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Fortifying compounds reduce starch hydrolysis of potato chips during gastro-small intestinal digestion in vitro

*Yudy Duarte-Correa, Oscar Vega-Castro, Nataly López-Barón, and Jaspreet Singh**

Potato chips are the most widely consumed snack in the world; therefore, they can be used as an ideal carrier for nutrient delivery. The objective of this study is to investigate the effect of impregnating vitamin E, vitamin C, and calcium through vacuum impregnation (VI) on the physico-chemical, microstructural, and starch hydrolysis (%) in vitro and estimated glycemic index (*eGI*) of potato chips from a variety grown in Colombia. The fortification process decreased the peak viscosity from 1428 ± 20 cP (unfortified) to 734 ± 27 cP (fortified) and increased pasting temperatures. The swelling power also decreased from 11.36 ± 0.32 g/g (unfortified) to 8.59±0.07 g/g (fortified) after the fortification. Approximately 42% and 63% of the starch is hydrolyzed in the first 10 min of small intestinal digestion for the fortified and control samples, respectively. At the end of in vitro digestion (120 min), fortified potato has 74% starch hydrolyzed, whereas the same is calculated at 95% for control samples. The microstructural characteristics of digesta obtained during in vitro digestion showed that fortified samples have well preserved cell structures. In conclusion, the addition of impregnation compounds led to a lower starch hydrolysis of potato chips with a consequent decrease in *eGI*.

1. Introduction

Potato is a promising food for the development of innovative food products with health benefits, due to its high consumption worldwide. Potatoes are nutrient-dense source of food and therefore able to provide a good proportion of daily recommended allowance of essential nutrients such as potassium, vitamin C, vitamin B6, and minerals like magnesium and iron.[1] Despite being rich in several nutrients, potatoes contain lower amounts of vitamin E and calcium, which are considered essential for several body functions. Vacuum impregnation (VI) has emerged as a non-invasive technique to incorporate physiologically active nutrients into the porous matrix of fruits and vegetables without destroying their cellular structure.[2–5] Some compounds have been previously incorporated into potato matrix using VI technique: ascorbic acid;[6] iron;[7] calcium, and zinc;[5,7,8] phenolic compounds from red root beet;[9] essential rosemary oil,[10] and vitamins and minerals.[11]

Starch is the major carbohydrate reserve in potatoes amounting approximately to 15–20% of their fresh weight.[12] Raw or uncooked potato starch is resistant towards enzymatic digestion,[13] however thermal processing dramatically increases its susceptibility to hydrolysis by amylolytic enzymes during digestion.[14] In vitro starch digestion is a technique used to study enzymatic hydrolysis of starch in terms of rate of glucose release under simulated gastro-small intestinal environments.[12] Predicting and controlling glucose absorption after consumption of starchy foods is important worldwide due to global health concerns, like diabetes and cardiovascular diseases.[15] The glycemic index (GI) is defined as the postprandial incremental glycemic area after a test meal, expressed as the percentage of the corresponding area after an equi-carbohydrate portion of glucose or white bread (reference food).[16] Estimated glycemic index (*eGI*) is a predictive method to measure the GI of a food product, which is calculated from the in vitro hydrolysis index (HI) pattern. Potatoes generally have higher starch digestibility and GI values compared to other foods,[1] which implies that its carbohydrates are presumed to be rapidly digested and absorbed, hence causing elevated glycemic responses. Evidence suggests that slow digestion and absorption of carbohydrates is desirable not only in individuals with diabetes but also in healthy individuals. Slow carbohydrate digestion has been linked to improved metabolic control, reduced blood lipid levels, improved glucose tolerance, protection against cardiovascular disease, prolonged satiety and reduced cariogenic potential.[17] Since potatoes are consumed worldwide, the development of potato food products formulated with bioactive ingredients and low glycemic features, will contribute to a growing demand coming from consumers looking for healthier food choices.[1]

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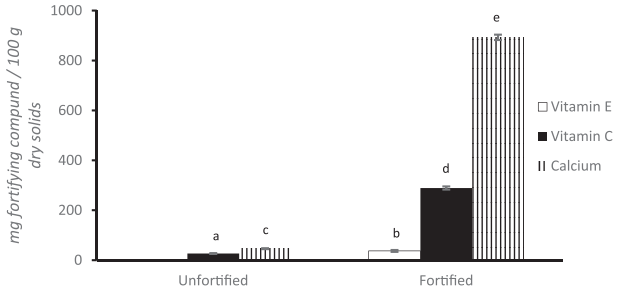

Figure 1. Content of vitamin E, vitamin C and calcium in fortified and unfortified potato chips.

The VI studies mentioned above have reported some characteristics of the fortified potatoes, but no reports are available about how this fortification affects their starch digestion behavior after consumption. The objective of this study was to fortify potato with vitamin C, vitamin E, and calcium using VI and to study the influence of this fortification on the rate of starch hydrolysis during gastro-small intestinal digestion and eGI of potato chips using an in vitro digestion model.

2. Results and Discussion

2.1. Determination of Vitamin C, Vitamin E, and Calcium

The contents of vitamin E, vitamin C, and calcium, in fortified and unfortified samples are shown in **Figure 1**. Vitamin E was not detected in unfortified samples. Similarly, vitamin E was no detected in the raw potato for the same variety.[19] The content of vitamin E in other cultivars of raw potato has been reported to range between 0.01 and 0.28 mg VitE/100 g fresh potato.[1,13,29] The content of vitamin C and calcium in unfortified potato chips was 26.43 ± 0.45 mg and 45.91 ± 1.78 mg 100 g^{-1} dry solids (ds), respectively. The value of vitamin C obtained was higher than that described by Burgos, Auqui, Amoros, Salas, and Bonierbale, (2009)[30] for microwaved Andean potato varieties (6.6–18.4 mg VitC/100 g (ds)), while calcium was within the range as reported by C. Andre et al. (2007)[31] for some Andean cultivars (27.10 -109.29 mg Ca/100 g (ds)). In contrast, fortified potato chips had 36.97 ± 3.73 mg VitE/100 g (ds), 289.10 ± 6.34 mg VitC/100 g (ds), and 892.90 ± 10.95 mg Ca/100 g (ds). In order to achieve high impregnation levels in potato tissue, it is necessary to carry out the impregnation process under vacuum rather than atmospheric conditions. Hironaka et al., (2011) used red ink to evaluate the impregnation levels in potato and concluded that no impregnation occurred at atmospheric pressure for 3–24 h of immersion.[6] Under atmospheric pressure, the plant cellular structure acts as a semi-permeable membrane, and the physiologically active compounds (PACs) are transferred from the concentrated solution to the cell by a diffusion driven process. In contrast, when a porous tissue is immersed in a PAC concentrated solution under vacuum conditions, air is extracted from the pores. When the atmospheric pressure is restored, the impregnation solution penetrates the intercellular spaces by capillary action and by the pressure gradients through hydrodynamic mechanism.[6] It has been reported that atmospheric impregnation is not sufficient to incorporate fortifying compounds into the potato tissue due to its thick periderm and densely packed cells, which are less permeable to water and gas.[6,4]

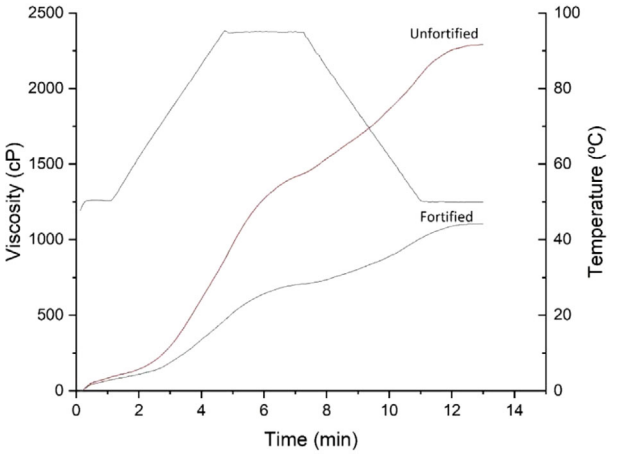

Figure 2. RVA pasting profiles of fortified and unfortified potato chips ($n = 3$).

The results show that the VI along with VMD were effective methods to increase the concentration of vitamin E, vitamin C, and calcium in potatoes. However, additional analyses to study their release and absorption in the gastrointestinal tract would be necessary to evaluate their bioaccessibility and bioavailability. Bioaccessibility has been defined as the quantity or fraction of nutrients released from the food matrix during digestion in the gastro-intestinal tract which later becomes available for absorption.[32] Therefore, it includes digestive transformations of food into nutrients ready for assimilation, the absorption/assimilation into intestinal epithelium cells, and finally to the pre-systemic metabolism (both intestinal and hepatic).[33] Bioavailability also includes the utilization of nutrients and therefore can be defined as the fraction of ingested nutrients that reach the systemic circulation and are utilized.[34] Overall, bioavailability includes gastro-intestinal digestion, absorption, metabolism, tissue distribution, and bioactivity.[33]

2.2. Pasting Properties

Pasting properties of fortified and unfortified samples are shown in **Figure 2** and **Table 1**. Powdered samples from unfortified chips showed significantly higher paste viscosities [peak (PV), trough (TV), final (FV), breakdown (BD), and setback (SB)] and also had significantly lower pasting temperatures (PT). Both samples showed absence of peak at maximum viscosity during heating, typical for native potato starches.[1,35]

The viscosity of starch products depends on the processing due to the changes in the starch structure, such as amylose leaching and the formation of a packed array of melted amylose and amylopectin molecules.[22] Fortification significantly decreased the PV of the samples from 1428 ± 20 (unfortified) to 734 ± 27 cP (fortified) ($n = 3$; $p < 0.05$). Since the only difference between fortified and unfortified samples is the addition of vitamin E, vitamin C, and calcium, it can be assumed that the lower viscosity developed by the fortified samples may be resulted due to the presence of these compounds, which may act as a physical barrier

Table 1. RVA pasting properties of fortified and unfortified potato chips. Different letters ("a" and "b") in the same column indicate significant differences ($p < 0.05$) ($n = 3$).

Potato chip	Peak viscosity (PV) [cP]	Trough viscosity (TV) [cP]	Breakdown viscosity (BD) [cP]	Final viscosity (FV) [cP]	Setback viscosity (SB) [cP]	Pasting temperature (PT) [°C]
Unfortified	1428 ± 20*a*	1318 ± 21*a*	111 ± 7*a*	2321 ± 39*a*	1004 ± 18*a*	74 ± 1*a*
Fortified	734 ± 27*b*	690 ± 31*b*	43 ± 8*b*	1138 ± 30*b*	448 ± 3*b*	80 ± 5*b*

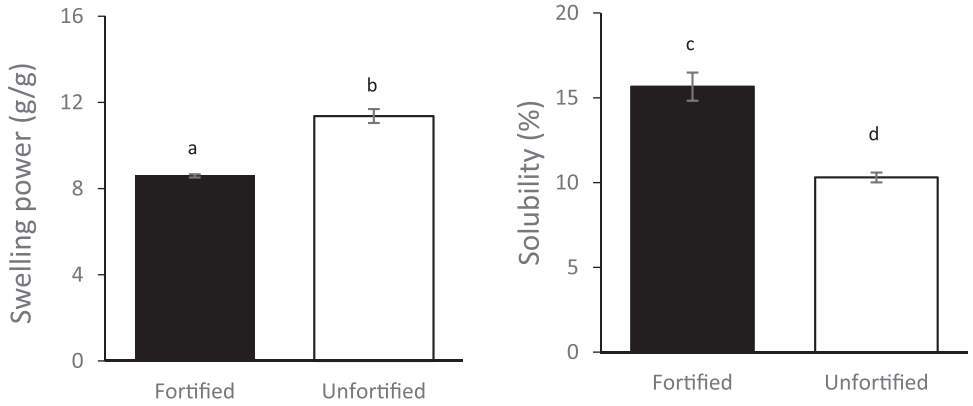

Figure 3. Swelling power (left) and solubility (right) of fortified and unfortified potato chips.

in the potato cell wall, thus restricts the swelling of starch.[28] The reduction in PV with the addition of calcium has been previously reported by T. Noda et al. (2014)[36] in the fortification of potato starch. A level of divalent cations (such as calcium) affected the starch pasting properties, presumptively by ionically crosslinking starch phosphate esters.[37] Also, the intact parenchyma cell walls appeared to delay or restrict swelling of starch granules.[35] Breakdown viscosity (BD = PV – TV) is regarded as an indicator of the disintegration extent of granules or paste stability,[36] which implies that fortified potatoes have less disrupted starch granules. Setback viscosity (SB = FV – TV) reflects the starch paste stability after cooling, indicating re-association of the amylopectin/amylose molecules during the cooling process.[38] These results indicate that fortified potato has a low rate of starch retrogradation or less tendency to retrograde. Finally, the PT reflects the heat energy required for structural disintegration of starch granules and paste formation.[39] The higher PT developed by fortified samples could be related to the greater effort needed for the water to act as plasticizer and break down intermolecular bonds of amylose and amylopectin molecules and thus, more heat was required to disintegrate the structure.[40]

2.3. Swelling Power and Solubility

Figure 3 shows the swelling power and solubility of the fortified and unfortified samples.

The hydration and swelling of starch during heating reveals the extent of interaction between the amorphous and crystalline regions within the starch chains.[41] The high swelling power of potato starches is a result of the weak internal organization, originated from negatively charged phosphate ester groups inside the starch granules.[24] The unfortified samples showed significantly higher swelling power (11.36 ± 0.32 g/g) compared to fortified samples (8.59 ± 0.07 g/g). This result was consistent with the pasting properties. The lower swelling power of fortified samples may be attributed to the ionic cross-linking of calcium with starch phosphate esters.[37] In potato, the solubility of the tuber, has been noted to be principally due to the swelling and solubility of the starch granules.[42] Higher swelling power of starch has been positively correlated with solubility.[43,44] In this case, the higher solubility of fortified samples (15.65 ± 0.07%) compared to unfortified (10.30 ± 0.29%), could be attributed to the addition of calcium in a soluble form. Calcium lactate has been reported to have relatively high solubility compared with other calcium salts.[4]

2.4. Gastro-Small Intestinal Starch Digestion in vitro

Starch hydrolysis (%) of fortified and unfortified samples is shown in **Figure 4**. During 30 min simulated gastric digestion, most of the starch remained undigested, due to the lack of amylases in the gastric fluid, and resulted in hydrolysis (%) between 0.41 and 4.97, which is attributed to the very low pH conditions and absence of amylases, as has been previously reported.[22,27,45] However, at 10 min of simulated small intestinal digestion (I10) the percentage of hydrolysis increased due to the action of pancreatic amylases, and approximately 42% and 63% of the starch was hydrolyzed for the fortified and control samples, respectively. At the end of the in vitro digestion (I120), fortified potato samples had significantly lower hydrolysis (74%) when compared to control samples (95%).

The observed differences among the samples could be attributed to the interaction between added compounds and digestive enzymes and/or the action of the added compounds on

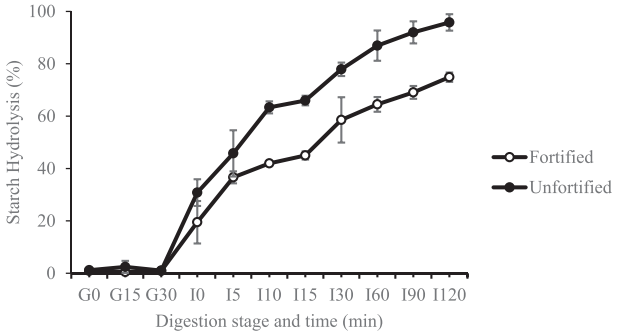

Figure 4. Starch hydrolysis (%) of fortified and unfortified potato chips.

the potato tissue, leading to a general decrease in the hydrolysis percentage. Although calcium is known to catalyze amylase activity,[15,46] ascorbic acid has been shown to be an effective inhibitor of porcine pancreatic and salivary α-amylases, and may decrease the enzyme activity by 100%.[47] Borah et al.[48] found a significant inhibitory activity exhibited by ascorbic acid against human pancreatic α-amylase via allosteric inhibition, so ascorbic acid may have the ability to not only act on the substrates but may act on the enzyme to influence enzymatic hydrolysis. On the other hand, the presence of ascorbic acid has been shown to enhance the calcium impregnation,[49] which may be related to permeability changes of the cellular wall and membranes. Regarding Vitamin E, it has been reported that both endogenous and added lipids during food processing may affect the starch digestibility as a consequence of the formation of a amylose-lipid complexes.[50] Hence, there is a possibility that the added Vitamin E may have interacted with the amylose in the starch to form inclusion complexes, thus leading to a reduction in the overall starch hydrolysis. On the other hand, the role of calcium on the starch digestibility can be related through its interaction with potato cell wall components. The plant cell wall is generally composed of three levels of organization: middle lamella, primary cell wall and secondary cell wall.[1] In potato parenchymal middle lamella, pectin is a major constituent and it is quite prone to react with calcium.[1,51] Thus, Ca^{2+} has a tendency to form cross-links between pectin molecules in the potato cell wall.[52] In the case of potato, it has been reported that the presence of Ca^{2+} is the constraining factor for the construction of the Ca-pectin cross-linkages within the cell wall of native potato tuber.[52] Therefore, the addition of calcium can overcome this limitation, and gives the cell wall strength,[8,53] resulting in a firmer potato product.

2.5. Estimated Glycemic Index (*eGI*)

The *eGI* for fortified potato was significantly lower than the unfortified sample (**Table 2**), this is related to the lower hydrolysis rate of the fortified chips discussed previously. Although the *eGI* has been reported to be highly correlated to the GI,[16] there are many factors such as botanical source, composition, starch gelatinization, food processing, texture, microstructure, storage, properties of cell wall polymers, particle size and granular structure,[1,50,54] and starch-non starch components interactions,[54–56] which determine the degree of starch digestion and its absorption in the body. In a recent study, Mason et al. (2019)[57] found that ascorbic acid supplementation led to a significantly lower postprandial glucose response among individuals with type 2 diabetes; they hypothesize that the improved response occurred due to decrease in oxidative stress.

Table 2. Hydrolysis index (HI) and estimated glycemic index (*eGI*) of fortified and unfortified potato chips. Different letters ("a" and "b") in the same column indicate significant differences ($p < 0.05$) ($n = 3$).

Potato chip	HI	eGI
Unfortified	80.57 ± 3.62*a*	83.94 ± 1.99*a*
Fortified	60.09 ± 2.69b	72.70 ± 1.47*b*

2.6. Microstructural Characteristics of Digesta

Figure 5 shows the microstructure of potato before and during the in vitro starch digestion. In both samples, potato tuber parenchyma showed retained cell wall layout to some extent, after blanching, fortifying, and drying processes. Also, it is observed some indentations and wrinkles on the surface that may have been caused during the freeze-drying process. After 30 min of gastric digestion, the micrographs (5c, 5d) showed little differences compared to undigested samples, i.e., small filamentary structures that could denote small particles like cell debris, glycoprotein, or polysaccharides.[58] After 10 min of intestinal digestion, the unfortified samples underwent hydrolysis of starch remnants, as reflected by the cells with homogenous background, with an indication of cell wall breakage (5e). The microstructural changes in fortified samples (5f) showed less damage of cell wall structures compared as those observed in unfortified samples, which showed breakage of cell structures (5e). The fortified samples showed well preserved cell wall structures, which are generally made up of middle lamella—mainly composed of pectic substances —and the primary cell wall—made of cellulose, hemicellulose, glycans, and pectins-.[14,27,59] This integrity of cell wall structures can also be observed after 120 min of simulated intestinal digestion (5g, 5h) where the cell walls of the fortified samples showed less rupture than those from unfortified samples. Therefore, in this case, the cell wall played an important role influencing the starch hydrolysis during digestion, as has been previously stated in other studies.[60,61] The addition of calcium in the fortification emulsion could enhance the firmness of the tissue making the cell wall stronger and interrupting the free access of amylolytic enzymes[59] and restricting the leaching of carbohydrates out of the cells,[60] thus decreasing the overall percentage of starch hydrolysis.

3. Conclusions

The VI technique along with VMD were effective methods to obtain dehydrated potato chips, fortified with calcium and vitamins C and E. These processes led to an increase in the levels of vitamin C and calcium than that usually exist in raw potato, and also allowed the enrichment with vitamin E, which is normally lacking in potatoes. The addition of fortifying compounds (vitamin E, vitamin C, and calcium) through VI technique, has been observed to delay as well as decrease the extent of starch hydrolysis

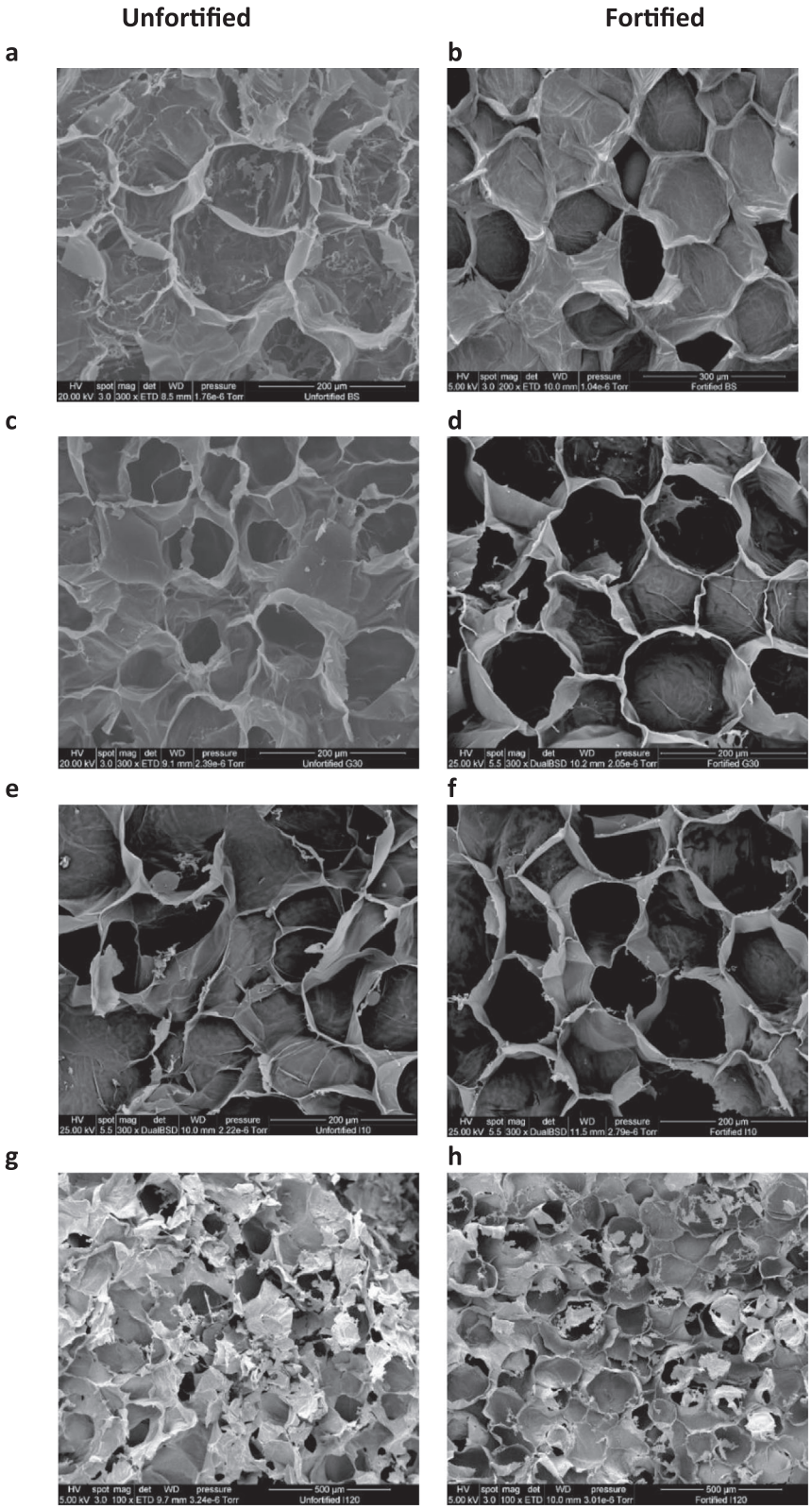

Figure 5. SEM micrographs of unfortified (A, C, E, G) and fortified (B, D, F, H) potato chips: before start digestion (undigested) (A, B); and after G30 (C, D); I10 (E, F) and I120 (G, H).

during gastro-small intestinal in vitro digestion of potato chips made with Diacol Capiro variety. The microstructure showed that the cell wall played an important role in influencing the starch hydrolysis during digestion. The fortified chips showed significantly lower paste viscosities, swelling power, and higher pasting temperatures when compared with unfortified samples. In order to understand the effect of individual fortifying compounds, it may be useful to impregnate the potatoes with them separately. However, the information obtained from this study could help the food industry to develop dehydrated potato products with low glycemic features. It is also important to note that the efficacy of impregnation process may be affected if applied on oil fried chips.

4. Experimental Section

Materials: Potatoes *(Solanum tuberosum)* of Diacol Capiro variety were used. Medium size tubers (45 –64 mm diameter)[18] were selected from the tubers grown in La Unión, Antioquia, Colombia. Potatoes were washed and cut into slices of 2.5 mm thick, blanched for 4 min at 80 ± 2 °C,[19] cooled and dried with an absorbent paper.

For the fortification process, Dl-*α*- tocopherol acetate (vitamin E), L-ascorbic acid (vitamin C), calcium lactate (calcium source), and the surfactants Tween 80 and Span 60 were obtained from Bellchem.

A total starch assay kit (Megazyme International, Ireland), DMSO method[19] was used to calculate the total starch content.

For the in vitro digestion, pepsin (porcine gastric mucosa, 800–2500 units mg protein^{-1}), pancreatin (hog pancreas, 4 USP), and invertase (Invertase, grade VII from baker's yeast, 401 U mg^{-1} solid) were acquired from Sigma–Aldrich Ltd (St Louis, USA). Amyloglucosidase (3260 U mL^{-1}) was acquired from Megazyme International Ireland Ltd.

Preparation of Dehydrated and Fortified Potato Chips: For the fortification process, an impregnation emulsion containing 0.2% Vitamin E, 0.6% Vitamin C, and 0.9% Ca^{2+}, the surfactants Tween 80 (0.051%) and Span 60 (0.049%), and an isotonic solution of Sodium chloride (NaCl) (0.8%) was prepared. The formulation of the impregnating emulsion was determined based on the VI parameters obtained for potato Diacol Capiro variety, as in our previous study.[19] The compounds of the impregnation emulsion were homogenized using an ultraturrax (Microdistech, MDT 1000, USA) at 20 000 rpm for four cycles. Each cycle consisted of 3 min of homogenization followed by 3 min of rest to avoid overheating.[19] The fortification process was carried out using the VI technique as follows: the potato slices were immersed in a desiccator containing the fortification emulsion. In order to fix the vacuum pressure, a vacuum pump (Welch, Gardner Denver Thomas, Inc. Sheboygan, WI, USA) was connected to the desiccator. The potato slices were subjected to vacuum pressure (77.3 kPa) during 3 min, and subsequently the slices remained submerged for an additional 4 min at atmospheric pressure.[11] The impregnated samples were then taken out from the desiccator. Finally, the dehydration was carried out in by Vacuum Microwave Drying (VMD) (CW 2450 MHz, Toshiba, China) described previously by Y. Duarte-Correa, Díaz-Osorio, Osorio-Arias, Sobral, & Vega-Castro, (2020).[11] The output power of dryer was 0.6 kW and the process was carried out at constant temperature (60 ± 2 °C) for 15 min. Blanched and microwaved dried potato samples without fortification were used as control (referred as unfortified). The sizes of the samples were approximately between 45 and 64 mm of major diameter and 2.5 mm thick. Fortified and unfortified samples were ground to powder using a ceramic mortar for further analyses of swelling power, solubility, and pasting properties.

Determination of Vitamin E, Vitamin C, and Calcium: Vitamin E, vitamin C, and calcium content were quantified for fortified and unfortified potato chips. Vitamin E was quantified by HPLC UV/vis detector (Knauer Azura).[19] The separation was performed at 285 nm and 30°C, with an Eclipse Plus C_{18} RP column, a mobile phase composed by methanol (53%), acetonitrile (45%), and water (2%) and a flow rate of 0.8 mL min^{-1}. Vitamin C quantification was carried out by titration method of indicator 2.6 dichlorophenolindophenol[20] in an acid environment, and the calcium analysis was carried out by atomic absorption,[21] where samples were previously calcined for 4 h at 550 °C and digested with nitric and hydrochloric acid.

Determination of Pasting Properties: The pasting profiles of fortified and unfortified samples were performed with the Potato Starch Method,[22,23] using a Rapid Visco-Analyzer (RVA, Newport Scientific, Sydney, Australia). The samples were equilibrated at 50°C for 1 min, heated and maintained at 95°C for 3 min, and then cooled down and maintained at 50°C for 2 min. The rotational speed through the process was kept at 160 rpm.

Determination of Swelling Power and Solubility: Swelling power and solubility of fortified and unfortified samples were determined according to Singh, McCarthy, Singh, Moughan, & Kaur (2007),[24] using potato flour suspensions. The flour suspensions were prepared containing ~2% w/v starch. Powdered samples (0.3 g) were heated in distilled water at 90 °C for 30 min, then cooled down to room temperature and centrifuged at 3500 x *g* for 20 min. The residue was weighted out to determine the swelling power using the ratio between the residue weight and the initial weight sample. To determine solubility, the supernatant was decanted and dried to a constant weight, in an oven at 110 °C. The ratio between the residue obtained after drying the supernatant (g) and the initial sample (g), represented the solubility that was expressed as a percentage.

Gastro-Small Intestinal Starch Digestion in vitro: An in vitro digestion model was used to evaluate the starch hydrolysis (%) of fortified and unfortified potato flour samples. The apparatus consisted of jacketed glass reactors with 500 mL capacity, connected to a water bath at 37±1°C .[25] In order to simulate the size reduction that occurs during mastication of potato chips, the chips were manually crushed four times to a particle size of approx. 3–4 mm using a pestle and mortar. One hundred seventy gram of prepared samples involving crushed potato chips (containing 4% starch) were placed in the digestion reactor and stirred mechanically at 300 rpm. The simulated gastric and intestinal fluids (SGF, SIF, respectively) were prepared according to United States Pharmacopeia.[26] At the beginning of the process, the pH was decreased until 1.2 using 3 M HCl and 0.5 M HCl to simulate gastric conditions, and SGF (25 mL) containing pepsin was added to start the enzymatic hydrolysis. After 0 (G0), 15 (G15), and 30 (G30) min of simulated gastric digestion, aliquots of 0.5 mL were taken, and directly placed on 2 mL of ethanol 96% and vortexed to stop the hydrolysis. After 30 min of simulating gastric conditions, the pH was increased to 6.8 using 3 M NaOH and 0.5 M NaOH in order to inactivate pepsin activity. SIF (22 mL) comprising pancreatin, amyloglucosidase, and invertase was then added to simulate small-intestine conditions. After 0 (I0), 5 (I5), 10 (I10), 15 (I15), 30 (I30), 60 (I60), 90 (I90), and 120 (I120) min of simulated intestinal digestion, aliquots of 0.5 mL were taken and directly placed on 2 mL of ethanol 96% and vortexed. After that, the samples were centrifuged (1800 g for 10 min) and 0.1 mL of ethanolic supernatant was incubated with 0.5 mL of amyloglucosidase-invertase solution.[25] Finally, the produced glucose was determined using GOPOD reagent (Megazyme International Ireland Ltd, Ireland). The percentage of starch hydrolysis was calculated as shown below:[27]

$$\%S_H = \frac{S_h}{S_i} \quad (1)$$

$$\%S_H = 0.9 \times \frac{G_p}{S_i} \quad (2)$$

where % S_H is the total starch hydrolysis (%), Sh is the hydrolyzed starch; Si the initial starch of the sample (g); and Gp the glucose produced (g).

Estimated Glycemic Index (eGI): The eGI of fortified and unfortified samples was calculated by following the equation:[16,22]

$$eGI = 39.71 + 0.549 \times HI \quad (3)$$

where HI is the hydrolysis index of the potato samples, calculated as the area under the curve throughout in vitro small intestinal digestion. White bread was used as a reference.

Microstructural Characteristics of Digesta: SEM was used to observe the modifications occurred in fortified and unfortified samples during in vitro digestion process. Samples were taken at fixed times as follows: before starting and at the end of simulated gastric digestion (BS, G30, respectively), and after 10 (I10), 60 (I60), and 120 (I120) min of simulated intestinal digestion. Samples taken were placed in 2 mL Eppendorf microcentrifuge tubes and immediately frozen by submerging in liquid nitrogen and then freeze dried. Finally, the freeze-dried samples were examined under a SEM microscope (FEI Quanta 200 FEI Electron Optics, Eindhoven, The Netherlands).[28]

Statistical Analysis: The results of each analysis were expressed as means ± standard deviation from three estimations. An analysis of variance (ANOVA) with Fisher's Least Significant Difference (LSD) test was used to determine significant differences among the means at a significance level of $p < 0.05$ using Statgraphics Centurion XVI software (Statistical Graphics Corporation, Ver. 16.0.07, Rockville, USA).

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Data available on request from authors.

Keywords

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