INTRODUCTION

Developing countries depend on potatoes as a significant source of food and nutrition, which is why it represents one of the essential Andean crops for both agriculture and food for many rural families (Casas and Daniel, 2017). Currently, Peru is the largest potato producer in Latin America, potatoes being the fourth most important food crop globally (Zhang et al., 2016). Across the world, potato consumption has changed from fresh to industrial products with added value (Cerón and López, 2013), which generates residues like peel that do not add value to the production chain and are destined for animal feed, fertilizer, and biofuel production (Sandoval et al., 2015). There is high demand throughout the world for snacks made of native processed potatoes. In 2017, Peru recorded an increase of 19.4% in sales of native potatoes processed products (ADEX, 2016); due to their unique variety of shapes, color, flavors, and textures (Peña, 2017). Peruvian farmers have managed to preserve diversity over many years (Flores, 2017) with breeding programs to improve the nutritional value of the potato in other highlands environments (André et al., 2009).
Research has been carried out with potato peel extract showing its potent antioxidant activity is due to the presence of phenolic compounds such as chlorogenic acid, caffeic acid, and vanillin (Sukrasno and Kusumardiyan, 2014). It has been reported that the polyphenol content in native potatoes is four times higher than in the improved varieties (Morales et al., 2015). Rojas-Padilla and Vásquez-Villalobos (2016) reported for Huagalina peel (mg/ 100 g dry weight): 476.82, 76.50 and 11.52 for chlorogenic acid, caffeic acid and vanillin respectively; and less concentrations in the cooking water of the whole tuber (Rojas-Padilla et al., 2018).

Numerous studies have examined chlorogenic acid (CGA) biological properties (Tajik et al., 2017), such as antibacterial, antioxidant (Naveed et al., 2018), reducing inflammatory damage (Wang et al., 2020), and anticarcinogenic (Siswanto et al., 2017; Bender and Atalay, 2011). Likewise, preclinical and clinical studies have shown that CGA treatment has beneficial effects on colon cancer, breast tumors, lung cancer, and chronic myelogenous leukemia (Bandyopadhyay et al., 2004).

Due to the importance of CGA, studies have been made of different purification techniques, which offer a high percentage recovery of the final product (Dutra-Molino et al., 2014). The application of an aqueous two-phase system (ATPS) offers advantages such as low cost, short times, and easy recovery of the phase-forming components, which generate a harmless environment for biomolecules due to their low interfacial tension and high water content. (Benavides and Rito-Palomares, 2008). It does not represent a health risk, so it may be possible to use it on an industrial scale (Aydogan et al., 2010). This technique involves constructing extractions formed by two polymers, a polymer, a salt, an ionic liquid, a salt, or alcohol of low molecular weight, and salt mixed in a concentration limit, resulting in two immiscible phases (Iqbal et al., 2016).

Genetic algorithms (GA) based on the mechanism of natural selection and population genetics constitute a stochastic method to optimize an objective function with linear or non-linear restrictions and are considered very efficient in solving large, discrete, non-linear optimization problems. A clear advantage of using them over other methods is the possibility of finding a general optimal or near-optimal solution without the need to investigate all parameters. GA operates on a population of potential solutions, applying the principle of "survival of the fittest" to increase the chances of a better approximation to a solution. In each generation, a new set of approximations is created by selecting individual parameters according to their aptitude level in the problem domain, using operators from natural genetics (Marijayaprakash et al., 2015). This research work aimed to use ATPS to extract and purify CGA from potato peel, using disodium phosphate (DSP) and ethanol (EtOH) and optimize CGA extraction by applying GA to create an industrially feasible system.

**MATERIALS AND METHODS**

**Raw material**

Native potato (Solanum tuberosum L.) variety Huagalinia, cultivated in Las Colpas (2,110 m.a.s.l.), Chugay - Sánchez Carrión (La Libertad - Peru). Geolocation: 7 ° 46'56"S and 77 ° 52'04"W (76% RH).

**Chemical reagents**

Chlorogenic acid (CGA) standard (≥ 95%) was obtained from Sigma-Aldrich (USA); ethanol absolute (EtOH), ammonium sulfate (AS), and disodium phosphate (DSP) (≥99%); phosphoric acid, acetic acid, boric acid was procured from J.T. Baker; deionized water obtained from a GenPure purification system (TermoFisher Scientific) was used in this study.

**Experimental methodology**

**Experimental design**

A Central Composite Design Rotatable (CCDR) was applied with two (2) levels 2^2 + 2*2 + 3 central points. The concentration of ethanol (EtOH) and disodium phosphate (DSP) were selected as factors capable of affecting CGA extraction. Each factor was tested at two levels with limits: upper (+) and lower (-).

Response Surface Methods (RSM) in R x 64 4.0.3 and RSM package was used to identify the regions of interest that resulted in the best extraction levels, which allows more significant degrees of freedom in the ANOVA and the ability to detect curvature in any quadratic effects (Gutiérrez and Vara, 2012). It was based on a 95% confidence level with a p-value <0.05 for each treatment. Likewise, it provides adjusted R^2 and R^2 values and the level of significance of the experimental values with those from the statistical model obtained using RSM.

**A sequence of the extraction and purification of CGA using an ATPS**

**Sample preparation**

The native Huagalinia variety potatoes were taken to the Laboratory three days after being harvested. They were immediately sliced, freeze-dried (Labconco Free Zone 3.5 Plus), and then the peel was carefully separated from the pulp. The peel was ground, and the powder was stored at 4 °C for later analysis.

**Construction of the phase diagram**

A phase diagram was elaborated by turbidimetric titration using ammonium sulfate (AS) and disodium phosphate (DSP) to compare the system's best formation (Nemati-Knade et al., 2012). To five mL of ethanol, 0.5 mL of a 25% DSP solution was added and then mixed by shaking. It was repeated fifteen times until the data...
to construct the phase diagram was obtained.

**Extraction of CGA from potato peel**

The extraction method was adapted from Narváez-Cuenca et al. (2012). An extraction solution (ES) containing 70% ethanol was used. Potato peel powder (40 g) was placed in a volumetric flask of 100 mL and filled up with ES, mixed with a magnetic agitator for 60 seconds, and given a subsequent ultrasonic treatment (Ultrasonic Bath 3800) of 40 Hz for 60 minutes at 30 °C, then filtered and concentrated using a rotary evaporator (Heidolph WB2000) at 40 °C, 1200 rpm and 80 mbar for 40 minutes. The CGA residue was diluted in 100 mL of deionized water and stored at 4 °C.

**Purification of CGA with ATPS**

0.5 mL of diluted CGA solution was centrifuged with 5 mL of deionized water; EtOH and DSP were also added for ATPS optimization. Once the solution was obtained, 1.5 mL of Britton-Robinson buffer was added to adjust the pH to 3.4 at 25 °C, then mixed using a vortex mixer (VWR Analog Vortex Mixer) until the DSP completely dissolved (~10 min). It was then centrifuged at 3500 rpm for 30 minutes and then kept at 10 °C for 18 hours, resulting in two phases (Figure 1). The EtOH-rich phase was separated and diluted with 250 mL deionized water to analyze the CGA concentration using a spectrophotometer (UNICO UV-VIS 4802) (López-Méndez et al., 2014). The absorbance of CGA was measured at a wavelength of 326 nm.

The phase relationship \( R \) was established using equation (1):

\[
R = \frac{V_t}{V_b} \quad \ldots \ldots (1)
\]

\( V_t \) and \( V_b \) are the volumes of the phase rich in EtOH and DSP, respectively. The partition coefficient \( K \) was established using equation (2):

\[
K = \frac{C_t}{C_b} \quad \ldots \ldots (2)
\]

\( C_t \) and \( C_b \) are the CGA concentrations in the EtOH-rich phase and the salt-rich phase.

The extraction efficiency (% EE) of CGA in the EtOH-rich phase was determined using equation (3):

\[
\% \text{ EE} = \frac{K}{(K + 1 / R)} \times 100 \quad \ldots \ldots (3)
\]

**Optimization**

The CGA extraction efficiency values for EtOH and DSP concentrations were optimized through genetic algorithms (GA) applying GA package with the free software R x64 4.0.3; the statistical model obtained by RSM was used as an objective function, with the restrictions of the limits established by the coded stationary points of the response surface. A population of 50 individuals with 200 iterations, elitism of 2, a crossover probability of 0.8, and mutation probability of 0.1 was used.

**RESULTS AND DISCUSSION**

**Selecting ethanol/salt system**

In Figure 2, the result of the selection phase is presented, showing two areas delineated by a curve. The upper phase contained mainly supernatant rich in EtOH and CGA, while the lower stage contained an aqueous liquid rich in salt (Cienfuegos et al., 2017). Likewise, it shows disodium phosphate’s superposition on ammonium sulfate as the best ethanol/salt ratio for an ATPS (Souza et al., 2015). For this reason, for extraction of CGA in potato peels, ethanol was chosen as a phase former for having advantages such as lower cost, no toxicity, and moderate boiling point, making it suitable for large-scale industrial production (Cienfuegos et al., 2017). Tan et al. (2014) obtained up to 93.44% efficien-
cy of CGA extraction from ramie (Boehmeria nivea L. Gaud) leaf, using disodium phosphate and 89.91% ammonium sulfate; Yang et al. (2016) used an ATPS formed by ionic liquid extract and salt to extract and purify CGA from ramie leaves. The maximum efficiency of 96.18% was obtained at pH 3.0 and temperature 37 °C. Wang et al. (2017) have used eutectic solvent coupled with the aqueous two-phase system (ATPS) for the negative pressure cavitation extraction and enrichment of chlorogenic acid (CGA) from blueberry leaves. Huang et al. (2019) evaluated hexafluoroisopropanol to develop novel alcohol-salt ATPS, which was applied to extract and purify CGA from ramie leaves. They reported optimum conditions with pH 3.0. The extraction efficiency was 99.3% in the salt-rich phase.

Chong and Su-Ling (2021) evaluated the effects of recycling aqueous two-phase extraction of phenolic components from haskao (Lonicera caerulea) leaves. The total average efficiencies across the two recycling stages were 91.4% for EtOH/AS and 99.6% for EtOH/DSP. Research's novelty is that the extraction and purification of CGA from native potato (Solanum tuberosum L.) peel was done with ATPS, and this method is always reported by researchers to extract and purify CGA in leaves.

The phase diagram data are necessary for designing an ATPS and developing models that may predict the distribution of CGA (López-Méndez et al., 2014). Tan et al. (2014) showed that acid systems formed by EtOH/DSP (pH 3.9) provide a better CGA extraction. Cheng et al. (2017) found that the recovery improved significantly when the mass fractions of DSP increased from 12.70% to 19.97%, because the water was descedned to the lower phase, and a higher concentration of impurities be transferred from the upper to the lower stage; this salt has the sodium [Na+] cation, the two-phase formation capacity is determined by the anion's hydration capacity.

Some of the properties that directly affect the particles' partition (ethanol/salt) are temperature, pH, types of salts, concentration, and molecular weight (Mu et al., 2017). This research used pH 3.4, a value lower than those reported (3.52-3.82) by Wu et al. (2014) with DSP solution.

**GA optimization of CGA extraction and purification using ATPS**

Table 1 shows the relationship of EtOH and DSP on the extraction efficiency through an ATPS, obtaining the highest yield values at 94.93% and 93.18% in treatments 3 and 8, respectively, which contain the highest levels of DSP, and would indicate that when an increase in their concentration occurs, the efficiency of extraction of phenolic compounds increases (Soto-Fig. 2. Ethanol/salt phase diagram.

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Table 1. Experimental results of the partition coefficient (K) and extraction efficiency in ATPS.

| Tests | Extraction conditions | Partition coefficient | Extraction efficiency |
|-------|-----------------------|-----------------------|-----------------------|
| X₁, EtOH (% w/w) | X₂, DSP (% w/w) | K | (% E.E.) |
| 1 | -1 (16.5) | -1 (22.2) | 2.98±0.04 | 82.26±0.10 |
| 2 | 1 (23.5) | -1 (22.2) | 2.56±0.07 | 84.74±0.14 |
| 3 | -1 (16.5) | 1 (32.8) | 6.73±0.09 | 94.93±0.08 |
| 4 | 1 (23.5) | 1 (32.8) | 2.02±0.07 | 83.86±0.05 |
| 5 | -1.41 (15.05) | 0 (27.5) | 5.75±0.05 | 91.41±0.08 |
| 6 | 1.41 (24.95) | 0 (27.5) | 1.43±0.05 | 79.62±0.11 |
| 7 | 0 (20) | -1.41 (20) | 4.09±0.04 | 86.81±0.12 |
| 8 | 0 (20) | 1.41 (35) | 4.38±0.11 | 93.48±0.09 |
| 9 | 0 (20) | 0 (27.5) | 5.22±0.05 | 93.36±0.09 |
| 10 | 0 (20) | 0 (27.5) | 5.21±0.07 | 93.04±0.10 |
| 11 | 0 (20) | 0 (27.5) | 5.18±0.11 | 92.76±0.11 |
Fig. 3. Extraction efficiency (% EE, represented by bars) and partition coefficient (K represented by circles and solid line).

García and Rosales-Castro, 2016). However, the opposite occurs when EtOH's mass fraction increases; the extraction efficiency spirals downwards.

The standard deviations (SD) of the partition coefficient (K) and % EE were minimal. Treatments 3, 8, 9 showed lower SD than 0.1%.

Figure 3 illustrates the concentration of CGA in ethanol-rich phase at different EtOH/DSP concentrations; the highest and lowest value of K is observed (7.6 and 1.42, respectively), which is defined as the quotient between the concentrations of the particle in the upper and lower phase of the system.

Higher temperatures are not favorable to induce ATPS's formation; CGA migrates mainly to the alcohol-rich phase (Malpiedi, 2014). As a consequence of the ionic interaction between the system and solutes, negative molecules will have a lower K (Cortés-Burgos, 2008).

Table 2 shows the coefficients that determine % E.E. molecules will have a lower K (Cortés-Burgos, 2008). While, positively charged ionic interaction between the system and solutes, negatively charged molecules have a higher Partition Coefficient (K) and % EE were minimal. Treatments 3, 8, 9 showed lower SD than 0.1%.

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Table 2. Coefficients of the independent variables and significance of the variables of the statistical model of the % EE level.

| Estimated value | Standard error | Value t | Pr (>|t|) |
|-----------------|----------------|---------|----------|
| (Intercept)     | 93.05333       | 0.92612 | 100.4766 | 1.851e-09 *** |
| x1: EtOH        | -3.15795       | 0.56713 | -5.5683  | 0.002572 ** |
| x2: DSP         | 2.65285        | 0.56713 | 4.6777   | 0.005445 ** |
| EtOH: DSP       | -3.38750       | 0.80204 | -4.2236  | 0.008299 ** |
| EtOH^2          | -4.11479       | 0.67502 | -6.0958  | 0.001720 ** |
| DSP^2           | -1.79979       | 0.67502 | -2.6663  | 0.044547 *  |

Significance codes: ‘***’ 0.001, ‘**’ 0.01, ‘*’ 0.05; R^2: 0.956, adjusted R^2: 0.9121; F-statistic: 21.74, 5 d.f., p-value: 0.002099
coefficients and % E.E. of CGA in the EtOH - rich
phase were observed at 25 °C. The Huagalina variety
had a yield of 443.7 ± 0.062 mg CGA / 100 g peel dry
weight; 7% lower value than those reported by
Rojas-Padilla and Vásquez-Villalobos (2016) with
476.82. ± 63.58 mg CGA/100 g peel dry weight, using
the UPLC MS-MS method with the same variety of na-
tive potato. It shows that the model is adequate, pre-
dicting the expected optimization. Therefore, it is shown
that the peels of native potato Huagalina constitute an
excellent food source with antioxidant potential. This
extraction method has all the necessary conditions re-
quired to scale up production at an industrial level.

Conclusion

The extraction and purification conditions of chlorogen-
ic acid (CGA) in the peel of native Huagalina variety
potato (Solanum tuberosum L.) were optimized. EtOH /
DSP was selected as an aqueous two-phase for the

Fig. 4. Contour surface and the three-dimensional response % EE of CGA.

Fig. 5. Iterations to maximize % E.E. with genetic algorithms.

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conflict of interest

The authors declare that they have no conflict of interest.

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