPH•‑SA assay were carried out in triplicate, and the results were reported as mean ± standard deviation. SPSS software was used for the statistical analysis. Duncan's multiple range test ($p \leq 0.05$) was used to determine the significant differences between the samples.

III. Results and Discussion

3.1. *Total Phenolic Compound (TPC)*

Figure 1 shows the total phenolic content in the chemical extract, buffer extract, the extract after simulated digestion, and the extract after simulated absorption. The total phenolic content in the chemical extract ranged from 2.24 to 5.47 mg GAE/g tablet, in the buffer extract ranged from 3.76 to 9.90 mg GAE/g tablet, in the extract after simulated digestion ranged from 5.69 to 15.43 mg GAE/g tablet, in the extract after simulated absorption ranged from 5.13 to 13.29 mg GAE/g tablet. The extracts after simulated digestion were superior to other extracts

followed by the extract after simulated absorption, buffer extract, and chemical extract respectively in all tablets formulations. The GMS tablets showed the highest phenolic content among all formulations. There were no significant differences ($p \leq 0.05$) between GSM and MSW formulations.

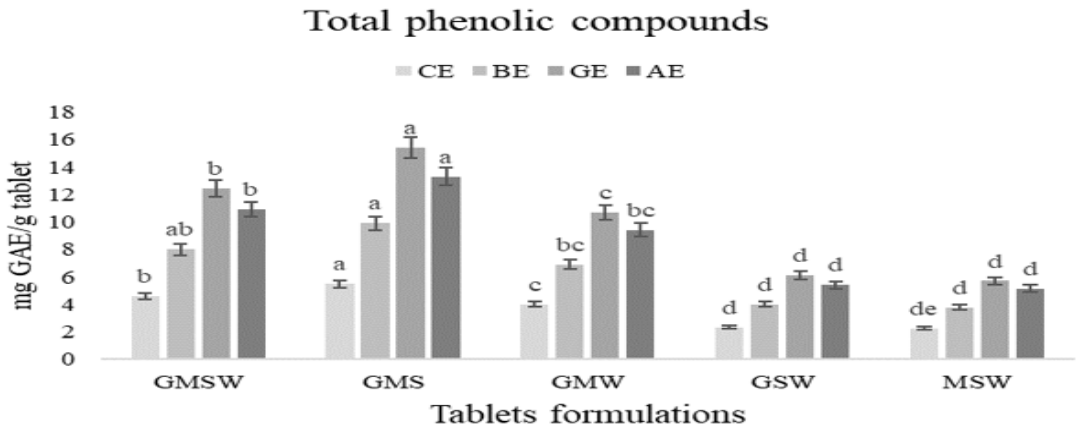

Fig. 1. Total phenolic content of the tablets formulations in the chemical extract (CE), buffer extract (BE), the extract after simulated digestion (GE), and the extract after simulated absorption (AE).

3.2. *Antioxidant Activity by DPPH•-SA Assay*

The antioxidant activity of the different extracts is shown in figure 2. The results showed that the extracts after simulated digestion had the highest antioxidant activity. The GMS tablets showed the highest antioxidant activity compared with the other formulations. The IC_{50} in the chemical extract where ranged from 18.06 to 24.23 mg tablet, in the buffer extract the IC_{50} ranged from 14.09 to 21.08 mg tablet, in the extract after simulated digestion the IC_{50} ranged from 7.40 to 11.39 mg tablet, and in the extract after simulated absorption the IC_{50} ranged from 10.66 to 17.20 mg tablet.

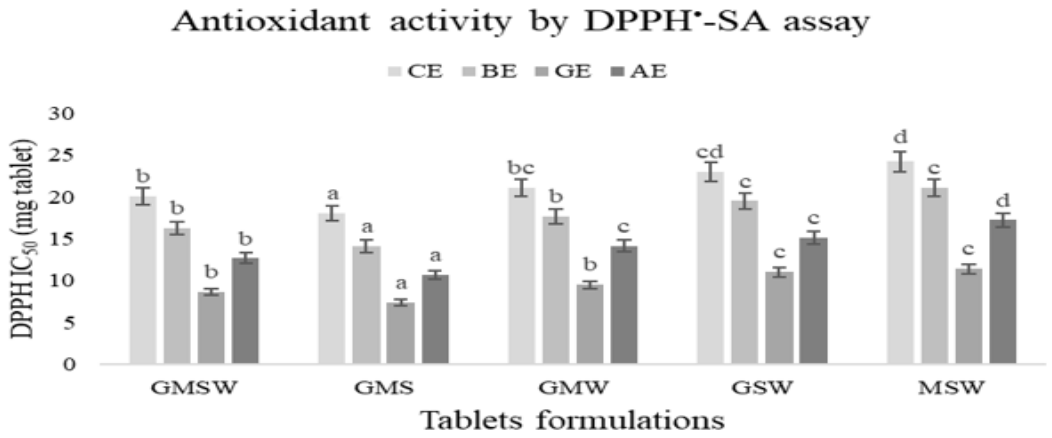

Fig. 2. Antioxidant activity by DPPH•-SA assay of the tablets formulations in the chemical extract (CE), buffer extract (BE), the extract after simulated digestion (GE), and the extract after simulated absorption (AE).

3.3. *Extractability Factor for Phenolic Compounds*

Extractability factor for phenolic compounds in buffer extract, the extract after simulated digestion, and the extract after simulated absorption are shown in figure 3. Extractability factor for phenolic compounds in buffer extract ranged from 1.68 to 1.81, in the extract after simulated digestion ranged from 2.54 to 2.82, and the extract after simulated absorption ranged from 2.29 to 2.43. The phenolic compounds extractability factor in GMS tablet was the highest among the other tablets formulations followed by GMSW, GMW, GSW, and MSW consecutively.

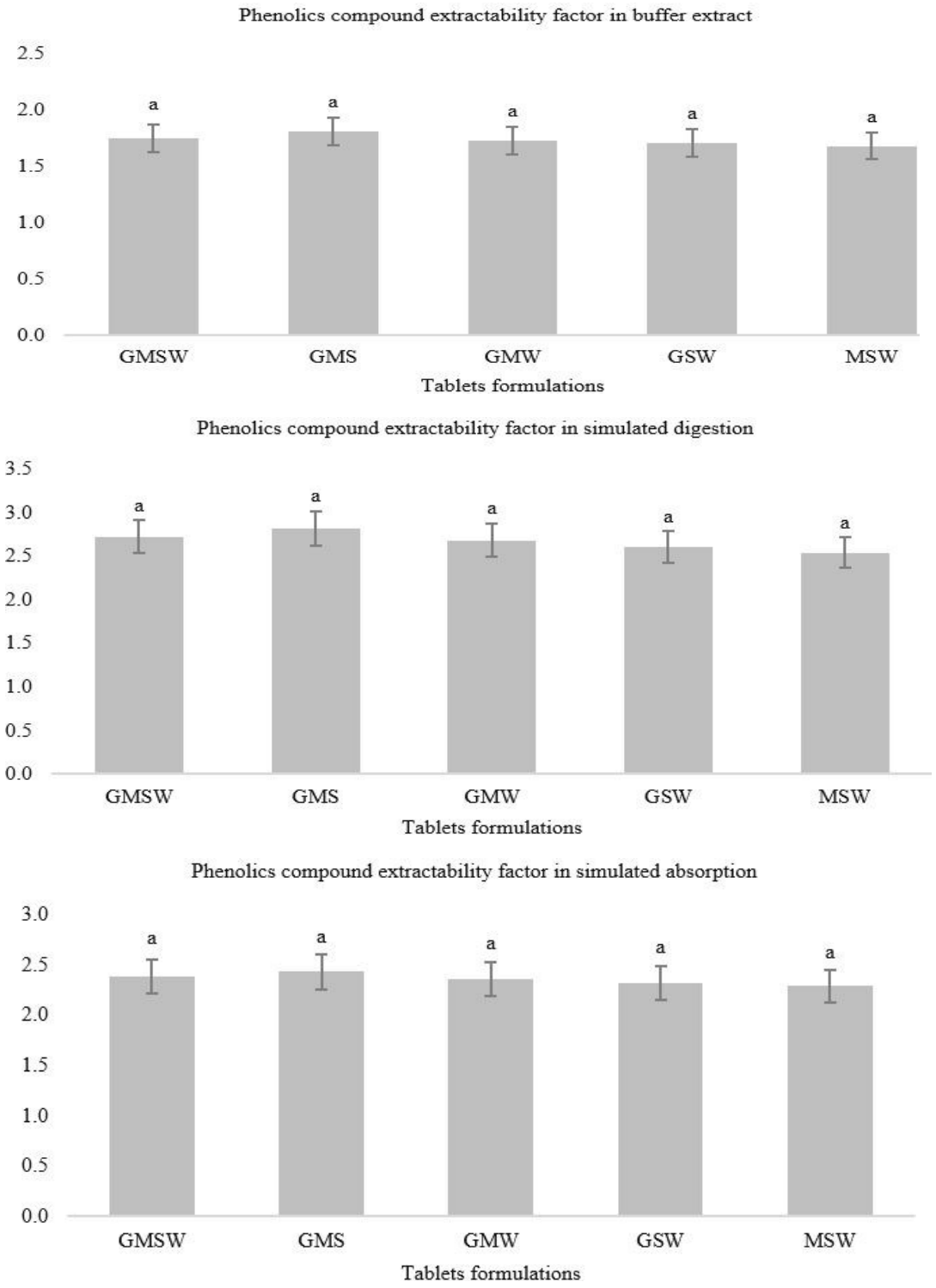

Fig. 3. Extractability factor for phenolic compounds the different tablets formulations, Gum arabic/ Maltodextrin/ Modified starch/ Whey protein (GMSW); Gum arabic/ Maltodextrin/ Modified starch (GMS); Gum arabic/ Maltodextrin/ Whey protein (GMW); Gum arabic/ Modified starch/ Whey protein (GSW); Maltodextrin/ Modified starch/ Whey protein (MSW).

3.4. *Extractability Factor for Antioxidant Activity*

Figure 4 shows the extractability factor for antioxidant activity in buffer extract, the extract after simulated digestion, and the extract after simulated absorption. The antioxidant activity extractability factor in GMS tablet was the highest among the other tablets formulations. In buffer extract, the extractability factor ranged from 1.15 to 1.28, the GMS tablet followed by GMSW, GMW, GSW and MSW respectively. On the other hand, the

extractability factor of the antioxidant activity in GMS tablet was the highest followed by GMSW, GMW, MSW and GSW respectively in the extract after simulated digestion and ranged from 2.08 to 2.44. Further, in the extract after simulated absorption ranged from 1.41 to 1.69, GMS tablet was the highest followed by GMSW, GSW, GMW and MSW.

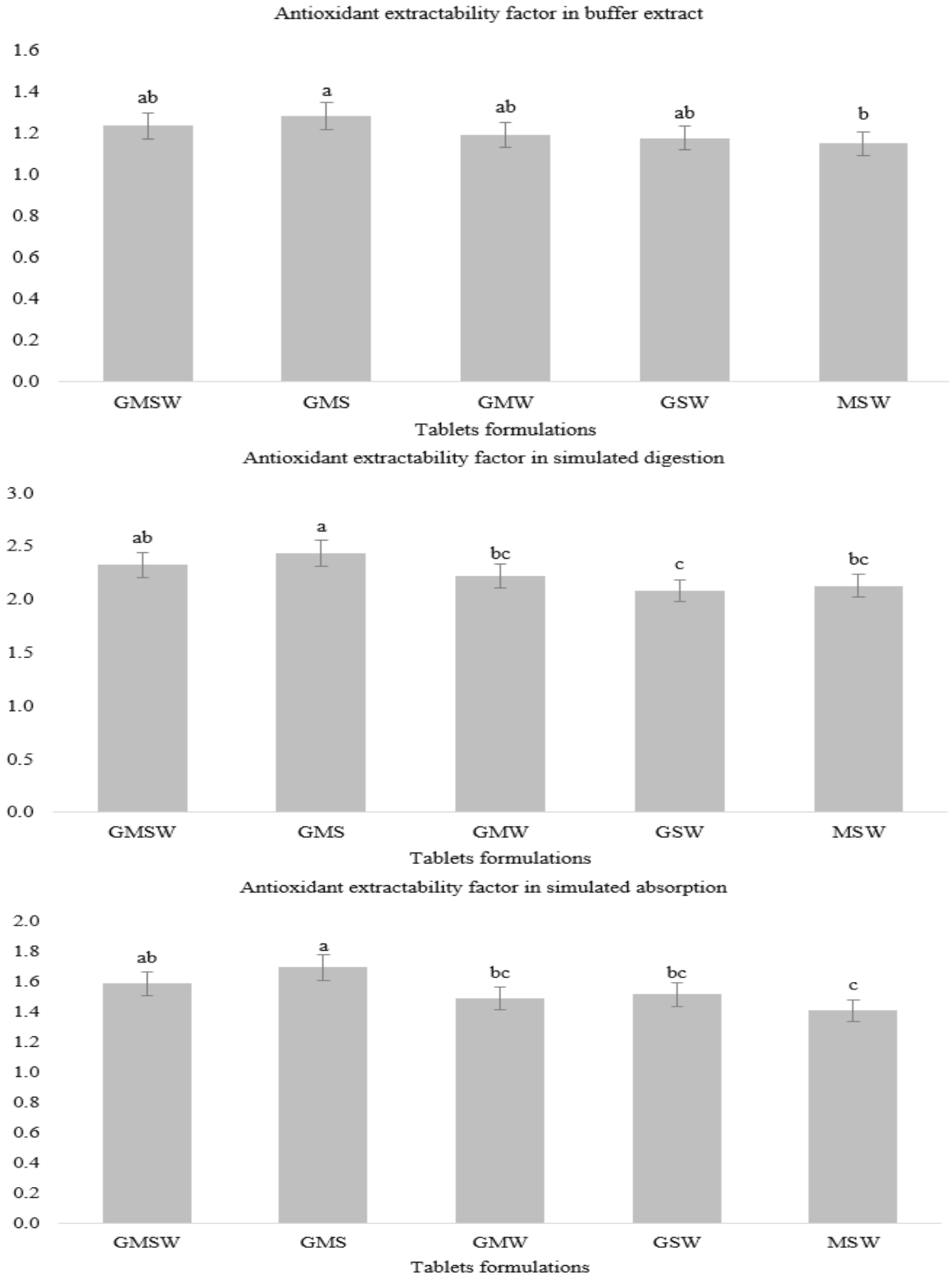

Fig. 4. Extractability factor for antioxidant activity the different tablets formulations, Gum arabic/ Maltodextrin/ Modified starch/ Whey protein (GMSW); Gum arabic/ Maltodextrin/ Modified starch (GMS); Gum arabic/ Maltodextrin/ Whey protein (GMW); Gum arabic/ Modified starch/ Whey protein (GSW); Maltodextrin/ Modified starch/ Whey protein (MSW).

3.5. *Bioavailability Index the Phenolic Compounds*

Bioavailability is defined as a part of a particular compound or its metabolite that reaches the systemic circulation without studying biological activity because the measurement of biological activity is subject to ethical and practical limitations (Ariza et al., 2018).

Bioavailability index of the phenolic compounds of the different tablets formulations is shown in figure 5. Bioavailability index ranged from 0.86 to 0.90 in terms of the phenolic compounds content in the tablets formulations. The MSW tablet showed the highest bioavailability index followed by GSW, GMW, GMSW and GMS respectively.

Bioavailability of phenolic compounds depends on several factors such as food matrix composition, chemical characteristics, pH, the interaction of phenolic compounds with proteins, carbohydrates and lipids, etc. (Karakaya, 2004).

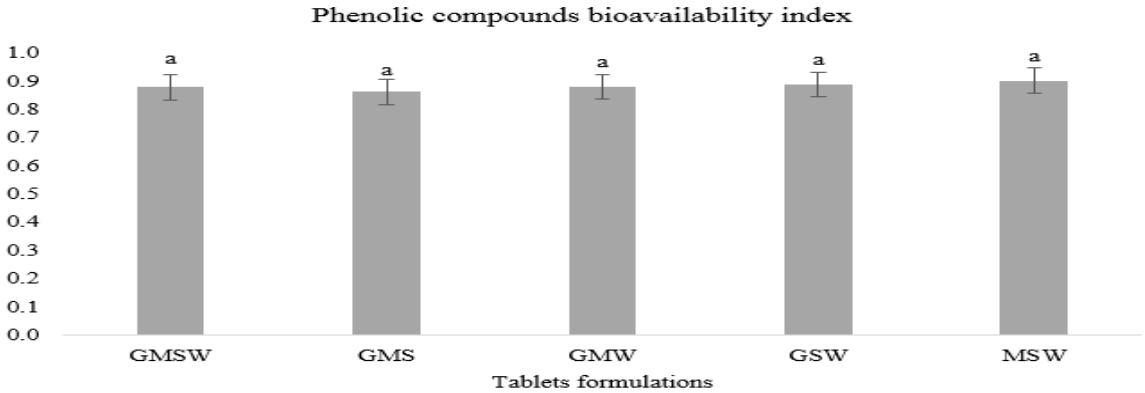

Fig. 5. Bioavailability index the phenolic compounds in the different tablets formulations, Gum arabic/ Maltodextrin/ Modified starch/ Whey protein (GMSW); Gum arabic/ Maltodextrin/ Modified starch (GMS); Gum arabic/ Maltodextrin/ Whey protein (GMW); Gum arabic/ Modified starch/ Whey protein (GSW); Maltodextrin/ Modified starch/ Whey protein (MSW).

3.6. *Bioavailability Index the Antioxidant Activity*

Figure 6 shows the antioxidant activity bioavailability index of different tablets formulations. Antioxidant activity bioavailability index in the tablets formulations ranged from 0.66 to 0.73. The GSW tablet showed the highest bioavailability index in terms of antioxidant activity followed by GMS, GMSW, GMW and MSW respectively.

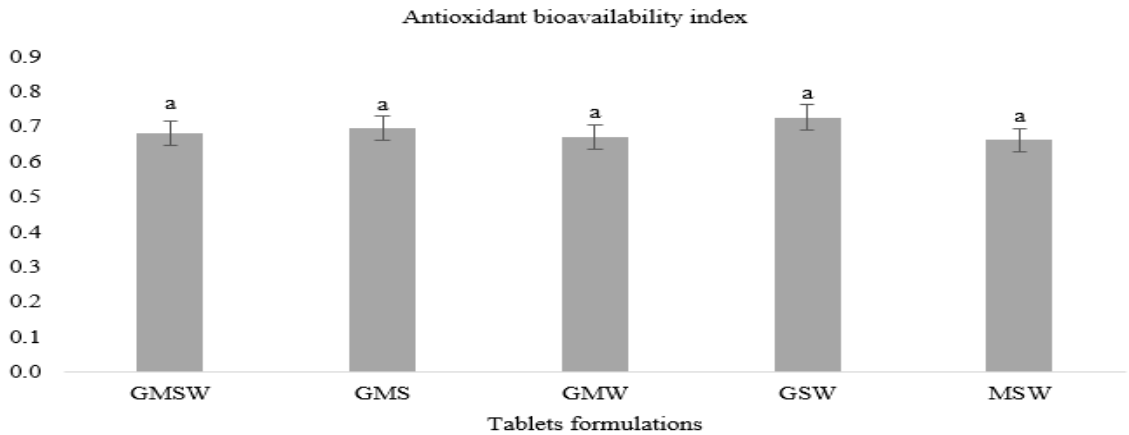

Fig. 6. Bioavailability index the antioxidant activity in the different tablets formulations, Gum arabic/ Maltodextrin/ Modified starch/ Whey protein (GMSW); Gum arabic/ Maltodextrin/ Modified starch (GMS); Gum arabic/ Maltodextrin/ Whey protein (GMW); Gum arabic/ Modified starch/ Whey protein (GSW); Maltodextrin/ Modified starch/ Whey protein (MSW).

IV. CONCLUSIONS

The results of the conducted research suggest that the encapsulation carrier agents have no significant effect on extractability factor / bioavailability index for phenolic compounds and bioavailability index for antioxidant activity in the microencapsulated Foshou fruit extract. In addition to the possibility of using effervescent tablets for oral delivery of microencapsulated Foshou fruit extract for improving the bioavailability of the phenolic compounds of the microencapsulated Foshou fruit extract.

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