Perigonium color and the antioxidant capacity of cañihua 
(*Chenopodium pallidicaule* Aellen)

Manuel Alfredo Callohuanca-Pariapaza; Evaristo Mamani-Mamani; Javier Mamani-Paredes; Ali William Canaza-Cayo

Currently, it is necessary to know the content of bioactive compounds, one of them is the antioxidant capacity of food, which has nutritional importance and functional properties, since these components are natural and play an important role in the prevention and treatment of several diseases, including cancer. Therefore, the objective of the investigation was to determine the nutritional quality and the relationship between the color intensity of the perigonium and the antioxidant capacity of the *Chenopodium pallidicaule* (*Ch. pallidicaule*).

As material of study, we used four accessions of *Ch. pallidicaule* with perigonium of defined colors such as light yellow, orange, purple and black. We developed the physical-chemical analyzes and the grain functional components in the Agroindustrial Engineering Laboratories of the National University of Altiplano Puno, and in the Laboratory of Chromatography and Spectrometry of the San Antonio de Abad National University of Cusco. The results were submitted to Pearson's correlation analysis, and they show that the flavonoid indices with the perigonium color intensity values express significant positive correlation. In addition, the antioxidant capacity equivalent to Trolox was significantly different between the perigonium color intensities, where the accession with black perigonium turns out to be the one that reached the highest value (5g eq. Trolox/100g sample). We conclude that the color of the perigonium exhibited antioxidant capacity, which kept a direct correlation with the flavonoid content.

Key Words: Antioxidant activity; phenols acids; flavonoids; grain color; cancer.

Perigonium color y la capacidad antioxidante de la cañihua
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Actualmente, es preciso conocer el contenido de compuestos bioactivos, entre ellos la capacidad antioxidante de los alimentos que tiene importancia nutricional y propiedades funcionales, ya que estos componentes son naturales y juegan un papel importante en la...
INTRODUCTION

*Ch. pallidicaule* originates in the Andes of southern Peru and northern Bolivia; the settlers of the Tiahuanaco culture domesticated it in areas near Lake Titicaca (Mujica, 2002; Vidaure et al., 2006). The fierce resistance of the highlands natives to the Spanish invasion attributed to their diet, so they contemptuously stigmatized it as Indian food, relegating its cultivation and consumption (Cabieses, 1995).

Currently, *Ch. pallidicaule* is considered a forgotten and underutilized species; it is cultivated at altitudes above 3860 masl, and it is very resistant to cold and prolonged droughts (Velásquez, 2018). Its protein content ranges from 13 to 19%, 18 out of the 20 main amino acids are found in *Ch. Pallidicaule*. It contains a good proportion of dietary fibers, polyunsaturated fats, good content of iron, and it is gluten-free, among other features (Repo-Carrasco, 1998).

Environmental pollution, global warming, the development of market economy, accelerated advance of science and technology, massive use of ICTs and globalization have caused a series of changes in today’s society, including changes in lifestyles, diet modifications and epidemiological transition, since the main causes of disease and mortality are not because of infectious diseases (Valenzuela et al., 2014). Cancer, diabetes, premature aging, coronary heart disease, Alzheimer’s, arthritis, depression, among others, known as “chronic non-communicable diseases” (CNCD) or “chronic degenerative diseases” (CDD), are more often accentuated in the population (Gonzalez et al., 2015).

Experts suggest following a healthy, varied and balanced diet. They consider the modification of eating patterns jointly with life habits of extremely importance, in order to reduce the risk of the presence of these chronic diseases, which is an alternative of great impact that has been gaining space and can minimize or prevent health problems. They are called “functional foods” (Salgado, 2017).

Functional foods can be of more varied types fromenriched or fortified foods to conventional and natural foods with additional bioactive components. The main bioactive or functional compounds present in food or additional ingredients are polyunsaturated fat acids,
with antioxidant activity, flavonoids, phenolic compounds, carotenoids, tocopherols, phytosterols, glycosinolates, organosulfur compounds, dietary fibers, probiotics, among others (Olagnero, 2007; Salgado, 2017).

All of these diseases are related to an “oxidative stress” increase, whose alterations are synthesized in the so-called FCA triad (Free radicals-oxidative stress, Chronic inflammation of low intensity-autoimmunity, and Apoptosis). These diseases present multiple comorbidities, whose treatment entails the strengthening of the biomolecular, cellular, tissue, organic, systemic, emotional, social and spiritual balance. The therapeutic proposals mainly seek to strengthen, reinstate-restore health, strengthening the homeostatic reserve (Gonzalez et al., 2015).

In cells and living organisms in normal conditions, the production of free radicals maintain a balance with the reactive species with systems of endogenous antioxidants or the homeostatic function of the organism. Therefore, that oxidation toxicity is limited, the same as the damage partially responsible for the natural aging of cells and organisms (Quintanar and Calderón, 2009). Nonetheless, when the balance is broken due to a deficit of antioxidants or an increase in the production of reactive species that generate oxidative chain reactions and cause several pathologies, this phenomenon is known as oxidative stress (Gonzalez et al., 2015).

Exogenous antioxidants come from the diet, such as vitamin E, vitamin C, β-carotene, lycopene, polyphenols, gallic acids, flavonoids, quercitin, hespiridin, catechins, and tannins (Quintanar and Calderón, 2009). The antioxidant capacity of foods or total antioxidant activity, in foods of plant origin, is attributed to the presence of phenolic compounds, especially flavonoids (Ciappini et al., 2013; Cartaya and Reynaldo, 2001). Antioxidant activity is the ability of a substance to inhibit oxidative degradation (Londoño, 2012).

Vegetable pigments are bioactive compounds that group four large groups: chlorophyll, carotenoids, flavonoids and betalains (Martínez et al., 2016). These pigments have therapeutic properties, and most of them provide a large amount of antioxidants to the body (Morales, 2012).

The main polyphenols as secondary plant metabolites are: flavonoids, phenolic acids, lignans and tannins, and they act as powerful antioxidants (Repo-Carrasco et al., 2010). Phenolic compounds in plants perform a series of metabolic functions in growth and reproduction and protect against external pathogens and ultraviolet radiation (UV); they are responsible for the color and characteristics of plants and food (Peñarrieta et al., 2014).

Flavonoids are polyphenolic substances composed by two phenyl rings (A and B), linked by a pyran ring (C) that share a common diphenylpyran skeleton: C6-C3-C6 and thanks to the variations of pyran, they are classified as: chalcones, flavonones, anthocyanidins, anthocyanins, flavan catechins, flavones and flavonols (Peñarrieta et al., 2014; Escamilla et al., 2009). They are very important for the development and proper functioning of plants, they act as protective agents against ultraviolet (UV) light or against infection by pathogenic organisms (Cartaya and Reynaldo, 2001). Likewise, in their antioxidant activity, they protect the body from damage caused by UV rays, environmental pollution, chemicals present in food, among others (Martínez-Flores et al., 2002).
The Anthocyanin pigments that belong to the group of flavonoids are present in almost all plants and in all their parts. Besides the coloring characteristics, they have powerful antioxidant properties (Kuskoski et al., 2004). Anthocyanins are interesting for two reasons: the first due to their impact on the sensory characteristics of food. The second due to their implication in human health and their therapeutic effects related to their antioxidant activity. The environment where they are cultivated is decisive on the anthocyanins composition and concentration (Aguilera et al., 2011).

According to different studies and experiences, Ch. Pallidicaule has great nutritional potential and the characteristics of a natural functional food. Due to the good content of flavonoids and phenolic acids Repo-Carrasco et al. (2010), affirm that indigenous Andean crops have excellent potential as a source of bioactive health-promoting compounds. The authors recommend carrying out further studies in order to identify the most promising varieties. On the other hand, the eye-catching and highly varied coloration of leaves, stems, inflorescences and grain, in physiological maturity, clearly show the antioxidant potential of their pigments. They improve the bioactive capacity, due to the formation of secondary metabolites, and the extreme environmental conditions that they endure during their cultivation.

Harvesting Ch. Pallidicaule grains in environmental conditions above 3820 m asl, supposes high incidence of solar radiation, low atmospheric pressure, little oxygen, dry and cold environment, frequent water stress and high temperature fluctuation between day and night, often endure frosts and hailstorms, typical climates of the Peruvian-Bolivian highlands (Callohuanca et al., 2019).

Given the described considerations, the present study has sought to determine the nutritional quality and the relationship between the color intensity of perigonium with the content of phenolic acids, flavonoids and antioxidant activity in the grains of four Ch. pallidicaule accessions.

MATERIALS AND METHODS

Study Material. Four representative accessions have been selected out of 26 Ch. pallidicaule accessions from the Andean Crops Germplasm Bank of the National University of the Altiplano Puno (UNA-Puno), corresponding to the 2017 - 2018 agricultural campaign harvest. They were cultivated at the Illpa Experimental Center and have molecular characterization by their grain yield potential (Mamani, 2013), assigning them ordinal values according to their perigonium color intensity (Table 1).

Physical-chemical Analysis. The physical-chemical analysis of the samples was developed in the Laboratory of Foods Nutritional Evaluation at the NUA-Puno Professional School of Agroindustrial Engineering using the method AOAC (1990). The analysis of the content of flavonoids, phenolic acid and antioxidant capacity, were developed in the Chromatography and Spectrometry Laboratory of the San Antonio de Abad National University of Cusco in the Faculty of Sciences, Cusco - Peru.
Bioactive compound analysis. The condition analysis using the HPLC (High Performance Liquid Chromatography) method for flavonoids were: Agilent 1200 Series Chromatograph; Zorbax Eclipse XDB-C18 Column 4.6 x 250mm, 5µm; Zorbax Eclipse XDB-C18 Pre-Column 4.6d x 12.5mm x 5µm; Column flow 1.0 ml/min; Solvent A Na3PO4, 21.6 mM at pH 3.3; Solvent B Acetonitrile. Gradient analysis system goes from 0% B to 100%; 370 nm DAD detection; oven temperature 40°C; analysis time 30 min; injection volume 1.0 µl. The results were expressed as quercetin equivalents (EQ) in mg/100 g.

The analysis conditions by HPLC phenolic acids were: Agilent 1200 Series Chromatograph; Zorbax Eclipse XDB-C18 Column 4.6 x 250mm, 5µm; Zorbax Eclipse XDB-C18 Pre-Column 4.6d x 12.5mm x 5µm; Column flow 1.0 ml/min; Solvent A Na3PO4, 21.6 mM at pH 3.3; Solvent B Acetonitrile. Gradient analysis system goes from 0% B to 100%; 285 nm DAD detection; oven temperature 40°C; analysis time 30 min; injection volume 1.0 µl and the results were expressed as gallic acid equivalents (EAG) in mg/100 g.

The results obtained by HPLC were developed in triplicate. However, the results from the corresponding laboratory consider only the average, in which the quantification was based using a quercetin and gallic acid standard. The areas and UV spectrum of the standard were compared with the samples. The results are expressed in milligrams equivalent of quercetin and gallic acid that are present in 100g of sample, according to the method described by Sakakibara (2003), with some modifications.

Determination of the antioxidant activity. The method used to determine the antioxidant activity was DPPH (2, 2-diphenyl-1-picrylhydrazyl). The conditions of analysis by Spectrophotometer were: Genesis 20 Thermo Electron Spectrophotometer; wavelength 517 nm; reading cell 1 cm glass cuvettes; where the equation of the standard curve is Y = 0.0467 X + 0.0479; R² = 0.9911.

The results obtained in the determination of antioxidant activity were carried out five times and express the Inhibition Coefficient at 50% (IC50 or IC50) in Trolox equivalent grams that are present in 100g of sample. This means that the concentration of the test sample produces a 50% inhibition of the free radical DPPH. The methodology used was based on Brand-Williams et al. (1997); A-Rahaman et al. (2013); Pugliese et al. (2013) and Molyneux (2004), with some modifications.

Statistical analysis. The results of the antioxidant activity were subjected to

| Accession key | Leaf color at physiological maturity | Color of Perogonium | Value of color |
|---------------|--------------------------------------|---------------------|----------------|
| 218           | Pink                                 | Golden             | 1              |
| 02            | Orange                               | Orange             | 2              |
| 27            | Purple                               | Purple             | 3              |
| 140           | Violet                               | Black              | 4              |

Table 1. Accessions under study with their respective leaf and grain colors.
a Completely Randomized Design with a classification criterion, considering the four accessions as treatments and with five repetitions each; analysis of variance and the respective significance test Tukey $\alpha = 0.05$ to contrast the differences of averages between each treatment, using the statistical software InfoStat.

**Correlation analysis.** In order to demonstrate the relationship of the content of phenolic acids and flavonoids with the ordinal values of the intensity of color of perigonium, we used the Pearson correlation technique.

**RESULTS AND DISCUSSION**

**Physical-chemical Analysis.** The results of the physical-chemical analysis (Table 2) show that the *Ch. pallidicaule* accessions have on average 91.35% dry matter, 8.65% moisture, 3.73% ash, 16.07% protein, 7.55% fat, 3.91% fiber and 60.09% carbohydrates. It is evident that they are rich in carbohydrates (58.89 - 61.98%), proteins (14.6 - 19.33%) and fats (6.59 - 8.57%).

These nutritional properties show that *Ch. pallidicaule* is one of the most nutritious Andean grains due to its protein content and quality. Its balanced composition of essential amino acids is similar to those of casein. Its high content of fats and carbohydrates are the main source of energy for the cells, tissues and organs of the body. Likewise, its dietary fiber content (3.91%) promotes positive effects on the health of consumers, such as reducing the level of cholesterol in the blood and improving digestion.

These values found are slightly lower than those indicated by De Bruin (1964), who reports for *Ch. pallidicaule* values of 67.6% carbohydrates, 16.9% protein and 8.8% lipids. The values also differ from those indicated by Huamaní (2018), who reports a carbohydrate content of 65.5 - 68.0%, proteins of 14.7 - 15.5% and lipids of 7.6 - 8.5%. A closer value of the protein content (15.2%) is that indicated by Repo-Carrasco et al. (2010), who obtained an average of five *Ch. pallidicaule* ecotypes from Puno and Cuzco. In this same study, moisture values (9.4 - 10.4%), fiber (4.8 - 14.4%) and ash (2.7 - 3.7%) are shown, which compared to the upper limit of our results show a slight percentage difference.

**Table 2.** Results of the physical-chemical analysis of four accessions of colored *Ch. pallidicaule* grains.

| Key | M.S.   | Humidity | Cinder | Protein | Fat | Fiber | Carbohydrates |
|-----|--------|----------|--------|---------|-----|-------|---------------|
| 218 | 90.20  | 9.80     | 3.88   | 14.78   | 7.80| 4.85  | 58.89         |
| 02  | 91.46  | 8.54     | 3.50   | 19.33   | 6.59| 2.23  | 59.81         |
| 27  | 91.27  | 8.73     | 3.79   | 15.58   | 7.23| 4.98  | 59.69         |
| 140 | 92.47  | 7.53     | 3.76   | 14.60   | 8.57| 3.56  | 61.98         |
| Average | 91.35 | 8.65     | 3.73   | 16.07   | 7.55| 3.91  | 60.09         |
These differences in the chemical content of *Ch. pallidicaule* could be due to the form of the crop, the state of maturation and the different environments in which the samples were cultivated and harvested. On the other hand, given that it is produced in extreme climatic conditions, such as in the highlands, it has probably developed a natural protection against oxidation. It constitutes an additional alternative to traditional cereals, as a fundamental food in the human diet. The results show that the color of perigonium is not a determining factor in protein synthesis.

**Active Compounds.** The levels of total phenolic compounds in the four *Ch. pallidicaule* accessions (Table 3) vary between 1.47 and 10.66 mg eq. gallic acid/100g of sample, with an average of 5.8 mg eq. gallic acid/100g sample; while the content of total flavonoids fluctuates between 14.64 and 32.58 mg eq. quercetin/100g of sample, with an average of 22.52 mg eq. quercetin/100g of sample, observing that the values are very different. Phenolic acids and flavonoids as important constituents of bioactive compounds are good indicators to identify health-promoting varieties of *Ch. pallidicaule* (Repo-Carrasco *et al.*, 2010).

The phenolic acid contents were dissimilar among the analyzed accessions, where the purple perigonium grains (Accession 27) reached the highest content of 10.661 mg eq. gallic acid / 100g, and the one with the lowest index obtained the black color accession. (Accession 140) with only 1,465mg eq. gallic acid/100g, these results show that *Ch. pallidicaule* has good bioactive qualities with a high antioxidant capacity. The *Ch. pallidicaule* accessions under study in the content of phenolic acids do not present a consistent correlation with the values of grain color intensity (*r* = -0.31; *p* <0.69), as they are secondary metabolites; their content may vary according to the environmental conditions during their cultivation.

The results of the phenolic acid content of the *Ch. pallidicaule* accessions obtained in the present study are higher than the values of *Ch. pallidicaule* ecotypes that vary between 1.4 and 1.9 mg eq. gallic acid/100g sample (Huamaní, 2018). They are lower than the values of 18.3 to 40.1 mg eq. gallic acid / 100 g reported by Repo-Carrasco *et al.* (2010). Both values are well below the results obtained by Luna (2005), who indicates that the content of total polyphenols in three varieties of *Ch. pallidicaule* varies between 233.13 and 253.80 mg eq. gallic acid/100g of matter. The results of ten *Ch. pallidicaule* ecotypes from Bolivia are already dry, where the total phenols vary from 2.1 - 8.0 mg eq. gallic acid / 100g sample (Peñarrieta *et al.*, 2014).

The factors that could affect the polyphenol content would be altitude, and climatic, edaphological and agronomic conditions. Likewise, the differences between the reported phenolic acid indices indicate that more research is still required on: the content of these acids, the factors that influence the greater or lesser synthesis such as environmental conditions during cultivation, the place of origin of the sample, color of the ecotype. In addition, it is required to standardize the analysis methods, analysis parameters such as total polyphenols, soluble phenolic acids and phenolic acids, among others.
The results of Table 3 show that the flavonoid content increases according to the intensity of grain color of the accessions under study, and presents a high and significant correlation (r = 0.99; p <0.0135) between flavonoid content and grain color intensity. The grains with value 1 (golden color) have a low content of flavonoids (14.64 mg eq. quercetin/100g), and as the value of the color intensity increases, the flavonoid indices are higher. Therefore, for the accession 140 of black grain color (with value 4) has a high content of flavonoids (32.58 mg eq. quercetin/100g), the lowest index corresponding to golden grain color and intermediate values to orange grains (18.05mg eq. quercetin/100g) and purple (24.80mg eq. quercetin/100g).

In addition, Repo-Carrasco et al. (2010), managed to identify ferulic acid as the most abundant phenolic acid (12.0±0.4 -29.8±0.2mg/100g), among the total phenolic compounds, for Ch. Pallidicaule. Likewise, quercetin is considered as the flavonoid with the highest concentration (21.4±1.4 - 84.3±1.2mg/100 g) together with isorhamnetin (average 30±20mg/100g).

The differences found in the flavonoid indices, in relation to the data reported in the present study, are probably determined by the same factors that influence the content of phenolic acids. Additionally, as Londoño (2012) affirms, the differences in the analysis methods can also influence the results.

### Table 3. Flavonoid and phenolic acid content of Ch. pallidicaule accessions by grain color.

| Accession | Color of Perigonium | Bioactive compounds (mg/100g) |
|-----------|---------------------|-------------------------------|
|           |                     | Phenolic acids EAG-Gallic Acid | Flavonoids EQ-Quercetin |
| 218       | Golden              | 6.66                          | 14.64                      |
| 02        | Orange              | 4.40                          | 18.05                      |
| 27        | Purple              | 10.66                         | 24.80                      |
| 140       | Black               | 1.47                          | 32.58                      |
| Average   |                     | 5.8                           | 22.52                      |

These values are below the values of Repo-Carrasco et al. (2010), who report an average index of total flavonoids in Ch. pallidicaule that varies between 46.10 to 144.30mg eq. quercetin/100g. It is attributed to the very showy and varied coloration of Ch. pallidicaule in maturation and, to the exceptionally high content of flavonoids. In the same way, it is below 1.5 and 2.0mg eq. catechin/g sample reported by Huamani (2018) of total flavonoid content.

### Antioxidant activity.

The antioxidant activity of the Ch. pallidicaule accessions (Table 4) show significant differences (p≤0.05) between them and present IC50 values between 2.32 ± 0.09 and 5±0.09g Trolox/100 g, highlighting accession 140 (black perigonium color) as the one with the highest antioxidant activity, and accession 02 (orange perigonium color) as the lowest antioxidant activity. This shows that the antioxidant activity is higher in the accessions
that presented greater color intensity (black and purple), which in turn were those with the highest concentration of flavonoids.

These results show that the perigonium color intensity values of accessions 218 and 02 (gold and orange) are not congruent in terms of antioxidant capacity, which is corroborated by Reyes et al. (2015) on the antioxidant activity of aqueous extracts of varieties of Jamaica (Hibiscus sabdarifff L) with calyces of different colors, which they attribute to anthocyanins.

In this regard, Repo-Carrasco et al. (2008) indicate that *Ch. pallidicaule* grains have high antioxidant properties that vary from 91.45 to 1509.80μg eq. Trolox/g. The indices expressed in the same units of the present work would be from 0.009145 to 0.15098g eq.Trolox/100g. In the same way, Luna (2005) obtained values between 3686.92 to 4178.65μg eq. Trolox/g of dry matter equivalent to 0.368692 to 0.417865g eq. Trolox/100 g when determining the antioxidant capacity of three varieties of *Ch. pallidicaule*, Also, Tacora (2010) reports the antioxidant capacity of the varieties "INIA-Illpa 406" and "Cupi", with 0.2174 and 0.1667g eq. Trolox/100g respectively. The cited reports do not specify the color of perigonium; they refer to results of a dry sample, while the results of this work refer to a wet basis.

Averages with different letters mean statistically significant differences (p <0.05) Marmouzi et al. (2015) report EC50 results for quinoa of 0.4mg extract/ml lower than the one found in the present study. Additionally, Nowak et al. (2016) reveal EC50 values (0.1 - 0.4mg extract/ml), for four species grains of Chenopodium (C. album, C. rubrum, C. hybridum and C. urbicum). Therefore, they have a lower antioxidant capacity, in contrast to the accessions studied. Huamani (2018) obtained EC50 values between 1.3 and 4.2mg extract/ml, with the red *Ch. pallidicaule* ecotype (red coloring grain) as the one that has the highest antioxidant activity and the Chilliwa ecotype (light lead coloring grain) as the one that has the lowest antioxidant activity.

Table 4. Average antioxidant capacity in colored *Ch. pallidicaule* (average ± SD, n = 5).

| Accession | Color of Perigonium | CI<sub>50</sub> Trolox g/100 g | CI<sub>50</sub> Trolox mmol/100 g |
|-----------|---------------------|-------------------------------|-------------------------------|
| 140       | Black               | 5.00<sup>a</sup> ± 0.09       | 19.89                         |
| 27        | Purple              | 3.08<sup>b</sup> ± 0.06       | 12.33                         |
| 218       | Golden              | 2.73<sup>c</sup> ± 0.03       | 10.89                         |
| 02        | Orange              | 2.32<sup>d</sup> ± 0.09       | 9.25                          |
| Average   |                     | 3.28                          | 13.09                         |
Fischer et al. (2013) affirms that the water limitation during the initial maturation phase of the grain until harvest, increases considerably the antioxidant activity. This happens due to the stress situation that the plant experiences during its development, as it occurs with the *Ch. pallidicaule* that grows naturally in an adverse-condition environment (climate and temperature). Thus, this would be a possible explanation for the high antioxidant capacity.

Finally, since it is a product with good nutritional and antioxidant potential, it is necessary to continue with further studies, and to standardize the measurement units of the laboratory analysis results. In the same way, it is necessary to identify accessions with high antioxidant potential and variables that can contribute to raise their qualities.

**CONCLUSIONS**

The nutritional analyses of the samples of *Ch. pallidicaule* indicates that it is rich in carbohydrates (58.89 - 61.98%), proteins (14.60 - 19.00%), and lipids (6.59 - 8.57%).

The concentration of total phenolic acid in the four *Ch. pallidicaule* accessions varies between 1.47 and 10.66mg eq. gallic acid/100g of sample, and the total flavonoids concentration varies between 14.64 and 32.58mg eq. quercetin/100g sample.

The phenolic acids content in *Ch. pallidicaule* is not associated with the values of color intensity of perigonium; conversely, the flavonoid indices express significant correlation and increase considerably in direct relation to the intensity of the color of the perigonium.

The average effective concentration (IC50) for the accessions of the *Ch. pallidicaule* antioxidant activity varies between 2.32 and 5g Trolox/100g of sample. The black accession of the perigonium color is the one with the highest antioxidant activity.

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**CONFLICT OF INTERESTS**

The authors declare that there is no conflict of interest.

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