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Ultrasound-assisted fortification of yellow sweet potato (*Ipomoea batatas*) with iron and ascorbic acid

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The objective of this study was to evaluate the effect of ultrasound on the incorporation of iron and ascorbic acid (AA) in sweet potato (*lpomoea batatas*) and to optimize the process parameters to obtain a fortified food. The incorporation was carried out using cubes of sweet potato submerged in 0.1% m/v ferrous sulfate and 1% m/v AA solutions, treated at different times and sonication frequencies (37 and 80 kHz), at 100 watts of power and $30 \pm 5^{\circ}$ C. ANOVA and Tukey's test at 5% significance were applied to establish significant differences and the process was evaluated using a factorial design. The results revealed that the application of ultrasound influences the content of iron and AA, incorporating greater amount of iron and AA compared to samples not treated with ultrasound. Similarly, longer times led to higher incorporation of iron and AA content in sweet potatoes; the frequency was not statistically significant. The highest iron content was 105.91 ± 0.03 mg/100 g and for AA, it was 392.65 ± 4.84 mg AAE/100 g. The defined ultrasonic process conditions produced an increase of 4928.99 and 610.65%, respectively, in iron and AA content in sweet potato.

KEYWORDS

ultrasound, iron, ascorbic acid, fortified sweet potato, food processing

1. Introduction

The need and consumption of fortified foods have increased significantly in recent years, there is great concern about the nutritional aspects and processed foods products consumed in the daily diet, this is due to the high nutritional deficiencies in the world population. Iron deficiency anemia is one of the most prevalent nutritional deficiencies worldwide, and is considered as a public health problem (Ministerio de Salud [MINSA], 2017). Worldwide, anemia affects 500 million women of reproductive age (15–49 years), 40% of pregnant women, and 42% of children under five years of age (Meilianti et al., 2023). In Peru, 40% of the population suffers from anemia (Instituto Nacional de Salud [INS], 2020), 33.6% of children under 5 years old, 20.6% of women between 15 and 49 years old by 2022 (Instituto Nacional de Estadística e Informática [INEI], 2023).

This issue can be approach by adhering to a strict diet, containing iron-rich foods, and consuming supplements or fortified and/or enriched foods. Iron fortification is considered the most effective option to prevent malnutrition on a large scale, and this strategy has been applied in different governmental programs (Joshi et al., 2019). Iron is a micronutrient necessary for the

human body; it participates in the production of hemoglobin and myoglobin, which are responsible for oxygen transport. Likewise, contributes to the metabolism of certain enzymes and in the synthesis and catabolism of neurotransmitters, therefore this deficiency has an impact on behavioral, mental and motor development, and also induce a slower speed of conduction of the sensory, auditory and visual systems (Rivera et al., 2012; Ministerio de Salud [MINSA], 2017).

Fortification is the addition of a micronutrient to food to increase the content of one or more essential micronutrients, correct or prevent a demonstrated deficiency (dietary, biochemical, functional, and/or clinical) of a nutrient in the population (Latham and FAO, 2002). Iron fortification is one of the most complex, there are different iron compounds concerning their solubility and chemical state; ferrous sulfate is the most widely used source of iron in the industry, due to its high bioavailability of the micronutrient, and its low cost (Shubham et al., 2020). To enhance absorption, iron is combined with vitamin C, which acts as a potentiator. Vitamin C in molar relations with iron greater than 1:1 can double the absorption of non-heme iron, despite the presence of dietary inhibitory factors (Tostado-Madrid et al., 2015). However, when iron incorporated directly into the food matrix, it can oxidize and cause undesirable changes in organoleptic properties (odor, taste, color) (Genevois et al., 2014). For this reason, the optimal fortification of foods with complex structures such as fruits and vegetables, requires a great deal of research, most of the traditional technologies used cause instability in the added nutrients; this is why new technologies such as ultrasound (US) are being used (Carvalho et al., 2021).

Ultrasonic technology has been the subject of numerous studies aimed at improving mass transfer in foods. These studies utilized different media, such as water, osmotic solutions and ethanol, to transmit acoustic waves into the food matrix. This method enables the efficient inclusion of compounds, such as iron, more effectively, improving their homogeneous dispersion of micronutrients (Rojas et al., 2019). Furthermore, the use of this technology aids in decreasing nutrient loss and processing times, favoring the quality of the sensory properties of the product (Bhargava et al., 2021).

Carvalho et al. (2021) demonstrated the efficacy of the combination of ultrasonic technology, microencapsulation, and convection drying in the production of fortified pineapple chips. The authors showed that optimal ultrasonic pre-treatment of ethanol (25kHz - 30min), significantly increases the iron content (up to 1,000% more than the control sample) in the final product. Similarly, Bonto et al. (2020) incorporated iron in rice with ultrasonic technology (40 kHz- 5 min). The absorption of iron in the rice sample was 321±13.43 mg/kg of rice, achieving a 28-fold increase when compared to the untreated rice sample. Furthermore, the experiment revealed efficient iron diffusion, and achieved 82.9% retention rate after washing and cooking. Similarly, Rojas et al. (2019) incorporated iron and carotenoid microencapsulates in pumpkin and apple, respectively. The application of ultrasound resulted in a more homogeneous distribution of iron increased iron content by over than 1,000% compared to the control samples.

Mashkour et al. (2018) incorporated iron into whole potatoes by vacuum impregnation in combination with ultrasonic technology (37 kHz - 45 min) as a pre-treatment. Their study showed that the combination of IV (vacuum impregnation) with the US (ultrasonic waves), resulted in higher iron incorporation of 210%. In addition, no significant effects on color and texture parameters were observed.

Miano and Augusto (2018) incorporated iron during the bean hydration process with ultrasound application (91 W/L; 25 kHz), after 510 min, the incorporation obtained with ultrasonic application was 60.1 mg Fe/100 g, compared to 34.4 mg Fe/100 g without ultrasonic application. Mason et al. (2015) mentioned that ultrasound generates positive effects on processed food, this effect occurs due to the collapse of cavitation bubbles that are caused by pressure fluctuations, exerted by the passage of acoustic waves from the US bath. This cell disruption can produce a higher mass transfer (solids gain and water loss), allowing better impregnation of the solvent and thus facilitating the incorporation of micronutrients in foods (Bonto et al., 2018; Yilmaz and Ersus Bilek, 2018).

The incorporation of iron in different foods has been investigated with positive results, reflected in the increase of the content of this micronutrient. To this extent, it is interesting to investigate the incorporation in food matrices, being sweet potato is a food of great interest and has great potential to become a food vehicle, since it ranks seventh in most produced food. Sweet potatoes are one of the most significant crops worldwide, producing over 104 million tons in 2014 (FAOSTAT data, 2017). In addition, production cost is relatively low (Grozo and INEI, 2021). Likewise, this food has valuable nutritional content and is highly rich in nutrients, such as vitamin C, Fe, K, and Ca. It is a member of the Convolvulaceae family, genus Ipomoea, and the type species Ipomea batatas L. The orange-fleshed sweet potato has antioxidant properties, anti-inflammatory effects, high iron and zinc content and a low glycemic index being a great ally in countries suffering food shortages (Oladejo and Ma, 2016; Renee et al., 2018; Nyathi et al., 2019). Although heme iron contained in animals is more bioavailable than non-heme iron (found in vegetables), many countries have low meat consumption and high prevalence of iron deficiency anemia, therefore, non-heme iron is an alternative option to increase iron intake and combat iron deficiency. Furthermore, vegetable sources may contain a significant content of AA, which favors a higher bioavailability of iron (Andre et al., 2018). Although studies on ultrasound-assisted food fortification are reviewed in the literature, there are no studies that propose the incorporation of iron and AA in sweet potato. Consequently, considering the great importance of iron-fortified foods for the world population, the objective of this work was to study the effect of ultrasound on the incorporation of iron and AA in sweet potato (Ipomoea batatas) and to evaluate the ultrasound parameters for obtaining an fortified food.

2. Materials and methods

2.1. Reagents

All the reagents were of analytical grade, for the determination of ascorbic acid (AA) we used 2,4-dinitrophenylhydrazine (Lobachemie, India), glacial acetic acid (Scharlau, Spain), bromine water, thiourea, sodium acetate, metaphosphoric acid, sulfuric acid (98%, m/v), the reagents were acquired from Movilab (Lima, Peru). For iron determination, 1, 10-phenanthroline (Biolab, Argentina), ammonium iron (II) sulfate (Fe(SO₄)(NH₄)₂(SO₄)6H₂O) (Movilab), hydroxylamine hydrochloride (Scharlau, Spain), hydrochloric acid (37% m/v) was purchased from (J.T.Baker, Mexico), nitric acid (65% m/v) was purchased from (Merck, Germany). AA $C_6H_8O_6$ and ferrous sulfate (FeSO₄) were purchased from Movilab.

2.2. Raw material and sample preparation

Yellow sweet potatoes (*Ipomoea batatas*) were acquired from a local supermarket in Sullana (Peru) and kept in storage at $27 \pm 3^{\circ}$ C for 2 to 3 days prior to utilization. The sample had a soluble solids content of $10 \pm 1^{\circ}$ Brix and a moisture content of $79.8 \pm 1.5\%$. The sweet potato samples were sliced into pieces measuring11 mm x 11 mm x 5 mm for further processing.

2.3. Ultrasound-assisted incorporation of iron and acorbic acid

Iron and AA were incorporated following the methodologies reported by Carvalho et al. (2021) and Rojas et al. (2019). First, the solution was prepared containing ferrous sulfate at 0.1% w/v and AA at 1% w/v (FA). Next, sweet potato slices were added to a beaker containing FA solution in a 1:5 w/v ratio. The above samples were placed in an the ultrasonic bath (Elmasonic P 30H, Germany) and treated at different frequencies (37 and 80 kHz) for 10, 20, 30, 40, 50, and 60 min., and ultrasound power of 100 watts and temperature inside the ultrasonic bath was 30°C. A control sample (without ultrasonic treatment) was also prepared in parallel. Subsequently, both the ultrasound-treated and control samples were drained, and dehydrated in a tray dryer (Dehydrator ST-04, 30–90°C, 0–15 h), at $45 \pm 2^{\circ}$ C for 8 h at air circulation speed (2.5 m/s). The Schematic diagram of the ultrasound-assisted incorporation of iron and AA in sweet potatoes is shown in Figure 1.

2.4. Determination of AA

Total AA (ascorbic acid + dehydroascorbic acid) was quantified using the UV spectrophotometric method described by Rahman et al. (2007). Ascorbic acid is oxidized to dehydroascorbic acid by the action of bromine water in an acidic medium, generated by the presence of acetic acid. The AA was extracted from the samples; for this, 1g of the sample was mixed with 20 mL of 5% w/v metaphosphoric acid solution and 10% v/v acetic acid centrifuged at 2500 rpm for 4 min. Next, the obtained mixture was filtered with Whatman No. 1 filter. Next, 3 mL of filtered solution (SF) was reacted with 0.15 mL of bromine water, and then gently stirred. Additionally, 0.80 mL of 10% m/v thiourea was added to remove excess bromine, then 1 mL of 2,4- dinitrophenylhydrazine solution was added and gently stirred. To finish the reaction, the samples were maintained in an incubator (Usamed, DNP-9052A) at 37°C temperature for 3 h; subsequently, the samples solutions were immediately cooled in an ice bath, added 1 mL of 85% m/v H₂SO₄ with constant stirring and made up to 5 mL with distilled water. The absorbance of the colored solution obtained was measured at 521 nm in a UV-visible spectrophotometer (Thermo Scientific, model Genesys 150). The calibration curves were obtained with standard solutions of AA ranging from 2.5 to 20 mg/L, following the same procedure used for the samples, and the concentrations were expressed in mg of AA (AAE) /100 g. All determinations were performed in triplicate and all steps were repeated for the blank sample.

2.5. Determination of total iron

The determination of total iron was performed according to the AOAC (1994) method (944.02) with adaptations. In this method, Fe²⁺ reacts with 1,10-phenanthroline to form a characteristic red-orange complex that absorbs strongly in the region around 510 nm of the visible spectrum. Hydroxylamine hydrochloride solution was applied as a reducing agent to reduce Fe³⁺ to Fe²⁺.

To perform the analysis, a crucible was used to weigh 1 g of the sample and which was subsequently placed in a muffle furnace (Sel-Horn "R-8L) at 550±15°C for a period of 5h. Next, 0.1 mL of nitric acid was added to the ashes obtained and evaporated on a hot plate. Next, 1 mL of hydrochloric acid was added and again evaporated on a hot plate. Finally, 0.2 mL of hydrochloric acid was added, and the volume was filled up to 10 mL with distilled water. For quantification, an aliquot of 2 mL of the above solution was reacted with 0.6 mL of hydroxylamine hydrochloride, then allowed to stand in a dark place for 5 min, followed by the addition of 1.5 mL of acetate buffer and 0.6 mL of 1,10-phenanthroline, stirred gently and distilled water was added to make up the 5 mL. Finally, the solution was agitated in a vortex (Cole-Parmer, model SA8) at 1000 rpm × 10s and kept at rest for 10 min. The absorbance was measured at 510 nm in a UV-visible spectrophotometer. The calibration curves were obtained with standard solutions of Fe²⁺ ranging from 0.5 to 5.0 mg/L, following the same procedure applied to the samples, and the concentrations were expressed in mg /100 g. All determinations were performed in triplicate and all steps were repeated for the blank sample.

2.6. Experimental design and statistical analysis

For screening purposes, a 2^2 full factorial design, with two levels (-1 and + 1) was applied to the two independent factors frequency (37-80 kHz) and ultrasound exposure time (10 and 60 min) in the responses of iron (mg/100 g) content and AA (mg AAE/100 g), in a total of 4 runs (Table 1). All the experiments were performed in triplicate.

Apart from the factorial design, aliquots were evaluated every 10 min (20, 30, 40, and 50 min) in 37 and 80 kHz to process control, in a total of 8 runs. For the analysis of results, the Analysis of Variance method (ANOVA) and Tukey's test were used to determine differences between different treatments. The process was evaluated using factorial design with Statgraphics Centurion XVI software and the analysis was performed with IBM-SPSS software.

3. Results and discussion

3.1. Incorporation of iron and AA

Table 2 presents the iron and AA content in the dehydrated sweet potato after the different treatments with and without ultrasound. The control dehydrated sweet potato (CT) presented an iron content of $2.15 \pm 0.01 \text{ mg}/100 \text{ g}$ and an AA content of $63.61 \pm 0.50 \text{ mg}/100 \text{ g}$, values higher than those found by Amagloh et al. (2022), who reported an AA content in fresh sweet potato ranging from 3.040 to 16.698 mg AAE/100 g. Peruvian food composition tables indicated an even lower



TABLE 1 Coded (-1 and + 1) and real parameters of the $2^{\rm 2}$ full factorial design.

Run	US Frequency (kHz)		US time (min)	
1	-1	37	-1	10
2	+1	80	-1	10
3	-1	37	1	60
4	+1	80	1	60

AA content of 0.80 mg/100 g was reported for dehydrated sweet potato; however, the iron values were 2.90 mg/100 g (María et al., 2017); which is in agreement with the findings of the present study. Both ultrasonic (US) and non-ultrasonic (NUS) treatments were effective in incorporating iron and AA into the sweet potato. Longer treatments resulted increased the content of both iron and AA content; however, ultrasonic frequency, as well as the interaction (frequency versus time), did not influence AA content; on the other hand, in the case of iron content, time and interaction were significant, as observed in Table 2.

According to Mashkour et al. (2018), the use of a 37 kHz frequency generated higher micronutrient incorporation compared to an 80 kHz frequency, due to the cavitation phenomenon induced by the US, the cavitational collapse produces the cellular rupture in the plant tissue, which allows the increased of cell permeability, resulting in better

mass transfer. Lower US frequencies generate longer wavelengths, and longer compression cycles and produce a violent cavitational implosion, resulting in greater cell disruption. In contrast, Yu et al. (2016) testing three different frequencies (25, 45, and 100 kHz) to enrich peanuts with resveratrol, used US as a pretreatment, the optimal frequency was found to be 100 kHz. The difference in these results may be attributed in the different in the food matrix, pretreatment methods, and frequencies utilized.

The iron content was incorporated in greater quantity in the treatments with longer exposure times (Figure 2B), for instance (Table 2) the treatment at 37 kHz for 50 and 60 min, showed an iron content of 72.96 and 105.91 mg/100 g. This represents an increase of 3,293 and 4,826% with respect to the control, and at 80 kHz the values were 60.52 and 77.98 mg/100 g in both times showing an increase in iron content of up to 2,715 and 3,427%, respectively. These results were higher than those observed at the same times in NUS. Incorporating iron in sweet potato with US at 30 min allows to obtain values equivalent to those demonstrated in the twice the duration in NUS, this demonstrates the effectiveness of the application of US. The iron content shown in each treatment studied, supplements the recommended daily intake of iron for pregnant women, which recommendation requires the consumption of 23 to 27 mg of iron/day. Additionally, the dietary iron requirement for adolescents is also covered, which ranges varies from 8 to 15 mg/day, depending on age and sex (Ministerio de Salud [MINSA], 2016).

TABLE 2 Total iron and ascorbic acid content in sweet potato, treated with and without ultrasound.

Treatments		Factors		Ascorbic acid	Total iron
		Frequency	Time US	mg AAE/100 g	mg/100 g
		US			
СТ				$63.61\pm0.50^{\rm m}$	$2.15\pm0.01^{\rm p}$
NUS	C1		10 min	$99.58 \pm 1.49^{\mathrm{jkl}}$	26.83 ± 0.18^k
	C2		20 min	$93.32 \pm 2.16^{\rm kl}$	$17.18\pm0.00^\circ$
	C3		30 min	107.38 ± 3.13^{ij}	32.75 ± 0.00^i
	C4		40 min	106.24 ± 1.38^{ij}	26.82 ± 0.00^k
	C5		50 min	$121.87 \pm 0.00^{\rm gh}$	24.30 ± 0.05^{1}
	C6		60 min	$152.73 \pm 0.46^{\rm f}$	30.43 ± 0.04^{j}
US	T1	37 kHz	10 min	103.71 ± 0.82^{jk}	$23.08\pm0.04^{\rm lm}$
	T2	37 kHz	20 min	$184.50 \pm 4.54^{\circ}$	$22.63\pm0.17^{\rm m}$
	T3	37 kHz	30 min	190.99±4.60°	$43.58 \pm 1.09^{\rm f}$
	T4	37 kHz	40 min	204.29 ± 4.37^{d}	$50.72 \pm 0.03^{\circ}$
	T5	37 kHz	50 min	$363.26 \pm 3.16^{\rm b}$	$72.96 \pm 0.05^{\circ}$
	T6	37 kHz	60 min	392.65 ± 4.84^{a}	105.91 ± 0.03^{a}
US	T7	80 kHz	10 min	91.82 ± 0.23^{1}	$42.70\pm0.75^{\rm f}$
	Т8	80 kHz	20 min	$127.91 \pm 0.86^{\rm g}$	20.56 ± 0.76^{n}
	Т9	80 kHz	30 min	$115.63 \pm 1.11^{\rm hi}$	$34.91\pm0.59^{\rm h}$
	T10	80 kHz	40 min	191.98±8.30°	$40.59\pm0.45^{\rm g}$
	T11	80 kHz	50 min	226.39 ± 8.78°	60.52 ± 0.06^d
	T12	80 kHz	60 min	235.70 ± 3.30°	$77.98 \pm 0.05^{\rm b}$
ANOVA				p-value	p-Value
Frequency (kHz)				>0.050	>0.050
Time (min)				<0.050	<0.050
Interaction			>0.050	< 0.050	

Values are mean \pm standard deviation, each treatment with three replicates. Means in the same column with different superscripts are significantly different (p < 0.05). AAE, AA equivalent; CT, control treatment.

Currently, there are no precedents for incorporating iron into sweet potatoes; however, in other foods, it has been possible to incorporate iron and other micronutrients into other foods using the same technology. Carvalho et al. (2021) propose the iron fortification of pineapple chips by using microencapsulation, ethanol, ultrasound, and convective drying. The authors mentioned that the longer time (30 min) of pre-treatment in ethanol and US increased the iron content up to 1157.5%; however, compared to the pre-treatment in ethanol, the US treatment did not significantly improve the iron incorporation. Rojas et al. (2019) showed a similar result for the incorporation of iron and microencapsulated carotenoids in pumpkin and apple. The authors found that by applying US treatment for 30 min, an increase of more than 1,000% of iron incorporation was found and its application helped to improve the dispersion of ethanol microparticles, despite this, the US did not significantly affect the results compared to the treatment without US. This is consistent with Miano and Augusto (2018) found that the iron content in beans increases significantly at longer exposure times, they obtained an increase of 1418.6 mg/kg of iron during 510 min of processing.

The amount of AA incorporation in sweet potato depends significantly on the time factor, as shown in Table 2, which ranges

from 93.32 to 152.73 mg AAE/100 g in NUS; US at 37 kHz from 103.71 to 392.65 mg AAE/100 g and US at 80 kHz from 91.82 to 235.70 mg AAE/100 g is observed; despite the frequency, factor did not evidence a significant statistical influence, at 37 kHz the highest AA ranges were observed (Figure 2A). Showing effective incorporation of AA from 63 to 517%, with respect to the control. In fact, 20 min of treatment was sufficient to exceed 60 min in NUS, achieving 50% higher AA. In similar studies, under the vitamin fortification approach in other food matrices, similar behavior was observed; for instance, Tiozon et al. (2021) incorporated folic acid (vitamin B) into rice using ultrasound as a pretreatment for 1, 3, and 5 min, found that longer sonication times resulted in greater incorporation and increased folic acid levels by 1,982% in brown rice and by 4,054 times in milled rice.; in addition, US improved the micronutrient retention capacity after cooking, by 93.53% (brown) and 86.52% (milled); however, the sonication time to be used should be evaluated according to the nature of the micronutrient, food matrix, among other factors. The incorporation of vitamin B5 in rice was study for different durations of 5, 15, 25, and 35 min; 25 min of sonication produced the greatest mass transfer by the rupturing of the cell walls (changes in cell microstructure), which provided an absorption of up to 140% more pantothenic acid than in



the untreated sample; because a long time produced the gelatinization due to temperature variation (42–45°C) (Bonto et al., 2018).

3.2. Ultrasound-assisted fortification

The aim of the optimization of the process was to obtain the parameters that allow a greater increase in the iron and AA content, as shown in the surface response plot in Figure 3. As observed, the lowest frequency (37 kHz) and the longest exposure time (60 min) were the optimal parameters for the incorporation of iron and AA, obtaining an iron content of 105.91 ± 0.03 , an increase of 4928.99%and an AA content of 392.65 ± 4.84 , an increase of 610.65% compared to its initial content. The basis for these results, as mentioned above, is due to the increased cell permeability of the feed tissue as a result of the cavitation collapse caused by the ultrasonic treatment. Similar results were obtained by Oladejo and Ma (2016), in their study optimized the US-assisted osmotic dehydration process in sweet potato (Ipomoea batatas) using methodology response surface (MRS); the optimized values were the following, frequency of 33.93 kHz, sucrose concentration 35.69% and the exposure time was 30 min, this time was the maximum value, concluding that the use of US allowed more mass transfer and that the lower frequency favors the results.

Similarly, Azarpazhooh et al. (2020) investigated the impregnation of grape pomace phenols in *aloe vera* using ultrasound-assisted osmotic dehydration (25 kHz, 200–400 W), and optimized the process using a MRS. The optimum values were 50% sucrose, 50°C, 59% amplitude, 20% grape pomace extract and 173 min exposure, with the maximum time value being 210 min. They study concluded that that a low frequency (25 kHz) with an exposure time of 173 min achieves a higher gain of solids, leading to increase the content and retention of phenolic compounds.

Sucheta et al. (2020), optimized the process of spice impregnation in black carrots assisted with US (37 kHz), they used a MRS design, whose optimum values were 8.18% NaCl, 4.30% spice mixture, and 14.47 min of ultrasound exposure. It should be noted that the maximum exposure time was 15 min, and the US improved mass transfer.

Mashkour et al. (2018) obtained different results, they optimized the process of iron fortification of potatoes by vacuum impregnation and US pre-treatment, the optimized values were; 37 kHz frequency and 24 min exposure time. The authors conclude that longer exposure time affect the nutritional content as they observed leakage of



compounds from the potato; however, this is a side effect of the vacuum impregnation technique which was enhanced by the US. Similar results were reported by Maleki et al. (2020), implying that prolonged ultrasound exposure time damages carrot 303 microtissues by reducing mass transfer, the optimum values were 25 kHz frequency and 10 min.

4. Conclusion

The study introduces a novel approach to AA and iron fortification, achieved satisfactory results, showed that both US and NUS applications were effective for the incorporation of AA and iron in sweet potato (*Ipomoea batatas*). However, US treatments with longer exposure significantly influenced the content of AA and iron content. The frequency factor and the interaction (frequency vs. time) did not have a significantly influence on AA content. For iron content, the time factor and the interaction (time vs. frequency) had a significant effect. The optimized conditions of incorporation favored an increase of 4928.99% in iron content and 610.65% in AA;

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in comparison with the NUS treatment during the same time. It is concluded that, on a laboratory scale, the US application significantly increased the iron content from the minimum time of treatment.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

KP-S: conceptualization, experimentation, literature review, and writing the original draft. LR-F: experimentation, writing original draft, literature review, and data analysis. ZS: conceptualization, data analysis, supervision, data curation, and review and editing. EC: conceptualization, supervision, review and editing. ME-D: conceptualization, supervision, formal analysis, and review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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