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Ancestral Peruvian ethnic fermented beverage “Chicha” based on purple corn (*Zea mays* L.): unraveling the health-relevant functional benefits

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Abstract

Chicha is a traditional ethnic corn-based fermented beverage with a profound cultural relevance in the Andean region, especially in Peru where it has been part of religious ceremonies since pre-Inca civilizations. *Chicha de Guiñapo* is made with purple corn and is a typical beverage from the Peruvian region of Arequipa (South of Peru) where some local people still preserve its traditional process and use, but no investigation has been focused on the study of its potential health-relevant functional properties linking the historical perspectives. The current research was aimed at advancing the ethnic cultural relevance of this traditional beverage with an understanding of deep historical and ecological roots and further by evaluating the differences in the traditional processing of *Chicha de Guiñapo* from five geographical zones in Arequipa (Peru). Furthermore, to understand the potential health relevance, the influence on the total phenolic content (TPC), the total anthocyanin content (TAC), the antioxidant capacity, the in vitro inhibition of α -glucosidase and α -amylase relevant for hyperglycemia management, along with the physicochemical characteristics, were evaluated. No significant differences were observed in the physicochemical parameters, the antioxidant capacity, and the phenolic contents among the *Chicha* samples from different origins. However, all samples showed a remarkable in vitro inhibition of α -glucosidase and α -amylase with a significant influence of the geographical zone of origin that links historical usage and contemporary health benefits. The Z2 *Chicha* sample that followed the most traditional preparation had the highest values of TAC, in vitro antioxidant capacity, and anti-hyperglycemia-relevant α -glucosidase and α -amylase inhibitory activities. Results from the current study reveal that *Chicha* has an important health-relevant functional potential and that the preservation of the traditional historical and ethnic knowledge about its processing is critical for its validation for wider use across all communities.

Keywords: *Chicha de Guiñapo*, Peruvian purple corn, Phenolic compounds, Ethnic relevance, Health

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Introduction

The word “*Chicha*” is used to describe a great variety of traditional ethnic fermented beverages highly consumed along the Andean region in South America, and made from different plant sources especially from maize (*Zea mays* L.) and among other grains such as some Andean pseudo cereals like quinoa (*Chenopodium quinoa*) (see Additional file 1, A). The origin of the word comes from the indigenous Panamanian word “*chibcha*” which means “maize” [1]. It is known that *Chicha* has been consumed for more than a thousand years in the Andean region, before the arrival of the Europeans and even before the rise of the empire of the Incas [2, 3].

The *Chicha* beverage was consumed as an integral part of daily food consumption and was used in religious ceremonies as a tribute for the dead along several important pre-Inca Andean cultures or larger civilizations, especially during the period of the *Chavin*, *Marcavalle*, *Churajon*, and *Tiwanaku* cultures (Fig. 1). Even though no written records exist about these practices during the pre-Inca period, several archeological studies have revealed that some ceramic plates and vessels were used for drinking *Chicha* [9, 10].

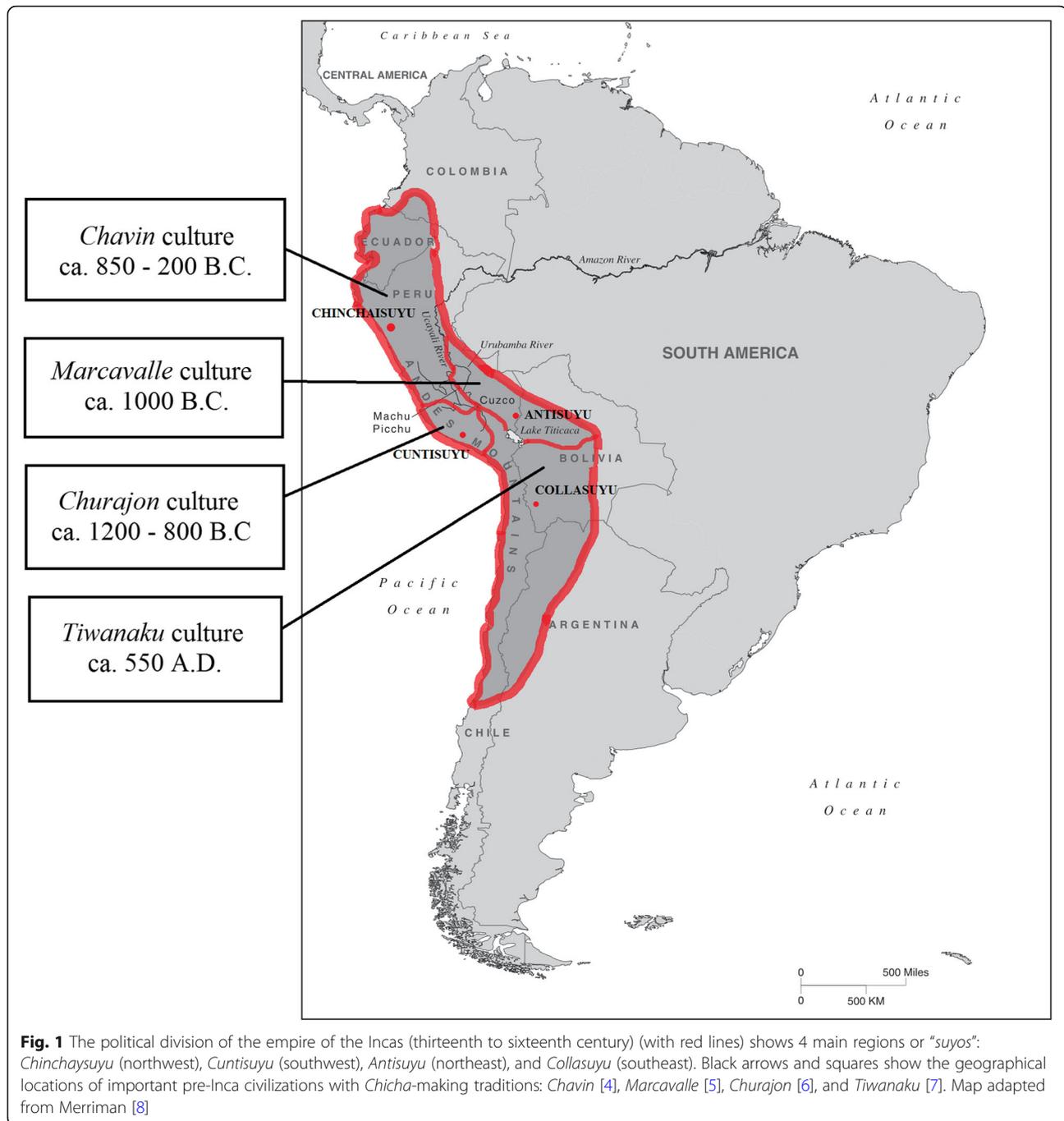
The geographical spread of the empire of the Incas (thirteenth to sixteenth century) extended to the present-day countries of Peru, Ecuador, Chile, and Argentina allowing the spread of *Chicha* preparation and consumption throughout the Andean region (Fig. 1). This beverage was used both as part of daily diet of the Inca population and as an essential element during the political and religious ceremonies. In this period, *Chicha* was not only made from corn but also from a variety of food plants such as oca (*Oxalis tuberosa*), cassava (*Manihot esculenta*), and quinoa (*Chenopodium quinoa*) [2]. The great extension of the empire of the Incas allowed the incorporation of the traditional practices of pre-Inca civilizations, and inhabitants across the empire therefore adapted the production of *Chicha* according to their own traditions and culture [10].

The expression “*Chicha de Jora*” was used for the first time during the colonial time when Spanish people invaded the territory of the Incas (sixteenth to nineteenth century). *Chicha de Jora* is a fermented beverage made only from maize and has a wide variety of colors and flavors depending on the type of maize used for its processing. The term “*Jora*” refers to the use of a completely germinated corn kernel as a raw material for *Chicha* preparation. During this period, the “*chicherias*” (places where *Chicha* was prepared and sold), “*pulperias*” (taverns), and “*picanterias*” (traditional places where people gathered to eat, drink *Chicha* and talk about the events of the day) appeared for the first time [2]. Currently, some local restaurants have preserved the tradition of *picanterias* in certain regions of Peru.

The region of Arequipa is in the south west of Peru and has eight geographical provinces with a capital city also named Arequipa which is located at an average altitude of 2335 m above sea level (m.a.s.l.) close to the Southern Andean mountains (Fig. 2). Ethnically, people from Arequipa city are mainly “mestizos” descendants of the native Americans and Spanish population that settled down in this area in the sixteenth century. This ethnic background has shown a marked influence on several cultural and historical aspects of Arequipa including its local gastronomy that has incorporated Andean foods and ingredients derived from foods introduced by the Spanish. However, the consumption of *Chicha* has been maintained during the colonial time until now, and its method of preparation in principle has been preserved since the pre-Inca ancestral and historical period with some modifications across time and depending on the Andean location [10].

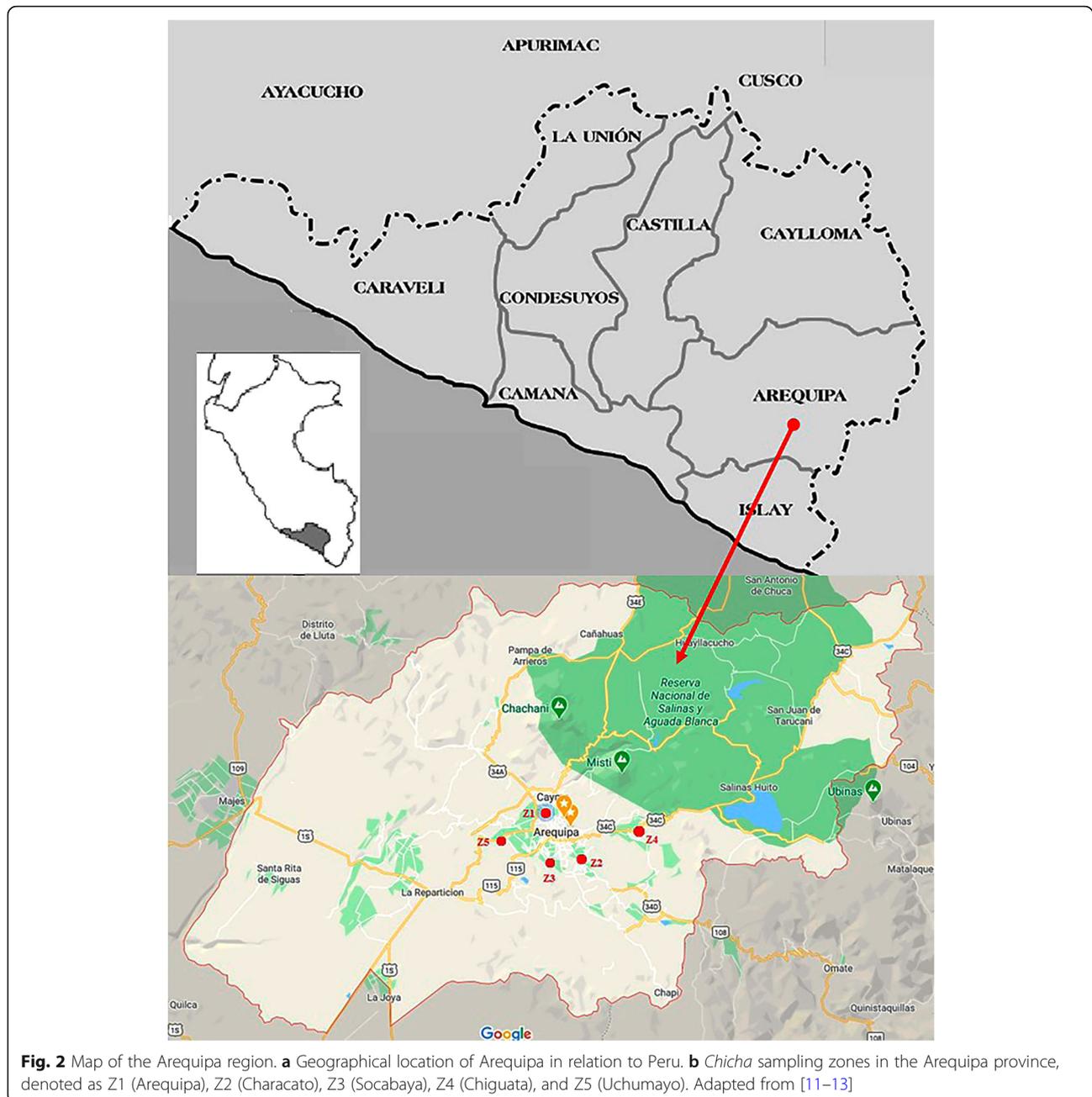
In Arequipa, the beverage is known as “*Chicha de Güiñapo*” and has become unique compared to *Chicha* from other regions of Peru due to the use of partially germinated purple corn (*Güiñapo*) as the main ingredient according to the oral tradition. This type of *Chicha* is produced by first germinating grains of local purple-black corn in a “*pooyo*” (a rectangular stone container) which is covered with straws and watered for 15 days approximately. After this step, partially germinated kernels are sun-dried and then milled into a coarse flour (Fig. 3). This flour is subsequently boiled with water for some hours to be then filtered. The insoluble cake is used to feed poultry and the filtered liquid is fermented overnight using “*concho*” or the residues of *Chicha* from the previous day [2] (Fig. 4). *Chicha de Güiñapo* was the historical basis of the ethnic food culture and gastronomy of Arequipa until the nineteenth century and the beverage was even more important than food itself [3]. The migration of people from the Peruvian coastal cities such as Lima and Trujillo influenced the *Chicha* processing replacing for example the use of locally grown purple corn by more commercial purple corn varieties which give a different flavor and texture to the final beverage. Slowly, its name and indication as *Chicha de Güiñapo* was changed to *Chicha de Jora* and “*chicherias*” lost importance giving prevalence to “*picanterias*.”

Currently, some places in Arequipa are trying to preserve the tradition of *picanterias* (where *Chicha* and typical food are prepared) and to reintroduce the name *Chicha de Güiñapo* specially for reinforcing the local cultural identity. Besides its use as a beverage, *Chicha* still has a deep cultural and religious meaning for native people from Andean locations in Arequipa. For example, in the present-day personal observations in the district



of Viraco (Castilla province in Arequipa) located at more than 3000 m.a.s.l, *Chicha* has been used as an offering together with flowers, coca (*Erythroxylum coca*) leaves, llama fat (*pichihuirra* in the native *quechua* language), corn kernels, and cigars to give thanks to the "Pachamama" (mother earth in *quechua*) during the months of February and the winter solstice in June. The offering is done in a selected sacred place within the *chacras* (local farms) and is directed by a "chaman" (a sort of

indigenous priest) in a massive fest with the objective to "pay the earth" or to give thanks to the earth for its generosity in the obtained harvest or the abundance of dairy and sheep cattle. After the *chaman* prayers in *quechua* language, all the offerings are buried, excepting *Chicha* which is poured on the ground. There is a strong belief among native farmers that if they do not give thanks to the earth in such ceremonies, next year's harvest and cattle will not be as fruitful as expected. Jennings [10]



has also described the use of *Chicha* in special events and feasts in other Andean regions in the country demonstrating that *Chicha* represents an important ethnic beverage in Peru.

Chicha de Jora which is more common in several regions of Peru differs from *Chicha de Gününapo* in several processing parameters. *Chicha de Gününapo* is made with partially germinated purple corn grains as stated above while *Chicha de Jora* is made with fully germinated purple corn kernels. Moreover, in the production of *Chicha de Gününapo*, the total fermentation time is

about 12 h, whereas in *Chicha de Jora*, the fermentation time can go up to several days [7]. These differences may influence the sensorial characteristics of *Chicha* such as the flavor and the chemical and bioactive composition along with the microbiome profile after fermentation. Other variations of *Chicha* across the Andean region are associated with the use of different types of maize (purple corn, white or other colored corn kernels), the addition of spices or sugar, and the application of different fermentation times, among other factors [10, 14].

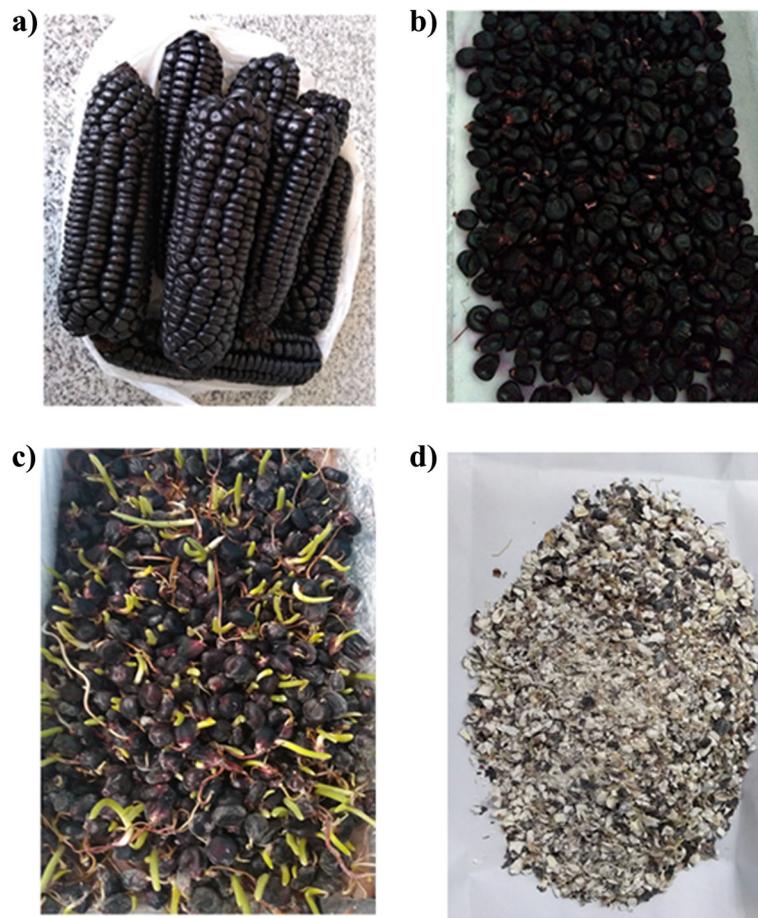


Fig. 3 Traditional processing of *Chicha de Güiñapo* (part 1): **a** mature purple corn ear, **b** purple corn kernels, **c** Partially germinated corn showing green seedlings, and **d** Milled germinated corn (*Güiñapo*)

Some types of *Chicha* from other countries or similar products derived from corn fermentation have been characterized mainly in terms of the microbiome composition [14–16]. However, the differences in current traditional processing of *Chicha* based on historical perspectives from several geographical areas in Peru and their association of the potential health-relevant bioactivity of *Chicha* based on fermentation of purple corn have not been investigated yet.

The current research was aimed at advancing the health relevance of this ethnic beverage treasure with deep historical relevance and perspectives by studying for the first time this purple corn-based fermented beverage from the Peruvian Andean region of Arequipa which has a well-known tradition in *Chicha* preparation based on deep historical knowledge. Main objectives were to document the traditional processing of *Chicha de Güiñapo* collected from different locations in Arequipa with historical knowledge and to evaluate its phenolic (total phenolics and anthocyanins) composition, the in vitro antioxidant capacity, and the potential

in vitro inhibitory activity of α -amylase and α -glucosidase bioactivity relevant for hyperglycemia management. This study would help to understand the potential of *Chicha* as a functional ethnic beverage based on historical perspectives and to target the improvement of the traditional processing of *Chicha* relevant for its validation and preservation of its ethnic roots and historical value.

Materials and methods

Chicha samples

Chicha samples were collected from five geographical zones (districts) located in the southern Peruvian region of Arequipa (Arequipa province): Arequipa, Characato, Socabaya, Chiguata, and Uchumayo and coded as Z1, Z2, Z3, Z4, and Z5, respectively. The samples were collected once a week for three consecutive weeks (each sample was considered a replicate per zone of collection). During the collection, a survey was applied to owners of “*picanterias*” for documenting the likely differences in *Chicha* processing.



Fig. 4 Traditional processing of *Chicha de Günüñapo* (part 2): **a** boiling of *Günüñapo*, **b** filtration using a special cloth, **c** *Chicha* before fermentation, **d** fermentation in traditional ceramic pots (*chombas*), **e** *Chicha de Günüñapo* ready for consumption and served in a “*capora*” (typical glass that mimics the shape of “*Kero*” which was a vessel used to drink *Chicha* in the Inca period)

Collected samples were transported in a refrigerated environment ($< 10^{\circ}\text{C}$), and once in the laboratory samples were centrifuged at 2000 rpm for 10 min, and supernatants were degasified by vortex at 1200 rpm for 5 min and then stored at -20°C until analysis.

Enzymes

α -Glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20) and α -amylase (EC 3.2.1.1) from porcine pancreas were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Physicochemical analyses

The pH was determined using a pH meter. Soluble solids were determined using a refractometer at 20°C . The alcohol content was determined as a percentage of ethanol according to the AOAC 935.19 method [17]. Titratable acidity (TA) was expressed as milligrams of lactic acid per 100 mL of *Chicha* according to the AOAC 942.15 method [17].

Total phenolic content (TPC)

The total phenolic content was determined by a modified Folin-Ciocalteu method based on the Singleton and Rossi method [18]. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per 100 mL of *Chicha*.

Total monomeric anthocyanin content (TMA)

The total monomeric anthocyanin content was determined by the differential pH method [19]. The results were expressed as milligrams of cyanidin-3-glucoside equivalents (C3G) per liter of *Chicha*.

Ultra-high performance liquid chromatography (UHPLC) analysis of total anthocyanin content (TAC)

The *Chicha* samples were centrifuged at 2000 rpm for 5 min and a 2-mL aliquot was vacuum evaporated at 40°C until dryness and resuspended in 1 mL of acidified water (0.01% HCl). The concentrated samples were then purified in a Strata solid phase extraction C18-E column ($55\ \mu\text{m}$, $70\ \text{Å}$) (Phenomenex Inc., Torrance, CA., USA)

previously pre-conditioned using 2 volumes of methanol (Sigma Chemical Co., St. Louis, MO, USA) followed by 2 volumes of acidified water. Samples were then eluted with acidified methanol (0.01% HCl), vacuum evaporated until dryness at 40 °C, and resuspended in 1 mL of acidified water [20].

Purified samples were filtered with a polyvinylidene difluoride filter (PVDF, 0.22 µm), and 5 µL was injected in an UltiMate 3000 RS UHPLC system (Thermo Fisher Scientific, Waltham, MA, USA), using an UltiMate 3000 RS quaternary pump and autosampler, a Vanquish diode array detection system, and controlled by the Chromeleon v7.2 SR4 software (Thermo Fisher Scientific). The anthocyanins were separated using a Kinetex C18 analytical column (100 × 2.1 mm i.d., 1.7 µm particle size; Phenomenex) protected with a Kinetex guard column (5 × 2.1 mm i.d., 1.7 µm particle size; Phenomenex). The sample vial tray was set at 5 °C, and a flow rate of 0.2 mL/min and a column temperature of 30 °C were applied. The solvents used for gradient elution were (A) 0.1% formic acid (pH 2.5) and (B) 100% acetonitrile. Initial conditions were 98% (A) and 2% (B), and the following gradient of solvent (B) was used: 2% for 2 min, then increased to 15% in 1 min and to 45% over the next 6 min, then increased to 98% over 1 min and maintained for 3 min, and finally set to initial conditions in 4 min with a re-equilibration time of 3 min (total run time of 20 min). The detection system was run from 200 to 600 nm, and the quantification was done at 525 nm. The sum of the areas of peaks detected at 525 nm was reported as TAC and expressed in milligrams of cyanidin chloride aglycone per liter (mg CC/L) using a calibration curve of this standard (Sigma Chemical Co., St. Louis, MO, USA) diluted in acidified methanol (0.5% acetic acid).

Total antioxidant activity

Total antioxidant activity was measured using two different methods: the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS+) free radical scavenging assays according to a modified method by Pérez-Jiménez [21].

The percentages of inhibitory activity obtained from the DPPH and ABTS radical scavenging assays were calculated and results were expressed as micromole of Trolox equivalents (TE) per 100 mL of *Chicha* using a curve of Trolox standard (100 to 900 µM) for both methods.

α-Glucosidase and α-amylase inhibition assays

The α-glucosidase and α-amylase inhibitory activities were determined using modified assay methods [22, 23]. The results were expressed as percentage of inhibition at different phenolic contents (GAE/mL).

Statistical analysis

All data were analyzed using the one-way analysis of variance (ANOVA) or the Kruskal-Wallis test when needed, following with the Tukey's test ($p < 0.05$) for multiple comparisons. The StatGraphics Centurion XVI software (Statgraphics Technologies, Inc., The Plains, Virginia) was used.

Results and discussion

Traditional *Chicha* processing

Figure 2 shows the geographical locations of *picanterias* (traditional restaurants) where *Chicha de Güiñapo* was sampled. A total of five zones corresponding to different districts (Z1:Arequipa, Z2:Characato, Z3:Socabaya, Z4:Chiguata, and Z5:Uchumayo) located in the province of Arequipa were selected based on the historical perspectives and ethnic antiquity of "*picanterias*" and because such places coincide with the location of pre-colonial "*curacazgos*" (local governments or provinces during the period of the empire of the Incas) where *Chicha* was likely prepared according to some historical records [6].

Table 1 summarizes the results of the surveys applied to owners of the five selected *picanterias* showing the different procedures for elaborating *Chicha de Güiñapo*. One of the most important difference is the type of raw material used. *Chicha* beverages from Arequipa and Characato districts (Z1 and Z2, respectively) are made with *Güiñapo* (partially germinated and milled purple corn kernel) elaborated with the purple corn cultivated in the farms located in the same districts; therefore, people in such *picanterias* elaborate their own *Güiñapo*. In addition, they still use the traditional instruments for preparing *Chicha* such as the "*batán*" (a special stone mill used for milling the germinated corn kernels into a coarse flour) and the "*chombas*" (ceramic vessels where *Chicha* is fermented) while the "*caporal*" (typical glass that mimics the shape of "*Kero*" which was a ceramic or wooden vessel used to drink *Chicha* in the Inca period) is used for serving the final *Chicha* (see Additional file 1, B,C).

Chicha from Socabaya, Chiguata, and Uchumayo (Z3, Z4, and Z5, respectively) are made with *Güiñapo* from commercial origin which is generally made with commercial purple corn kernels (likely of varieties from other regions in Peru) and once germinated and dried, the final product might be mixed with *Güiñapo* from different local suppliers. This reveals that "*picanterias*" are increasing the use of commercial *Güiñapo* which is not always processed using neither native purple corn varieties nor standardized conditions leading to potential differences in *Chicha* flavor and texture. Producers from these zones sometimes use the traditional tools for *Chicha* preparation or combine the use of plastic or

Table 1 Sampling locations and key points in the elaboration process of *Chicha de Güiñapo* in the Arequipa region

Zone	Arequipa (Z1)	Characato (Z2)	Socabaya (Z3)	Chiguata (Z4)	Uchumayo (Z5)
Latitude	– 16.457194	– 16.464156	– 16.457194	– 16.402603	– 16.424996
Longitude	– 71.543811	– 71.493390	– 71.543811	– 71.391579	– 71.663230
Name of the <i>Picanteria</i>	<i>La Nueva Palomino</i>	<i>Charito</i>	<i>Andenes del Koricancha</i>	<i>Doña Leo</i>	<i>Picanteria-Bar Marilú</i>
Raw material	Purple corn	Purple corn	Commercial Güiñapo ¹	Commercial Güiñapo ¹	Commercial Güiñapo ¹
Germination	15 days	20 days	No	No	No
Drying	3 days	5 days	No	No	No
Milling	Yes	Yes	No	No	No
Güiñapo/water rate (kg/L)	Not informed	1/33	1/20	1/16	1/18
Boiling (h)	3	1	1	1	3
Filtration	Yes	Yes	Yes	Yes	Yes
Fermentation	Overnight with addition of sugar	Overnight with addition of sugar	12 h	12 h	4–5 h with addition of sugar
Starter	<i>Chicha</i> from the previous day				
Additional ingredients after fermentation	Sugar ⁴	None ²	Sugar ³	Sugar ³	Sugar ⁴

¹Milled and partially germinated purple corn kernels

²Sugar added at the beginning of the fermentation

³Sugar added at the end of the fermentation

⁴Sugar added at the beginning and the end of the fermentation

metal pots for the fermentation step (see Additional file 1, D-F).

Both the boiling and fermentation times differ between the sampling locations. Since *Chicha* is made in artisanal conditions, it is most likely that these parameters are not exactly supervised, and therefore, it is difficult to determine their influence in the final quality and composition of *Chicha*. Overall, the time of fermentation ranges from 10 to 12 h with the addition of sugar at the beginning of the fermentation step (Z2), at the end of the process (Z3 and Z4), or both (Z1 and Z5) (Table 1). In contrast, the *Chicha* in Uchumayo (Z5) had the lowest fermentation time (4–5 h) which may explain its lowest contents in the total phenolic compounds and total monomeric anthocyanins as shown in Table 3. Differences concerning the type of raw material and the processing parameters would likely influence the bioactive composition and health-relevant functional properties of *Chicha*.

Chicha is the most representative fermented beverage made from corn in the Andean region, but there are other important corn-based fermented products across Central and South America. In Mexico, a beverage called *Pozol* is produced based on the fermentation of both corn and cacao beans. *Pozol* production includes a direct boiling of these grains without a previous germination process. The fermentation time is also increased to around 12 days which increases the alcohol content in the beverage thus making its flavor quite different from

Chicha [16]. In Ecuador, *Chicha* is produced using yellow, white, or purple corn. The grains are left in water for a day, after which the germination time is set to around 13 days similarly as in the case of *Chicha de Güiñapo* [15].

Physicochemical properties

The physicochemical characteristics measured in *Chicha* samples from different origins are shown in Table 2. The pH value ranged from 3.21 to 3.72 whereas the ethanol content (% v/v) and the titratable acidity varied from 1.63 to 3.43 and from 0.23 to 0.36 g lactic acid/100 mL, respectively. The Z5 *Chicha* showed the highest ethanol concentration among samples whereas Z1 had the highest acidity. The alcohol content showed a high variability (up to 67% for Z4) indicating differences in the fermentation time or other parameters such as the type and composition of the starter used in *Chicha* processing at different weeks. Likely, the starter used every time varies in microbiome composition and activity which in turn affects the final alcohol content. The soluble solids were overall similar among all *Chicha* samples ranging from 9.57 to 11.70 °Brix and Z5 sample showed the lowest value. This sample also showed the lowest titratable acidity which may be associated to its lowest time of fermentation among the other samples (Table 1).

Table 2 Physicochemical properties of *Chicha de Güiñapo*

Zone	pH	Ethanol (%)	Titrateable acidity (g lactic acid/100 mL)	°Brix
Z1	3.21 ± 0.04	2.30 ± 1.32	0.36 ± 0.04	11.70 ± 1.57
Z2	3.43 ± 0.11	2.07 ± 0.98	0.31 ± 0.03	10.70 ± 2.98
Z3	3.72 ± 0.44	1.67 ± 0.25	0.24 ± 0.11	11.47 ± 0.99
Z4	3.39 ± 0.08	1.63 ± 1.11	0.30 ± 0.04	10.43 ± 1.62
Z5	3.60 ± 0.04	3.43 ± 1.45	0.23 ± 0.03	9.57 ± 0.93

No significant difference at $p < 0.05$

Although no statistical differences were found in evaluated parameters, some *Chicha* samples such as Z3 and Z5 exhibited differences in terms of ethanol concentration, titrateable acidity, and °Brix. The processing parameters such as the type of raw material (corn or *Güiñapo*), fermentation time, the composition of the starter, and the use or not of sugar during and after the fermentation might explain observed differences. The standardization of the *Chicha* processing and the potential interaction of sugar with *Chicha* microbiome and its effect on physicochemical properties and bioactivity needs further research.

Other authors have reported similar physicochemical results for other corn-based fermented beverages. Barbosa et al. [15] have reported a pH value of 3.65, a mean titrateable acidity of 0.24 g lactic acid/100 mL, and an average ethanol concentration of 2.16% when evaluating an Ecuadorian *Chicha* made with white, yellow, and purple corn. Nuraida et al. [24] found a pH value of 4.05 and a mean titrateable acidity of 0.40 g lactic acid/100 g in *Pozol* samples after a 6-day fermentation period.

Total phenolic content, anthocyanin content, and antioxidant capacity

The total phenolic contents (TPC), the total monomeric anthocyanins (TMA), and the total anthocyanin contents (TAC) measured by UHPLC-DAD along with the antioxidant capacity assayed with two in vitro methods are shown in Table 3. No statistical differences were found

among results based on the *Chicha* origin; however, certain variability was observed in the results of TPC, TMA, TAC, and the antioxidant capacity. The TPC varied from 28.09 to 41.79 mg GAE/100 mL whereas the TMA and the TAC ranged from 16.81 to 31.09 mg C3G/L and from 39.45 to 63.16 mg CC/L, respectively. The Z2 sample had the highest values of TPC, TMA, and TAC among evaluated *Chicha* samples whereas Z5 sample had the lowest concentrations of these parameters.

A low variability in the antioxidant capacity results was observed among the *Chicha* samples from different origins. The Z2 sample had the highest antioxidant values with the ABTS and DPPH methods (235.78 and 131.94 $\mu\text{mol TE}/100\text{ mL}$, respectively). This sample also showed the highest TPC, TMA, and TAC values as stated previously. A significant positive correlation was found between the TPC and ABTS results, and between the TMA and TAC values, indicating that phenolic compounds from purple corn mostly anthocyanins or their microbial biotransformation-derived phenolic metabolites likely contributed to the antioxidant capacity in *Chicha*.

Flores-Calderon et al. [25] have reported similar TPC and TMA values (48 mg GAE/100 mL and 19 mg C3G/L, respectively) as those reported in the current study when malt beers made with blue corn were evaluated. However, the same authors reported higher ABTS and DPPH antioxidant capacity results (2600 $\mu\text{mol TE}/100\text{ mL}$ and 4800 $\mu\text{mol TE}/100\text{ mL}$, respectively), which may

Table 3 Total phenolic content (TPC), total monomeric anthocyanin (TMA), total anthocyanin content (TAC), and antioxidant capacity by the ABTS and DPPH methods in *Chicha*

	TPC [†] (mg GAE/100 mL)	TMA** (mg/L) [‡]	TAC (UHPLC)* (mg CC/L) [‡]	ABTS [†] ($\mu\text{mol TE}/100\text{ mL}$)	DPPH ($\mu\text{mol TE}/100\text{ mL}$)
Z1	33.45 ± 0.01	18.37 ± 2.59	42.22 ± 3.34	187.21 ± 7.89	106.76 ± 24.74
Z2	41.79 ± 0.08	31.09 ± 9.73	63.16 ± 13.59	235.78 ± 41.50	131.94 ± 14.77
Z3	30.63 ± 0.05	25.73 ± 8.95	52.78 ± 12.69	177.07 ± 26.18	122.44 ± 32.24
Z4	35.54 ± 0.06	27.06 ± 6.71	57.31 ± 9.07	213.98 ± 31.87	120.99 ± 32.24
Z5	28.09 ± 0.03	16.81 ± 6.07	39.45 ± 9.25	203.28 ± 80.09	108.47 ± 22.32

No significant difference at $p < 0.05$

*Expressed as cyanidin chloride aglycon

**Expressed as cyanidin-3-glucoside equivalents.

[†]Significant correlation (Pearson) between TPC and ABTS; $r = 0.62$

[‡]Significant correlation (Pearson) between TMA and TAC; $r = 0.78$

be due to the longer fermentation time of malt beer likely associated to increasing biotransformation of molecules with antioxidant capacity compared to the fermentation time applied in *Chicha* samples evaluated in the current research (16 days versus 1 day) [25]. The fermentation process has been reported to improve the antioxidant capacity of different fermented foods and beverages through the biotransformation of polyphenols by lactic acid bacteria [26, 27]. In addition, TPC ranges from 30 to 35 mg GAE/100 mL and in vitro antioxidant capacity values of 300 and 180 $\mu\text{mol TE}/100\text{ mL}$ have been found by Zou et al. [28] for a beverage made from persimmon (*Diospyros kaki*) vinegar fermentation process for 2 weeks. These values are comparable to ranges found in the current research.

Alpha-glucosidase enzyme inhibitory activity

The α -glucosidase inhibitory activity was determined for the undiluted and 1/2, 1/5, and 1/10 diluted *Chicha* samples, and the different doses were expressed as milligrams of GAE/mL of *Chicha*. According to Fig. 5, all samples inhibited the α -glucosidase enzyme in a dose-dependent trend. No significant differences ($p < 0.05$) were found among the α -glucosidase inhibitory activity results for the undiluted *Chicha* and the 1/10 dilution. Z2 and Z4 samples showed the highest inhibitory activity (74.26–75.73% and 44.47–52.51%, at 1/2 and 1/5 dilutions equivalent to 0.034 and 0.014 mg GAE/mL, respectively).

The inhibition of α -glucosidase enzyme was significantly correlated to the TPC at all assayed dilutions ($r = 0.6\text{--}0.75$, $p < 0.05$) indicating that phenolic compounds likely reflecting anthocyanins may be related to observed bioactivity. Polyphenols are known to have a high antioxidant capacity and a medium to high in vitro α -glucosidase and α -amylase inhibitory activity [29]. Several studies have found a high correlation between the TMA or specific types of anthocyanins with the inhibition of α -glucosidase and other enzymes relevant for the metabolism of polysaccharides [30, 31].

Other phenolic or non-phenolic compounds likely released or metabolized during the mixed lactic acid bacteria and yeast-based fermentation in *Chicha* may have also contributed to the high α -glucosidase inhibition shown in the current investigation, but more research is needed to elucidate this possibility. Simamora et al. [32] have reported α -glucosidase inhibitory activity of $65.53 \pm 0.75\%$ for a 100-day fermented-based juice made with *Morinda citrifolia* at a 0.035 mg GAE/mL dose. *Chicha* samples with lower fermentation time (12 h) had comparable ranges of inhibition (53.31–75.73%) at a similar sample dose (0.034 mg GAE/mL).

Alpha-amylase enzyme inhibitory activity

The results of the α -amylase inhibitory activity determined at different dilutions of *Chicha* samples are shown in Fig. 6. The Z2 undiluted sample showed the

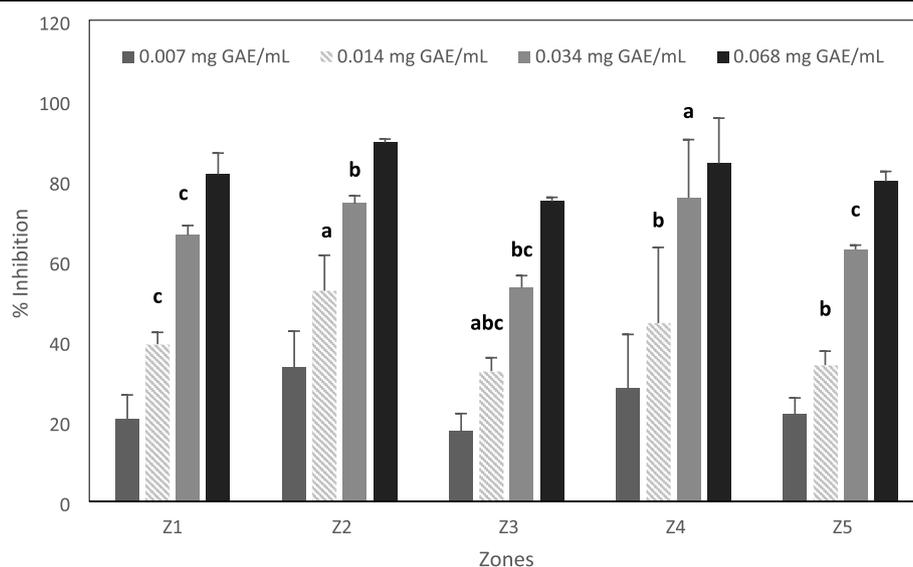


Fig. 5 Dose-dependent (0, 1/2, 1/5, and 1/10 dilutions corresponding to 0.068, 0.034, 0.014, and 0.007 mg GAE/mL doses, respectively) response in α -glucosidase inhibitory activity (%) of *Chicha* from five different locations in Arequipa. Different lower-case letters in bars with similar pattern indicate significant differences at $p < 0.05$

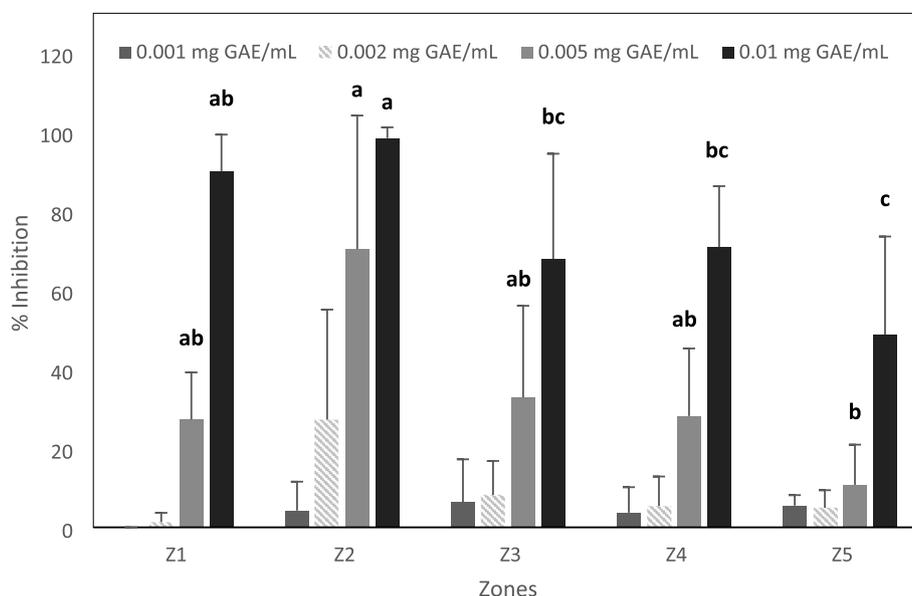


Fig. 6 Dose-dependent response (0, 1/2, 1/5, and 1/10 dilutions corresponding to 0.01, 0.005, 0.002, and 0.001 mg GAE/mL doses, respectively) response in α -amylase inhibitory activity (%) of *Chicha* from five different locations in Arequipa. Different lower-case letters in bars with similar pattern represent significant differences at $p < 0.05$

highest α -amylase inhibitory activity (98.41%) followed by Z1 (89.99%). At a lower sample dose (0.005 mg GAE/mL), Z2 sample also exhibited the highest inhibition among evaluated samples. Pearson coefficients revealed a positive correlation between the TPC levels and the α -amylase inhibitory activity at 1/2 and 1/5 dilutions ($r = 0.84$ and $r = 0.70$, $p < 0.05$, respectively) indicating that anthocyanins and likely other fermentation-derived phenolic compounds might be associated with the inhibition of α -amylase.

Johnson et al. [33] have reported a high α -amylase inhibitory activity (92.21% at a dose of 0.59 mg GAE/mL) for yeast fermented berry juice with high anthocyanin contents (up to 71.4 ± 10.4 mg C3G/L). Ranges from 35 to 38% and from 0 to 25% have been reported in undiluted and partially diluted fermented beverages such as tea and wine which are lower than results reported here [34]. This may be related to differences in the phenolic profile and the type of processing associated with tea and wine compared to *Chicha*.

Conclusion

The traditional knowledge based on historical perspectives of the ethnic beverage *Chicha de Güiñapo* is being preserved in evaluated districts of Arequipa (Peru). Linking historical perspectives this study found some differences in the type of raw material and processing parameters depending on the geographical localization of *picanterias*. In terms of

contemporary chemical analysis of physicochemical parameters, the phenolic contents and the in vitro antioxidant capacity have not been affected by such processing differences; however, the inhibition of the health-relevant α -amylase and α -glucosidase enzymes was significantly influenced and correlated to the TPC. The Z2 sample which followed the most traditional ethnic production process showed the highest potential for health associated hyperglycemia management. The current study shows for the first time the health-relevant potential of the traditional ethnic Peruvian purple corn-based fermented beverage *Chicha de Güiñapo*. Further follow-up studies are being conducted from the foundations of this research to understand the changes in phenolic profiles and probiotic microbiome likely developed during the fermentation of *Chicha* for current health applications which in turn would help to preserve the historical perspectives and ethnic knowledge of its processing and cultural relevance among people from the Andean region.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s42779-020-00063-3>.

Additional file 1. Supplementary figures. Figure A: *Chicha* made from different cereals in Peru. Figure B: *Picanteria* of Zone 1. Figure C: *Picanteria* of Zone 2. Figure D: *Picanteria* of Zone 3. Figure E: *Picanteria* of Zone 4. Figure F: *Picanteria* of Zone 5.

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Authors' contributions

Diego Vargas-Yana drafted the paper and together with Brigitte Aguilar-Morón and Nelly Pezo-Torres conducted the experimental work. Lena Gálvez Ranilla and Kalidas Shetty designed the study, edited the paper, and critically reviewed the manuscript. All authors contributed as members of the research group and approved the manuscript.

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Availability of data and materials

All data and materials are available and presented in the manuscript.

Competing interests

The authors declare that they have no competing interests.

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