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Arqueología

Importance of glycoalkaloids analysis (α-solanine and α-chaconine) derived from potato consumption in pre-Hispanic inhabitants of the Americas

Importancia del análisis de glicoalcaloides (α-solanina y α-chaconina) por consumo de papa en los habitantes prehispánicos de América

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Abstract

In the pre-Columbian societies of the Americas, a wide range of food practices was observed. However, several countries shared certain staple foods, such as the potato, which has held a significant place in the diet since ancient times. It is important to note that potatoes contain glycoalkaloids, a class of alkaloids with potential toxicity risks when consumed in high concentrations by both humans and animals. This study aims to offer guidance on the presence of glycoalkaloids in potatoes and proposes further research into these compounds in archaeological remains that were utilized as food across all American countries. This recommendation stems from the dearth of studies on this subject, particularly in cases where archaeological discoveries include potato starch granules. In this study, various databases were examined to discover historical insights into the potato and to elucidate the primary aspects of glycoalkaloid chemistry associated with this tuber. The findings underscore the crucial role played by the potato in pre-Columbian cultures of the Americas, particularly in the regions situated along the Andes mountain range. Furthermore, the analysis of its nutritional composition unveiled the prevalence of two key glycoalkaloids in the tuber: α-solanine and α-chaconine. In this research, a comprehensive review of the chemical properties, botanical functions, human metabolism, potential health effects, toxicity thresholds, and available analytical techniques for the detection and quantification of toxic compounds was conducted. The significance of identifying these molecules in archaeological contexts was highlighted, as their presence may prompt investigations into prevalent diseases among historical populations.

Keywords: Diseases; Food; History; Cultivation.

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Resumen
En todo el continente americano, las sociedades precolombinas mostraron prácticas alimentarias diversas; no obstante, diferentes países compartían alimentos comunes. Un ejemplo es la papa, un tubérculo que ha sido un alimento básico en la dieta desde la antigüedad. Las papas contienen glicoalcaloides, una clase de alcaloides que, cuando se ingieren en concentraciones elevadas, pueden plantear riesgos de toxicidad tanto para los seres humanos como para los animales. El objetivo de este estudio es ofrecer una guía sobre glicoalcaloides presentes en papas, y recomendar que se realice investigación de estos compuestos en materiales arqueológicos que fueron destinados para alimentos en todos los países de América. Esta sugerencia surge de la ausencia de estudios de esta naturaleza, particularmente cuando los hallazgos arqueológicos incluyen gránulos de almidón de papa. Se revisaron varias bases de datos para encontrar información histórica sobre la papa e indicar los principales aspectos de la química de los glicoalcaloides relacionados con el tubérculo. Los principales hallazgos indican la importancia que representó la papa en las culturas precolombinas de América, especialmente en las regiones situadas a lo largo de la cordillera de los Andes. La investigación de la composición nutricional reveló que en el tubérculo se pueden encontrar dos glicoalcaloides predominantes: α*-solanina y* α*-chaconina. Se realizó un análisis bibliográfico de estos compuestos tóxicos para comprender su importancia, propiedades químicas, funciones botánicas, metabolismo en el ser humano junto con los posibles problemas de salud, umbrales de toxicidad y las diversas técnicas analíticas disponibles para su detección y cuantificación. La identificación de estas moléculas adquiere importancia en contextos arqueológicos, ya que su presencia puede dar lugar a investigaciones sobre posibles enfermedades prevalentes en la población de la época.*

Palabras clave: Enfermedades; Alimentos; Historia; Cultivo.

Introduction

Nutrition in antiquity significantly influenced the evolutionary trajectory of humanity. However, it is important to recognize that food derived from the Earth may contain toxic substances, posing potential health risks and even mortality to (Urugo & Tringo, 2023). Present global research initiatives are dedicated to comprehensively unraveling the paleodiets of our ancestors by employing rigorous chemical analytical techniques. This approach is crucial due to the subjective nature of data on dietary practices obtained from several archaeological studies. Furthermore, there is an imperative need to explore potential toxins encountered by individuals in the past, with the goal of comprehending the possible health implications associated with their dietary exposures (Song *et al.*, 2020).

Evidence suggests that the consumption and cultivation of diverse potato varieties in the American continent can be traced back 10,000 years. This tuber played a significant role as the primary source of sustenance in the diets of various populations (Jorgensen *et al.,* 2023; Pearsall, 2008). Over millennia, the cultivation and utilization of potato starch as a food source have been documented in various regions of the Americas, ranging from the United States to Chile and Argentina (Gavrilenko *et al.,* 2023). Notably, the significance of potato consumption is prominent in present territories of Peru, Ecuador, and Bolivia (Melton *et al.,* 2020; Ordoñez-Araque *et al.,* 2022; Rumold & Aldenderfer, 2016). Research conducted in the Andes Mountains region provides valuable insights into the historical consumption of potatoes. Archaeological settlements in this area have undergone thorough analysis, including the examination of residues, starch analysis, and scrutiny of skeletal remains from refuse deposits, in order to determine the potential diets of the inhabitants.

The inhabitants of the Ecuadorian highlands during the Formative period had a diet comprising potatoes, maize, various types of beans, chili peppers, oca, quinoa, avocado, custard apple, as well as rabbit, deer, and fish (Idrovo, 2002; Pearsall, 2008; Stahl, 2003; Zeidler, 2008). Investigations at the Cotocollao archaeological site in Quito revealed that the residents primarily consumed potatoes, maize, and quinoa (Mesia-Montenegro, 2014; Pearsall, 2003; Villalba, 1988; Zeidler, 2008). Additionally, Molestina (2006), indicated that maize and potatoes were the predominant foods among the population residing in the contemporary capital of Ecuador.

It is important to note that in archaeological contexts where the presence of potato starch has been identified in tools, researchers have overlooked investigating the potential existence of glycoalkaloids in order to determine the extent of exposure of the inhabitants to this alkaloid. Despite the identification of starch from various potato varieties in archaeological fragments, a specific analysis for α-solanine and α-chaconine, which are potato glycoalkaloids, has been notably absent. For example, Louderback & Pavlik (2017) identified starch from a papata variety, the oldest documented in the Utah region, yet did not conduct an analysis for glycoalkaloid content. Although it has been suggested in the discussion section that wild potato varieties may have higher glycoalkaloid content, explicit recommendations for their analysis have been lacking in research efforts.

In the context of this introduction, this study seeks to

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provide a comprehensive overview of the key aspects of glycoalkaloids present in potatoes. The primary objective is to underscore the significance of analyzing these compounds and shed light on the potential health implications encountered by pre-Hispanic inhabitants of the Americas, given the substantial role of tubers, particularly potatoes, in their dietary habits. This research is targeted towards professionals working with archaeological materials for the analysis of food biomolecules, aiming to encourage the examination of α-solanine and α-chaconine in archaeological artifacts or utensils such as lithics and ceramics.

Nutritional Aspects and Toxic Compounds of Potatoes

The dietary composition of ancient American settlements varies and is not consistently determined through chemical methods. However, it is evident that potatoes and other tubers were essential components of their daily diet (Shoji *et al.,* 2023). The potato, a consumable tuber indigenous to South America, is derived from the herbaceous species *Solanum tuberosum* within the *Solanum* genus of the Solanaceae family. Historically and currently, the potato is one of the most crucial global crops, exhibiting diverse shapes and colors, and demonstrating resilience to challenging climatic conditions. The worldwide estimate of potato varieties surpasses five thousand, with approximately 4500 found in South America, spanning from Venezuela to Chile. It is noteworthy that 100 species are classified as wild among these varieties (Rondon *et al.,* 2022).

Potatoes contain approximately 20% starch and are a rich source of various nutrients, including vitamins such as thiamin, riboflavin, vitamin C, and niacin, as well as minerals like iron, phosphorus, potassium, and calcium. The consumption of potato skin also provides dietary fiber and a variety of bioactive compounds, including carotenoids and phenolic compounds. However, it is important to note that potatoes may also contain toxic compounds known as glycoalkaloids, which are bitter-tasting alkaloids produced as a defense mechanism against parasites, insects, and pathogenic microorganisms. Consumption of these toxic compounds can potentially lead to health issues (Sharma *et al.,* 2020). Alkaloids are nitrogenous organic compounds that typically arise from the secondary metabolism of plants and are synthesized from amino acids or N-heterocyclic compounds. They can be categorized based on their structure into indoles, quinolines, isoquinolines, pyrrolidines, pyridines, pyrrolizidines, steroids, tropanes, and terpenoids (Kurek, 2019).

Glycoalkaloids are a group of alkaloids known for their steroid structure with nitrogen and their connection to one or more carbohydrate molecules. After the removal of the carbohydrate, the glycoalkaloid transforms into a molecule referred to as aglycone. Potatoes have the ability to produce multiple glycoalkaloids, such as α-solanine, β-solanine, γ-solanine, α-chaconine, β-chaconine, γ-chaconine, α-solamarines, β-solamarine, demissidine, and 5-β-solanidan-3-aol. The primary glycoalkaloids, α-solanine (consisting of the sugar solatriose, comprising D-glucose, D-galactose, and L-rhamnose) and α-chaconine (composed of chacotriose, which is formed by D-glucose and two L-rhamnose units), hold particular significance as they collectively make up 95% of the total glycoalkaloids found in potatoes (Zarins & Kruma, 2017).

Chemistry of glycoalkaloids

The glycoalkaloids are a group of steroidal alkaloids with insolubility in water, chloroform, and ether. They are categorized based on the plant family to which they belong, including *Veratrum* (a genus of poisonous perennial herbs in the Melanthiaceae family), *Solanum* (a genus of herbaceous, shrubby, or climbing plants in the Solanaceae family), steroidal alkaloids of *Apocynaceae* (a Dicotyledon family), and Buxus (a genus of plants in the Buxaceae family) (Ginzberg *et al.,* 2008). This paper focuses on the glycoalkaloids produced in plants of the Solanaceae family, which are derived from non-nitrogenous precursors. These steroidal alkaloids are synthesized through an ester bond containing nitrogen in its structure, usually linked to one or several sugar molecules, commonly known as solanidin (aglycone).

The glycoalkaloids α-solanine and α-chaconine are synthesized from cholesterol via cyclization to form solanidine, followed by glycosylation. These compounds are associated with tetracyclic triterpenoids of cyclopentanoperhydrophenanthrene and contribute to a bitter taste. In addition to potatoes, glycoalkaloids can be found in other plants from the Solanaceae family, including those yielding vegetables such as tomatoes or eggplants (Dey *et al.,* 2019). As mentioned previously, the primary glycoalkaloids in potatoes are α-solanine and α-chaconine. These compounds are composed of a polar segment (consisting of variable monosaccharides binding at carbon 3), a lipophilic steroid (non-polar), and a nitrogen-containing heterocyclic compound (Ginzberg, 2008; Zarins, 2017).

The role of glycoalkaloids in plants

In plants of the Solanaceae family, both α-solanine and α-chaconine serve as secondary metabolites, acting as defense mechanisms against a range of environmental stresses including pest attacks, inadequate irrigation, and extreme temperatures. During times of stress, there is an observed increase in glycoalkaloid production in the crop. These compounds are distributed across various plant parts, including the stems, leaves, skin, and potato flesh. Synthesis of these compounds commences during germination and peaks during the flowering stage. Additionally, research suggests that post-harvest, potatoes can continue to produce glycoalkaloids,

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dependent on storage conditions.

Exposure to ultraviolet light after harvesting can create stress conditions. Uncontrolled humidity during storage may lead to the emergence of pests such as fungi, causing the potato to activate its defense mechanisms (Haddadin *et al.,* 2001).

In a study conducted by Romanucci *et al.* (2016) in Italy, the levels of α-solanine and α-chaconine in potatoes from a market were investigated. The analysis was performed at the time of cultivation and after a 20-day storage period in a dark room at ambient temperature. The results indicated that during storage, four potato varieties showed increased α-solanine levels, while all varieties displayed elevated α-chaconine concentrations, surpassing the recommended limits by the end of the study. These findings raised concerns as the storage took place without sunlight. This suggests that metabolic processes in potatoes persist post-cultivation, potentially leading to the generation of more toxic compounds over time, regardless of the storage conditions.

Human metabolism

The compounds α-solanine and α-chaconine both contain solanidine. Several studies have detailed the synthesis of the chaconine molecule from solanine. Additionally, it has been proposed that these two glycoalkaloids could be formed simultaneously, potentially exerting a synergistic effect on the plant (Friedman *et al.,* 2002). This is notable as it suggests that the consumption of potatoes containing both glycoalkaloids may have a more pronounced impact on health. The presence of α-solanine and α-chaconine has been observed to inhibit the activity of acetylcholinesterase (EC 3.1.1.7), an enzyme primarily found in nervous tissues and red blood cells responsible for the breakdown of the neurotransmitter acetylcholine. This inhibition leads to an increase in acetylcholine concentration, thereby impacting the parasympathetic nervous system with characteristic cholinergic symptoms such as heightened fluid secretion and nerve transmission inhibition (McGehee *et al.,* 2000). Additionally, these glycoalkaloids exhibit irritant properties towards the gastric mucosa and esophagus. Moreover, in the presence of somatic cells, they disrupt the active transport of sodium and modify the relationship between phospholipids and liposomes (Simões, 2008).

Health issues

The ingestion of potatoes containing elevated concentrations of α-solanine and α-chaconine can result in a range of health implications, contingent on the quantity consumed. Notably, these implications are pertinent to both humans and animals (Mensinga *et al.,* 2005). Numerous documented pathologies are associated with the consumption of glycoalkaloid-containing foods, with the most severe outcomes including teratogenic effects. Furthermore, these alkaloids may contribute to gastrointestinal disorders, neurological alterations, chronic gastrointestinal bleeding, edema, and potentially, fatality depending on the dosage. Concerning teratogenicity, empirical evidence suggests an increased risk of birth defects, particularly neural tube defects. A correlation has been established between the consumption of these toxins and the incidence of anencephaly and spina bifida (Martín, 2011; Ni *et al.,* 2018). In human beings, the consumption of vegetables containing glycoalkaloids has the potential to elicit toxic effects such as nausea, vomiting, and diarrhea (Schrenk *et al.,* 2020). A study conducted by Friedman & Rasooly (2013), investigated the teratogenic effects of α-solanine, α-chaconine, and various aglycones on frog embryos. The results indicated that α-chaconine demonstrates higher teratogenic and embryotoxic effects in comparison to α-solanine, as evidenced by the mean lethal concentration LC50 following a 96-hour exposure to the compounds. Furthermore, it was established that the synergistic combination of α-solanine and α-chaconine leads to more severe malformations and mortality than the effects observed with isolated solanidine. This suggests a trend that these compounds, in the absence of the carbohydrate side chain, induce fewer toxic effects, underscoring the potential significance of this chain in regulating teratogenicity (Yamashoji & Matsuda, 2013).

Research has revealed the existence of inhibitors for α-solanine and α-chaconine, such as folic acid, methotrexate (a structural analogue of folic acid), glucose-6-phosphate (a glucose molecule phosphorylated at carbon 6), and the oxidized form of nicotine adenine dinucleotide (NADP). The aforementioned compounds have exhibited the capability to protect frog embryos from induced malformations, particularly severe anencephaly in the brain and less pronounced malformations in other organs, subsequent to exposure to α-chaconine (McWilliams *et al.,* 2000). When externally administered, α-solanine demonstrates sedative and analgesic effects by inducing paralysis of sensitive nerves.

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There are reports suggesting that glycoalkaloids found in potatoes might have a positive effect on reducing human immunodeficiency virus (HIV) infection and intestinal infections associated with acquired immunodeficiency syndrome (AIDS). Nevertheless, further research is required to substantiate this hypothesis (Barceloux, 2009; Martín, 2011).

Thresholds for toxicity

The World Health Organization (WHO), in collaboration with the Food and Agriculture Organization of the United Nations (FAO), has established the safe threshold for human consumption of glycoalkaloids from potatoes to be in the range of 2 to 10 mg per 100 g of fresh weight of the tuber. As previously mentioned, the quantity of glycoalkaloids is influenced by various factors, including the type of potato, as alkaloid levels are genetically determined. It is also noteworthy that the peel serves as the primary site for the concentration of glycoalkaloids in the potato (Kuete, 2014). The presence of high levels of α-solanine and α-chaconine has been linked to potential health risks. Administration of doses ranging from 2 to 5 mg per kg of body weight may induce symptoms of toxicity, while doses exceeding 6 mg per kg of body weight can result in fatal consequences. Adverse health effects have been reported with doses of 200 to 400 mg for adults and 20 to 40 mg for children. It is expected that a standard commercial potato contains less than 0.2 mg of glycoalkaloids, although this quantity can significantly escalate if the crop is subjected to inadequate care, such as insufficient water, pesticide use, or lack of fertilizer. Furthermore, despite adherence to concentration levels during cultivation, the content of α-solanine and α-chaconine may undergo an increase during storage (Izawa *et al.,* 2010; Kuete, 2014). In instances where a potato exhibits a bitter taste, it may signal a potential high concentration of glycoalkaloids, often accompanied by an intensified green coloration as they accumulate. Despite being thermostable and bioaccumulative compounds, certain post-harvest processes such as washing, blanching, and cooking treatments might lead to a partial reduction of their concentration. It is noteworthy that boiling does not entirely eliminate these glycoalkaloids; however, significant denaturation occurs during frying. Consequently, some research studies have recommended avoiding regular consumption of potatoes with skin in light of these findings (Crews, 2014).

Analytical evaluation of glycoalkaloids

In the examination of glycoalkaloids in archaeological samples, it is crucial to discern the materials utilized in both food preparation and storage. Based on the expertise of the research group involved in this study, it is recommended to first conduct a starch analysis to establish whether the utensil was employed in the preparation of a potato-based dish. For instance, historical pots, traditionally utilized for cooking and food storage, retain substances capable of absorbing diverse organic compounds from the foods they housed. The innate porosity of ceramic and stone materials allows for the gradual absorption of molecules over time. Throughout the cooking process of specific foods, such as potatoes, certain constituents, including glycoalkaloids, can permeate the cellular structure of these materials. This phenomenon contributes to the prolonged preservation of the food over time (García-Granero *et al.,* 2022).

Starch recovery and identification can be conducted on small archaeological fragments using minimal sample amounts. It is recommended to directly scrape the archaeological sample, whether ceramic or lithic, using gloves and a sterile scalpel (Ciofalo *et al.,* 2018; Melton, 2020).

The procedure for recovering ancient starch involves the following steps: First, disinfect the area with a 5% acetic acid solution. Obtain at least 0.25 grams of the sample and place it in a sterile tube. Add 1.25 milliliters (ml) of cesium chloride with a specific gravity of 1.79 g/ cm^3 to the tube. Agitate the tube and centrifuge it at 3000 rpm for 20 minutes. Transfer the floating fraction to another sterile tube. Subsequently, add distilled water and centrifuge at 9000 rpm for 8 minutes. Discard part of the floating fraction, add distilled water again, and centrifuge at 5000 rpm for 5 minutes. Repeat this step. Discard the floating liquid and use the final fraction remaining in the tube. Place the final fraction on a slide plate with a drop of glycerol. Finally, the analysis of the plate should be carried out using an optical microscope capable of photography in both bright-field view and polarized light. For additional details, please see our research on starch and fatty acid analysis: (Ordoñez-Araque *et al.,* 2024).

Figure 1 displays a fragment of a vessel discovered at the Tagshima settlement in Quito, dating back to the Formative period (1500 - 500 BC) of the pre-Hispanic era in the city (Ordoñez-Araque, 2022). Starch analysis was performed on this fragment, resulting in the recovery of various starch granules, including potato starch (*Solanum Tuberosum* and *Solanum* spp.), following a previously described extraction protocol. The identification of starch granules from different species should be carried out using starch samples available in the laboratory where the analysis is conducted, alongside research on both ancient and contemporary starch. This can be exemplified by the works of Mesía-Montenegro & Weber (2023) and Pagán-Jiménez (2015).

After confirming that the archaeological artifact has been in contact with potatoes, a qualitative analysis can be conducted, followed by a quantitative analysis to determine the presence of glycoalkaloids. However, if there is enough sample available, the quantitative analysis can be performed directly without the need for a separate qualitative phase.

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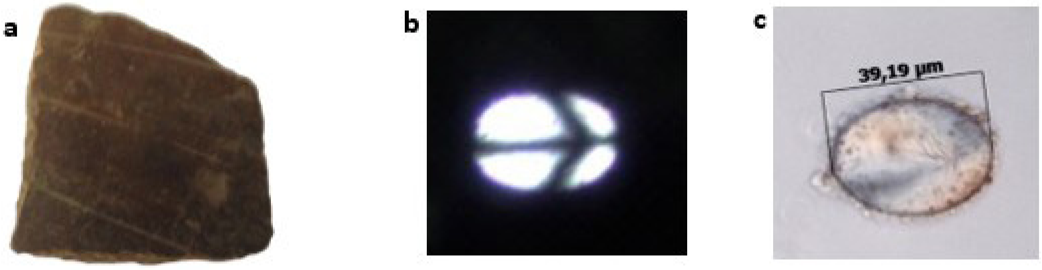

Figure 1. Archaeological evidence of potato starch. a: Fragment excavated from the Tagshima settlement in Quito. b-c: Images showing potato starch recovered from the archaeological fragment using optical microscopy. b: Starch under polarized light. c: Starch under bright light.

Figura 1. *Evidencia arqueológica de almidón de papa. a: Fragmento excavado en el asentamiento de Tagshima en Quito. b-c: Imágenes que muestran el almidón de papa recuperado del fragmento arqueológico mediante microscopía óptica. b: Almidón bajo luz polarizada. c: Fécula bajo luz brillante.*

<u>Qualitative phase</u>

In this phase, we can swiftly ascertain the potential presence of alkaloids in a sample. Through various tests, we can observe whether the residue under analysis is positive for any alkaloids in general. It is worth noting that this stage is recommended when there is a sufficient sample size. Otherwise, the analysis of starch alone should be conducted. For expeditious analyses, it is imperative to consider that if the sample is dissolved in an organic solvent, the solvent must be evaporated beforehand. The sample should be dissolved in 1 ml of hydrochloric acid (1%), and the addition of 0.15 ml of the reagent should align with the specific assay being conducted (Bermejo *et al.,* 2014). An outline of the diverse tests employed to determine the presence of alkaloids in a given sample is provided in Table 1.

A method alternative to qualitative alkaloid detection is thin-layer chromatography (TLC). Various studies have investigated the presence of solanine in different foods. For instance, Vélez-Terreros (2016) conducted research on the analysis of solanine in eggplant (*Solanum melongena*). This study involved the extraction of glycoalkaloids through maceration using solvents such as acetic acid, absolute ethanol, or a combination of acetic acid and ethanol.

In the process of Thin-Layer Chromatography (TLC) identification, it is imperative to have access to specified standards, such as α-solanine, featuring a purity level of 99%. Silica gel chromatoplates are utilized and introduced into the chromatographic chamber. The identification of the particular compound is accomplished through the comparison of retention factors. However, it is crucial to recognize that while this methodology has proved effective, it has been executed with a 10-gram food sample, a quantity that may not be viable within archaeological contexts.

<u>Glycoalkaloid identification</u>

Test	Positive alkaloid reaction indicator	Comments
Dragendorff	Opalescence Definite turbidity Precipitate	This assay is widely employed in research, involving the addition of Dragendorff's reagent.
Mayer	Opalescence Definite turbidity Heavy precipitacion	Mayer's reagent is employed. Prior to applying the reagent, sodium chloride powder is added, shaken, and filtered.
Wagner	Opalescence Definite turbidity Heavy precipitacion	Wagner's reagent is employed.
Liebermann-Burchard	Red, brown or deep green color	The Liebermann-Burchard reagent, comprising acetic anhydride, chloroform, and sulfuric acid, is employed.

Table 1. Tests for detecting alkaloid presence. Positive results for alkaloids are determined if any of the three reactions occur during each test. It is worth noting that in Mayer's assay, the presence of opalescence alone may lead to a false positive for certain alkaloids. Bermejo, 2014; Vélez-Terreros & Pilaquinga (2016).

Tabla 1. *Pruebas para detectar la presencia de alcaloides. Los resultados positivos para alcaloides se determinan si cualquiera de las tres reacciones ocurre durante cada prueba. Vale la pena señalar que en el ensayo de Mayer, la sola presencia de opalescencia puede dar lugar a un falso positivo para ciertos alcaloides. Bermejo, 2014; Vélez-Terreros & Pilaquinga (2016).*

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Several technologies are utilized for the quantification of α-solanine and α-chaconine. However, it is important to acknowledge the impracticality of quantifying these compounds in archaeological samples due to the limited sample size. While these methods allow for the identification of glycoalkaloid presence, determining precise concentrations may be unfeasible when dealing with minimal sample quantities. Nevertheless, these analytical techniques contribute to understanding the existence of these glycoalkaloids and provide insights into the potential health implications associated with their historical consumption. In the industry, the AOAC methodology, particularly Official Method 997.13 (Glycoalkaloids α-Solanine and α-Chaconine in Potato Tubers), is commonly relied upon for glycoalkaloid analysis using high-performance liquid chromatography (HPLC-PDA). This methodology involves the construction of standard curves for individual glycoalkaloids, the establishment of detection and quantification limits, and the calculation of peak areas obtained during the analysis (Aziz et al., 2012; Prasad et al., 2020). Nevertheless, alternative methodologies exist that can produce superior results, as they require minimal sample quantities (ranging from 0.25 to 0.5 grams) to detect various compounds, including traces of glycoalkaloids in the order of parts per billion. For this analysis, techniques such as liquid chromatography coupled with mass spectrometry (LC-MS) can be employed, which is one of the most commonly used approaches for determining α-solanine and α-chaconine glycoalkaloids.

An additional notable methodology involves the utilization of extraction and ultra-performance liquid chromatography in conjunction with an electrospray ionization triple-quadrupole tandem mass spectrometer (UPLC-MS/MS). This approach has the potential to generate optimal results, particularly in the examination of samples containing archaeological sediments spanning hundreds or thousands of years, owing to its capability to handle minute sample quantities (Liu et al., 2014). This method was utilized to detect the presence of theobromine alkaloid from cocoa in vessels dating back to 3500 B.C. in the Ecuadorian Amazon, as demonstrated by Zarrillo et al. (2018). While it is feasible to quantify the quantity of glycoalkaloids using these technologies, it is recommended to conduct preliminary experiments prior to analyzing archaeological samples. These experiments should replicate the process of cooking potatoes in pots, followed by scraping and analyzing small amounts. Furthermore, the experiments should involve combining traces of genuine potatoes with material from a pot. The objective is to establish which technique yields the most accurate results, even when glycoalkaloids are present in minimal quantities in the sample. This recommendation is crucial as there is currently no research guiding the selection of the optimal technique for analyzing glycoalkaloids in archaeological samples.

Conclusions

The analysis of glycoalkaloids in archaeological specimens has not been extensively documented in studies focused on the identification of potato starch. Given the historical evidence of potato consumption in the Americas and the longstanding cultivation of potatoes in the Andes mountain range of South America for millennia, it is pertinent to recommend the investigation of these compounds in archaeological materials from the predecessors of diverse settlements.

The excessive intake of glycoalkaloids has been associated with various health issues. Considering the historical absence of stringent controls in the cultivation and storage of tubers such as potatoes, it is reasonable to hypothesize that ancient populations were exposed to potatoes containing elevated concentrations of α-solanine and α-chaconine. This exposure may have resulted in severe health implications and potential pathologies.

The collaboration among professionals analyzing archaeological food samples, including archaeologists, anthropologists, chemists, and nutritionists, is paramount for ensuring high-quality and unbiased results. A comprehensive understanding of the chemistry of substances to which our ancestors were exposed is also crucial. Consequently, articles of this nature aim to serve as a guide for individuals lacking a background in this field, providing a simplified comprehension of the chemistry involved in different molecules.

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References

Aziz, A., Randhawa, M., Butt, M., Asghar, A., Yasin, M., & Shibamoto, T. (2012). Glycoalkaloids (α-chaconine and α-solanine) contents of selected Pakistani potato cultivars and their dietary intake assessment. *Journal of Food Science*, 77(3), 58–61. https://doi.org/10.1111/J.1750-3841.2011.02582.X

Barceloux, D. (2009). Potatoes, tomatoes, and solanine toxicity (Solanum tuberosum L., Solanum lycopersicum L.). *Disease-a-Month : DM*, 55(6), 391–402. https://doi.org/10.1016/J.DISAMONTH.2009.03.009

Bermejo, A., Pereira, S., Cintra, J., & Morales, G. (2014). Determinación de parámetros químico- físico de las tinturas al 20% obtenidas de las hojas, tallos y frutos de Melia azedarach L (Pursiana). *Revista Habanera de Ciencias Médicas*, 13(5), 670–680.

Ciofalo, A., Keegan, W., Pateman, M., Pagán-Jiménez, J., & Hofman, C. (2018). Determining precolonial botanical foodways: starch recovery and analysis, Long Island, The Bahamas. *Journal of Archaeological Science: Reports*, 21, 305–317. https://doi.org/10.1016/J.JASREP.2018.07.022

Crews, C. (2014). Natural Toxicants: Alkaloids. *Encyclopedia of Food Safety*, 2, 251–260. https://doi.org/10.1016/B978-0-12-378612-8.00175-X

Dey, P., Kundu, A., Chakraborty, H., Kar, B., Choi, W., Lee, B., Bhakta, T., Atanasov, A., & Kim, H. (2019). Therapeutic value of steroidal alkaloids in cancer: Current trends and future perspectives. *International Journal of Cancer*, 145(7), 1731–1744. https://doi.org/10.1002/IJC.31965

Friedman, M., & Rasooly, R. (2013). Review of the Inhibition of Biological Activities of Food-Related Selected Toxins by Natural Compounds. *Toxins,* 5, 743–775. https://doi.org/10.3390/toxins5040743

García-Granero, J., Suryanarayan, A., Cubas, M., Craig, O., Cárdenas, M., Ajithprasad, P., & Madella, M. (2022). Integrating Lipid and Starch Grain Analyses From Pottery Vessels to Explore Prehistoric Foodways in Northern Gujarat, India. *Frontiers in Ecology and Evolution*, 10, 840199. https://doi.org/10.3389/FEVO.2022.840199/BIBTEX

Gavrilenko, T., Chukhina, I., Antonova, O., Krylova, E., Shipilina, L., Oskina, N., & Kostina, L. (2023). Comparative Analysis of the Genetic Diversity of Chilean Cultivated Potato Based on a Molecular Study of Authentic Herbarium Specimens and Present-Day Gene Bank Accessions. *Plants*, 12(1). https://doi.org/10.3390/PLANTS12010174/S1

Ginzberg, I., Tokuhisa, J., & Veilleux, R. (2008). Potato Steroidal Glycoalkaloids: Biosynthesis and Genetic Manipulation. *Potato Research* 52:1, 52(1), 1–15. https://doi.org/10.1007/S11540-008-9103-4

Haddadin, M., Humeid, M., Qaroot, F., & Robinson, R. (2001). Effect of exposure to light on the solanine content of two varieties of potato (Solanum tuberosum) popular in Jordan. *Food Chemistry,* 73(2), 205–208. https://doi.org/10.1016/S0308-8146(00)00279-X

Idrovo, J. (2002). El formativo en la Sierra Ecuatoriana. In P. Ledergerber -Crespo (Ed.), *Formativo en la Sierra Ecuatoriana Jaime Idrovo Urigüen* (3era., pp. 114–123). Abya-Yala.

Izawa, K., Amino, Y., Kohmura, M., Ueda, Y., & Kuroda, M. (2010). Human–Environment Interactions – Taste. *Comprehensive Natural Products II: Chemistry and Biology*, 4, 631–671. https://doi.org/10.1016/B978-008045382-8.00108-8

Jorgensen, K., Garcia, O., Kiyamu, M., Brutsaert, T., & Bigham, A. (2023). Genetic adaptations to potato starch digestion in the Peruvian Andes. *American Journal of Biological Anthropology*, 180(1), 162–172. https://doi.org/10.1002/AJPA.24656

Kuete, V. (2014). Health Effects of Alkaloids from African Medicinal Plants. *Toxicological Survey of African Medicinal Plants*, 611–633. https://doi.org/10.1016/B978-0-12-800018-2.00021-2

Kurek, J. (2019). Introductory Chapter: Alkaloids - Their Importance in Nature and for Human Life. *Alkaloids - Their Importance in Nature and Human Life.* https://doi.org/10.5772/INTECHOPEN.85400

Liu, W., Zhang, N., Li, B., Fan, S., Zhao, R., Li, L. P., Wu, G. H., & Zhao, Y. (2014). Determination of α-chaconine and α-solanine in commercial potato crisps by QuEChERS extraction and UPLC-MS/MS. *Chemical Papers,* 68(11), 1498–1504. https://doi.org/10.2478/S11696-014-0617-8/MACHINEREADABLECITATION/RIS

Louderback, L., & Pavlik, B. (2017). Starch granule evidence for the earliest potato use in North America. *Proceedings of the National Academy of Sciences of the United States of America*, 114(29), 7606–7610. https://doi.org/10.1073/PNAS.1705540114/SUPPL_FILE/PNAS.201705540SI.PDF

Martín, I. (2011). *Determinación de glicoalcaloides: α-solanina y α-chaconina en patata mediante cromatografía de líquidos de ultra presión acoplada a espectrometría de masas de triple cuadrupolo.* [Universidad de Almería]. http://hdl.handle.net/10835/491

McGehee, D., Krasowski, M., Fung, D., Wilson, B., Gronert, G., & Moss, J. (2000). Cholinesterase inhibition by potato glycoalkaloids slows mivacurium metabolism. *Anesthesiology*, 93(2), 510–519. https://doi.org/10.1097/00000542-200008000-00031

McWilliams, M., Blankemeyer, J., & Friedman, M. (2000). The folic acid analogue methotrexate protects frog embryo cell membranes against damage by the potato glycoalkaloid alphachaconine. *Food and Chemical Toxicology : An International Journal Published for the British Industrial Biological Research Association*, 38(10), 853–859. https://doi.org/10.1016/S0278-6915(00)00090-9

Melton, M., Biwer, M., & Panjarjian, R. (2020). Differentiating Chuño blanco and Chuño negro in archaeological samples based on starch metrics and morphological attributes. *Journal of Archaeological Science: Reports*, 34, 102650. https://doi.org/10.1016/J.JASREP.2020.102650

Mensinga, T., Sips, A., Rompelberg, C., Van Twillert, K., Meulenbelt, J., Van Den Top, H., & Van Egmond, H. (2005). Potato glycoalkaloids and adverse effects in humans: an ascending dose study. *Regulatory Toxicology and Pharmacology*, 41(1), 66–72. https://doi.org/10.1016/J.YRTPH.2004.09.004

Mesia-Montenegro, C. (2014). El periodo formativo en los andes septentrionales y sus relaciones con los andes centrales. *Arqueología y Sociedad*, 0(27), 111–130.

Molestina, M. (2006). El pensamiento simbólico de los habitantes de La Florida (Quito-Ecuador). *Bulletin de l'Institut Français d'études Andines*, 35 (3), 377–395. https://doi.org/10.4000/bifea.3931

Ni, W., Tian, T., Zhang, L., Li, Z., Wang, L., & Ren, A. (2018). Maternal periconceptional consumption of sprouted potato and risks of neural tube defects and orofacial clefts. *Nutrition Journal* 2018 17:1, 17(1), 1–8. https://doi.org/10.1186/S12937-018-0420-4

Ordoñez-Araque, R., Ramos-Guerrero, L., Vargas-Jentzsch, P., Romero-Bastidas, M., Rodríguez-Herrera, N., Vallejo-Holguín, R., Fuentes-Gualotuña, C., & Ruales, J. (2024). Fatty Acids and Starch Identification within Minute Archaeological Fragments: Qualitative Investigation for Assessing Feasibility. *Foods* 13(7), 1090. https://doi.org/10.3390/FOODS13071090

Ordoñez-Araque, R., Ruales, J., Vargas-Jentzsch, P., Ramos-Guerrero, L., Romero-Bastidas, M., Montalvo-Puente, C., & Serrano-Ayala, S. (2022). Pre-Hispanic Periods and Diet Analysis of the Inhabitants of the Quito Plateau (Ecuador): A Review. *Heritage,* 5(4), 3446–3462. https://doi.org/10.3390/HERITAGE5040177

Pearsall, D. (2003). Plant food resources of the Ecuadorian Formative: an overview and comparison to the Central Andes. In Raymond & L. Burger (Eds.), *Archaeology of Formative Ecuador* (pp. 213–257). Dumbarton Oaks Research Library and Collection.

Pearsall, D. (2008). Plant Domestication and the Shift to Agriculture in the Andes. In *The Handbook of South American Archaeology* (pp. 105–120). Springer, New York, NY. https://doi.org/10.1007/978-0-387-74907-5_7

Prasad, A., Patel, P., Pandey, S., Niranjan, A., & Misra, P. (2020). Growth and alkaloid production along with expression profiles of biosynthetic pathway genes in two contrasting morphotypes of prickly and prickleless Solanum viarum Dunal. *Protoplasma,* 257(2), 561–572. https://doi.org/10.1007/S00709-019-01446-3/METRICS

Romanucci, V., Pisanti, A., Di Fabio, G., Davinelli, S., Scapagnini, G., Guaragna, A., & Zarrelli, A. (2016). Toxin levels in different variety of potatoes: Alarming contents of α-chaconine. *Phytochemistry Letters*, 16, 103–107. https://doi.org/10.1016/J.PHYTOL.2016.03.013

Rondon, S., Carrillo, C., Cuesta, H., Navarro, P., & Acuña, I. (2022). Latin America potato production: pests and foes. In *Insect Pests of Potato: Global Perspectives on Biology and Management* (pp. 317–330). Academic Press. https://doi.org/10.1016/B978-0-12-821237-0.00019-6

Rumold, C., & Aldenderfer, M. (2016). Late Archaic-Early Formative period microbotanical evidence for potato at Jiskairumoko in the Titicaca Basin of southern Peru. *Proceedings of the National Academy of Sciences of the United States of America*, 113(48), 13672–13677. https://doi.org/10.1073/PNAS.1604265113/SUPPL_FILE/PNAS.201604265SI.PDF

Schrenk, D., Bignami, M., Bodin, L., Chipman, J. K., Mazo, J. del, Hogstrand, C., Hoogenboom, L. (Ron), Leblanc, J., Nebbia, C. S., Nielsen, E., Ntzani, E., Petersen, A., Sand, S., Schwerdtle, T., Vleminckx, C., Wallace, H., Brimer, L., Cottrill, B., Dusemund, B., … Grasl-Kraupp, B. (2020). Risk assessment of glycoalkaloids in feed and food, in particular in potatoes and potato-derived products. *EFSA Journal*, 18(8), 6222. https://doi.org/10.2903/J.EFSA.2020.6222

Sharma, S., Jaiswal, A., & Jaiswal, S. (2020). Chapter 21 - Potato. *Nutritional Composition and Antioxidant Properties of Fruits and Vegetables,* 339–347. https://doi.org/10.1016/B978-0-12-812780-3.00021-0

Shoji, K., Vásquez S., V. F., & Rosales T., T. E. (2023). Starch grains on human teeth as evidence for 4000 BCE potato consumption at the Cruz Verde site, northern coast of Peru. *Journal of Archaeological Science: Reports,* 51, 104152. https://doi.org/10.1016/J.JASREP.2023.104152

Simões, J. (2008). Evenenamento por glicoalcalóides da batata (solanum tuberosum) em bovinos. *Veterinaria,* 1–6.

Song, J., Wang, X., Wang, Y., Zhang, Y., & Yu, Y. J. (2020). High-throughput identification of volatile and semi-volatile organic compounds in archaeological samples by gas chromatography–mass spectrometry combined with advanced chemometrics methodology. *Microchemical Journal*, 158, 105289. https://doi.org/10.1016/J.MICROC.2020.105289

Stoessel, L., Martínez, G., & Constenla, D. (2015). Preliminary analysis of fatty acids recovered from archaeological pottery of the lower course of the Colorado River (North-eastern Patagonia): Contributions to the hunter-gatherers subsistence patterns. *Magallania*, 43(1), 231–249. https://doi.org/10.4067/s0718-22442015000100013

Urugo, M., & Tringo, T. (2023). Naturally Occurring Plant Food Toxicants and the Role of Food Processing Methods in Their Detoxification. *International Journal of Food Science*, 2023. https://doi.org/10.1155/2023/9947841

Vélez-Terreros, P., & Pilaquinga, F. (2016). Extracción e identificación de la solanina obtenida del fruto de la berenjena (Solanum melongena L.). *InfoANALÍTICA,* 4(1), 21–32. https://doi.org/10.26807/IA.V4I1.8

Villalba, M. (1988). *Cotocollao: una aldea formativa del valle de Quito* : Vol. Serie Monográfica 2 (Museo del Banco Central del Ecuador, Ed.). Miscelánea antropológica Ecuatoriana.

Yamashoji, S., & Matsuda, T. (2013). Synergistic cytotoxicity induced by α-solanine and α-chaconine. *Food Chemistry,* 141(2), 669–674. https://doi.org/10.1016/J.FOODCHEM.2013.03.104

Zarins, R., & Kruma, Z. (2017). *Glycoalkaloids in potatoes: a review.* https://doi.org/10.22616/FOODBALT.2017.002

Zarrillo, S., Gaikwad, N., Lanaud, C., Powis, T., Viot, C., Lesur, I., Fouet, O., Argout, X., Guichoux, E., Salin, F., Solorzano, R. L., Bouchez, O., Vignes, H., Severts, P., Hurtado, J., Yepez, A., Grivetti, L., Blake, M., & Valdez, F. (2018). The use and domestication of Theobroma cacao during the mid-Holocene in the upper Amazon. *Nature Ecology & Evolution*, 2(12), 1879–1888. https://doi.org/10.1038/s41559-018-0697-x

Zeidler, J. (2008). The Ecuadorian Formative. *The Handbook of South American Archaeology*, 459–488. https://doi.org/10.1007/978-0-387-74907-5_24