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Original Article

Effect of different concentrations of stem juice of Oxalis tuberosa Mol. and juice of the fruit of Gaultheria glomerata (Cav.) Sleumer on the antioxidant activity of the heat-treated functional beverage

Efeito de diferentes concentrações de suco do caule de Oxalis tuberosa Mol. e de suco do fruto de Gaultheria glomerata (Cav.) Sleumer sobre a atividade antioxidante da bebida funcional tratada termicamente

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Abstract

Functional beverages with added health benefits are popular among peoples and athletes because they help them recover faster from intense workouts and perform better overall. This research set out to determine how well heat-treated stem juice from Oxalis tuberosa Mol. "oca" and fruit juice from Gaultheria glomerata (Cav.) Sleumer "laqa-laqa" performed as an antioxidant in a functional drink. The "oca" stems and the "laqa-laqa" fruit were collected to obtain the juice. For this study, 30 semi-trained panellists used sensory evaluation to rate four treatments (Bo, B1, B2, and B3) with varying quantities of "oca" and "laqa-laqa" juice. The results concluded that the treatment B2, which included 300 ml of "oca" stem juice, 800 ml of "laqa-laqa" juice, 1000 ml of treated water, and 220 g of refined sugar, was given the highest score after a physicochemical evaluation of its colour, smell, taste, and overall appearance. Similarly, the results showed that the protein content increased by 1.38%, the fat content by 1.08%, the moisture percentage by 99.5%, the ash content by 1.82%, and the carbohydrate content by 6.22% after B2 treatment. Similarly, results revealed significant enhancement in antioxidant profiling such as total polyphenols: 1825 mg of gallic acid/100 g and antioxidant Activity: 89.56% µmol of trolox /100 g. In conclusion, due to its high energy content and antioxidant activity, it may be a viable nutritional option for athletes who engage in rigorous, frequent physical exertion.

Keywords: "Oca", "Laqa-laqa", antioxidants, energy, polyphenols.

Resumo

As bebidas funcionais com benefícios adicionais à saúde são populares entre as pessoas e os atletas porque os ajudam a se recuperar mais rapidamente de exercícios intensos e a ter um desempenho geral melhor. Esta pesquisa teve como objetivo determinar o quão beneficamente o suco do caule tratado termicamente de Oxalis tuberosa Mol. "oca" e o suco de fruta de Gaultheria glomerata (Cav.) Sleumer "laqa-laqa" atuaram como antioxidante em bebida funcional. Os caules da "oca" e os frutos da "laqa-laqa" foram coletados para a obtenção do suco. Para este estudo, 30 provadores semitreinados usaram a avaliação sensorial para avaliar quatro tratamentos (B0, B1, B2 e B3) com quantidades variadas de suco de "oca" e "laqa-laqa". Os resultados indicaram que o tratamento B2, que incluía 300 mL de suco de caule de "oca", 800 mL de suco de "laqa-laqa", 1.000 mL de água tratada e 220 g de açúcar refinado, obteve a maior pontuação após avaliação físico-química e avaliação de cor, cheiro, sabor e aparência geral. Da mesma forma, os resultados mostraram que o teor de proteína aumentou em 1,38%, o teor de gordura,

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em 1,08%, o percentual de umidade, em 99,5%, o teor de cinzas, em 1,82%, e o teor de carboidratos, em 6,22%, após o tratamento B2. Da mesma forma, os resultados revelaram aumento significativo no perfil antioxidante, como polifenóis totais: 1.825 mg de ácido gálico/100 g e atividade antioxidante de 89,56% µmol de trolox /100 g. Em conclusão, devido ao seu alto teor energético e à atividade antioxidante, pode ser uma opção nutricional viável para atletas que praticam esforço físico rigoroso e frequente.

Palavras-chave: "Oca", "Laqa-laqa", antioxidantes, energia, polifenóis.

1. Introduction

People all around the world are turning to functional foods as a kind of treatment for a wide range of illnesses (Gondal, 2023; Brown et al., 2018). Many different types of food, such as those high in bioactive chemicals or probiotic bacteria, fall under the umbrella term "functional foods," which has a positive impact on health. Nowadays the demand towards the healthiest drinks is increasing mainly due to the presence of antioxidants, vitamins and minerals (Žuntar et al., 2020; Enriquez Paredes and Ore Areche, 2021). Similarly, the rising popularity of fruit juices can be attributed to the positive connotations that the drinks hold among modern customers (Odriozola-Serrano et al., 2022). From a practical point of view, they can be natural or of synthetic origin (Heredia, 2016). Different plant species are propitious for their use in the preparation of these drinks (Flores-Aguilar and Flores-Rivera, 2022). The stem of the *Oxalis tuberosa* Mol. which presents a broad spectrum of phytochemical ingredients and also show antioxidant activity. Therefore, the development of functional drinks based on wild fruit juices may provide a middle ground solution, as these products can be viewed as a novel class of functional food due to the wide range of bioactive compounds (including peptides, amino acids, antioxidants and vitamins) they contain and the synergistic health benefits they can provide when consumed (Mantzourani et al., 2018).

Oxalis *tuberosa* Mol. also known as potato, oca or ibia, is a very old crop. It develops rapidly (Oré et al., 2019) and is cultivated annually as a tuber in the Andean, reaches a height of 0.20 to 0.40 metres (Cruz Amaya and Tubay Lucero, 2019), and can be used in its whole due to the fact that it is rich in protein, carbs, and vitamin C in particular (Cáceres Pereda and Valerio Molina, 2021). Most people eat it parboiled, and it tastes great, especially if it has been left out in the sun, when it develops a wonderful sweetness. The *Oxalis tuberosa* Mol. act as an excellent raw material option for the preparation of foods with functional properties (Güemes-Vera et al., 2019). There is a wide range of nutritional quality in oca; some varieties are superior to potatoes, while others are just as good. This is because oca has the features of colour, phenolic compounds, and antioxidant capacity, which can be changed depending on storage or prepared conditions (Baldeón Aliaga, 2013; Cotrina Cabello et al., 2023).

Native fruits with natural hues, such as *Gaultheria glomerata* (Cav.) *Sleumer*, often known as "laqa-laqa," provide an alternative to meeting the rising demand for functional foods with a high amount of bioactive chemicals. The Americas, Oceania, and Asia are the original homes of these plants. Once divided into the genus Pernettya, the Southern Hemisphere species are now considered to be part of the single genus Gaultheria due to a lack of credible morphological or genetic differences between the two. The laqa-laqa is a subfructescent, semi-prostrate, lithophyte that can grow to a height of 30 centimeters and produces fruit once yearly in the months of March and April. The berries that make up the fruit have a diameter of 0.5 to 1.8 centimeters and a height of 1 to 1.5 centimeters, and they are distributed in a cluster inflorescence. Local people consume the fruit as an alternative medicine, to reduce headaches, stress, stomach pain, they are also consumed for its pleasant aroma and sweet taste *Gaultheria* fruit *glomerata* (Cav.) *Sleumer* contains 112.88 mg of anthocyanins per 100 g of fruit, and they are considered the main metabolites that provide color to plants. In the same way, it has 344.37 mg of phenolic compounds and 13.87 mg of vitamin C (Zapata et al., 2014). Due to its content of bioactive compounds, the fruits of *G. glomerata* (Cav.) *Sleumer* could be used as a healthy functional food, with the best state of conservation being frozen with reference to nuts (Barragan-Condori et al., 2021). *Gaultheria* fruit *glomerata* (Cav.) *Sleumer*, due to its attractive natural coloration and solubility, it can be considered an alternative source of anthocyanins instead of the synthetic colorants and currently using for the processing of functional foods and beverages [16]. In this sense, it is a viable, natural option with antioxidant potential that can be incorporated, thanks to its anthocyanin content, as a colorant in the formulation of functional beverages.

Anthocyanins are a class of flavonoids extracted from water-soluble plants, they show antioxidant, anti-inflammatory and anti- apoptotic activities (Zhang et al., 2019). The anthocyanins located in the different organs of the plant, are of the utmost importance, because they play a role in physiological adaptation in the process of plant propagation, in attraction of pollinating organisms and are part of the defense mechanisms against elements of biotic and environmental stress (Menzies et al., 2016). They are commonly synthesized from various species, causing the presence of red, blue, orange and purple colors in a wide variety of flowers, fruits and vegetables at pH differentials (Navas et al., 2012).

Due to their superior activity against microorganisms, heat treatments continue to be at least one of the most employed types of stabilization for the conservation and prolonging of the usable life of juices (Alaqeel, 2024). Many of the juice's healthful ingredients (Nechytailo et al., 2024), however, can be destroyed by the high processing temperatures. The food business has been pushed to innovate by consumer demand for healthier options, leading to the development of innovative technologies that reduce the degree of degradation to food's nutritional integrity (Gondal et al., 2023a; Odriozola-Serrano et al., 2022; De La Cruz-Marcos et al., 2023; Khan et al., 2024; Gondal and Tayyiba, 2022; Areche et al., 2022a, b).

The body produces antioxidants, but a diet rich in antioxidants can supplement the action of these sweeping enzymes, making native products like oca and laqa-laqa particularly beneficial. Bioactive compounds, in particular phenols and antioxidants, are substances found in certain foods that fulfil the function of protecting against free radicals that cause ageing processes and some other diseases. In order to take advantage and give added value and analyze the possibility of obtaining new products from the stem of *Oxalis tuberosa* Mol. "oca" and the fruit of *Gaultheria glomerata* (Cav.) *Sleumer* "laqa-laqa", the formulation of the functional drink has been carried out from the mixture of both products, with laboratory tests. In this sense, in the present investigation, the effect of the juice of the stem of *Oxalis tuberosa* Mol and juice of the fruit of *Gaultheria glomerata* (Cav.) *Sleumer on the* antioxidant capacity of the functional drink was evaluated. The research carried out contributes to increase the information of the functional drink in obtaining a new product.

2. Materials and Methods

2.1. Collection of raw material and preparation of juices

The oca stem was collected after the oca harvest with an estimated amount of 10 kg and on the other hand, the 5kg laqa-laqa fruit was collected in a ripe state. Both raw materials were collected from the place called "Torowishqana" of the district and province of Acobamba in Huancavelica – Peru. After that, they were brought to the Laboratory of Agroindustrial Processes at the National University of Huancavelica, which is part of the Professional School of Agroindustrial Engineering for further analysis. The stems and fruits were cleaned, drained, and the stems were chopped before being stored at 4 °C for processing. The oca stem juice was extracted using an Imaco brand extractor, obtaining 6 liters of juice and the laqa-laqa fruit was blended in an Oster brand blender, obtaining 3 liters of juice. The extracted juices were centrifuged at 24,000 rpm for 15 minutes, and the resulting supernatants were filtered through a 2-mm steel mesh sieve. Before the treatments were applied, the juices were frozen at -16 °C and stored in the dark. The experimental crops are depicted in broad strokes in Figures 1 and 2.

2.2. Process of obtaining the drink

To obtain the functional drink from the stem juice of "oca" Oxalis tuberosa Mol. and juice of "laqa-laqa" Gaultheria glomerata (Cav.) Sleumer, three treatments (formulations) were carried out, with 3 repetitions each, the quantities are described in Table 1. Based on the results of the one-way analysis of variance (ANOVA), we employed a completely randomized design (CRD) with three repeats to compare the means of the treatments (Younas et al., 2022; Jiang et al., 2022; Gondal et al., 2023b), employing the TUKEY test with a 95% confidence interval

2.3. Procedure

The juices of the "oca" stem and the "laqa-laqa" fruit were measured in test tubes and the treated water was measured in measuring jugs, while the sugar was weighed on an analytical balance (see Table 1) for each of the treatments, which were added to a stainless-steel pot where they were homogenized. The homogenized mixture was subjected to a heat treatment at 90 °C for a period of 1 min. After the heat treatment, the functional drink was filtered through a 1 mm mesh stainless steel sieve, in order to obtain a drink without particles. The packaging was carried out at 75 °C in glass containers of 250 ml capacity, which were immediately sealed and submerged in a container with cold water, in order to obtain their vacuum sealing. The functional drink was stored for 14 days in a refrigerator at 4 °C in order to preserve it. After 14 days, the sensory evaluation was carried out.

2.4. Sensory evaluation

The sensory evaluation was carried out with 30 semi-trained panelists, determining the attributes of color, smell, flavor, general appearance and texture; using the hedonic scale from 1 to 5, with 1 being the lowest score and 5 the highest score.

Figure 1. a) *Gaultheria* fruit *glomerata* (Cav.) *Sleumer* "laqa-laqa" on your plant. b) Fruit collected from "laqa-laqa".

Figure 2. a) Plant of Oxalis tuberosa Mol. b) Stem of Oxalis tuberosa Mol. after the harvest.

Table 1. Formulations of the functional drink of the stem extract of *Oxalis tuberosa* Mol. "oca" and *Gaultheria juice glomerata* (Cav.) *Sleumer* "laqa-laqa".

Component	Treatments			
	Bo	B1	B2	B3
"Oca" juice	500ml	400ml	300ml	200ml
"Laqa-laqa" juice	400ml	1000ml	800ml	600ml
Water	1000ml	1000ml	1000ml	1000ml
Sugar	300g	270g	220g	200g

2.5. Determination of total polyphenol content

The total polyphenol content of *Stevia rebaudiana* stem extracts were determined using the Folin-Ciocalteau method (mixture of phosphotungstic and phosphomolybdic acid) and reported as gallic acid equivalents, through a calibration curve (Lester et al., 2012). 20 µL of sample diluted with distilled water were taken, or standard solution of gallic acid in the case of the curve, 1580 µL of water, 100 µL of Folin-Ciocalteu reagent and 300 µL of 20% sodium carbonate solution were added. (m/w). The mixture was shaken and incubated for 60 min in the dark. The absorbance was measured at 725 nm using water as blank. Aqueous solutions of gallic acid (between 0 and 1000 ppm) were used for the calibration curve. The results were exciting as mg gallic acid equivalents (GAEs) per gram of dry sample.

2.6. Determination of antioxidant activity

The in vitro antioxidant capacity was studied by evaluating the free radical scavenging effect in the DPPH, TEAC, FRAP and Folin-Ciocalteu. Final antioxidant activity determined through all methods was calculated using a regression equation between Trolox concentration (0–20 µM) and absorbance changes. The final results were expressed in equivalent micromoles of Trolox per liter of juice.

2.7. Statistical analysis

Statistical analysis was performed using the MS Excel 2021 program. Based on the results of the one-way analysis of variance (ANOVA), We employed a completely randomized design (CRD) with three repeats to compare the means of the treatments, employing the TUKEY test with a 95% confidence interval. Differences were considered significant at the $p<0.05$ level.

2.8. Correlation among various selected attributes

Correlation analysis showed that there was a highly significant relationship among all the parameters including physiochemical evaluation suggesting that the optimal availability of nutrients from different juices may improve human health that eventually contribute in enhancing the overall health of peoples. The score plot describes the distribution of various treatments of stem juices. As a result, some remarkable variations across all treatments of stem juices can be seen. The loading plot provides a more visual representation of the relationship and variance across all parameters evaluated in this study. Figure 3 revealed a correlation matrix graphically by corrplot. A highly significant association was observed in all parameters of physiochemical analysis of the performed manuscript.

3. Results

3.1. Sensory evaluation

Sensory evaluation of the functional drink of the stem juice of *Oxalis tuberosa* Mol. and *Gaultheria* juice

glomerata (Cav.) *Sleumer* heat-treated was carried out with 30 panelists, who evaluated the attributes of color, smell, flavor and general appearance. As a result of the sensory evaluation of the functional drink, it was determined by means of the averages obtained from the Likert scale (see Figure 4, where treatment B2 was determined as the treatment with the greatest acceptability, which was formulated with 300 ml of stem juice. of "oca", 800 ml of "laqa-laqa" juice, 1000 ml of treated water and 220 g of refined sugar. The averages obtained with the highest score were 4.07 for color, 4.00 for odor, 3.87 for taste, and 3.67 for general appearance.

3.2. Physicochemical analysis

In the sensory evaluation, treatment B2, formulated with 300 ml of "oca" stem juice, 800 ml of "laqa-laqa" juice, 1000 ml of treated water and 220 g of refined sugar, obtained the highest acceptability among the panelists; for which it was submitted to the proximal chemical analysis, the results are shown in Table 2, while Table 3 shows the results of the physicochemical analysis of the functional drink.

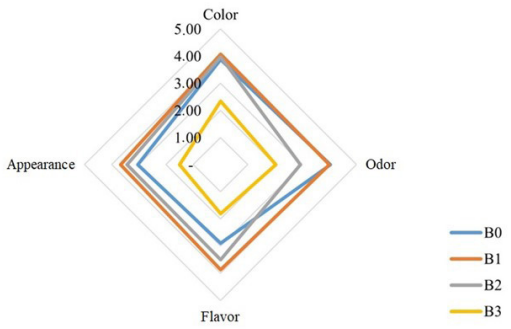

Figure 3. Sensory evaluation of the functional drink of the stem juice of *Oxalis tuberosa* Mol. "oca" and "*Gaultheria juice glomerata* Cav" *Sleumer* "laqa-laqa" heat treated.

4. Discussion

The proximal chemical composition of the functional drink of the stem juice of *Oxalis tuberosa* Mol. "oca" (Figure 2a and 2b) and the *Gaultheria glomerata* (Cav.) *Sleumer*

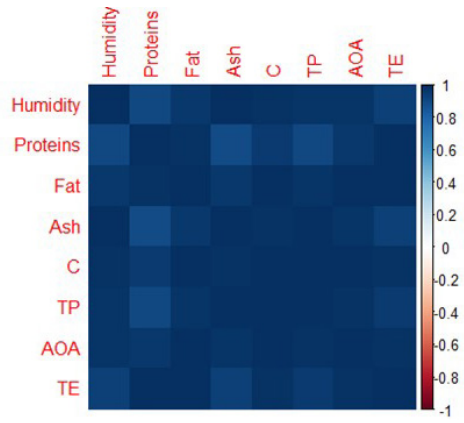

Figure 4. Corrplot (Correlation plot) represents correlation matrix among different attributes of functional juices. The dark blue color shows a high positive correlation while light blue and sky blue represent less association among measured parameters. The color legend on the right-hand side of corrplot shows the correlation coefficient and corresponding colors. The abbreviations are as Total Energy (TE), Antioxidant Activity (AOA), Total Phenol (TP), Carbohydrates (C).

Table 2. Proximal chemical composition of the functional beverage of the stem juice of *Oxalis tuberosa* Mol. "oca" and *Gaultheria juice glomerata* (Cav.) Sleumer "laqa-laqa".

Treatment	Humidity (%)	Proteins (%)	Fat (%)	Ash (%)	Carbohydrates (%)
Bo	74.3±0.01c	1.29±0.034d	0.99±0.004d	1.72±d	6.17±0.006c
B1	82.3±0.08b	1.32±0.033c	1.04±0.022c	1.80±c	6.23±0.024b
B2	89.5±0.51a	1.49±0.048a	1.17±0.234a	1.88±a	6.34±0.006a
B3	85.4±0.03ab	1.38±0.093b	1.10±0.043b	1.85±ab	6.29±0.026ab

The LSD test (P<0.05) reveals that means with different letters are substantially different.

Table 3. Physicochemical composition of the functional drink from the stem juice of *Oxalis tuberosa* Mol. "oca" and *Gaultheria juice glomerata* (Cav.) Sleumer "laqa-laqa".

Treatment	Total phenols (mg Ga/100 g)	AOA (% μmol To /100 g)	Total Energy (kcal/100 g)
B0	1783±0.02c	76.92±0.012d	28.93±0.028d
B1	1800±0.16b	81.23±0.032c	31.22±0.042c
B2	1825±0.01a	89.56±0.010a	40.15±0.030a
B3	1818±0.02ab	85.34±0.207b	35.30±0.010b

Gallic acid: Ga; Trolox: To; Antioxidant activity: AOA. The LSD test (P<0.05) reveals that means with different letters are substantially different.

(Figure 1a and 1b) contains 1.38% protein, this value is much lower than that obtained by Enriquez Paredes and Ore Areche (2021) who obtained 8.53%. The nutritional profile, combined with a hypocaloric diet, makes the functional drink an extremely beneficial for patients with non-alcoholic fatty liver disease who need this measure (Vázquez-Frías et al., 2020).

The differences in the content of total polyphenols and antioxidant capacity of the functional beverage evaluated show that the chemical properties of the *Gaultheria fruit glomerata* (Cav.) Sleumer "laqa-laqa" affect the composition and physicochemical activity of the functional drink (M'hiri et al., 2015). Differences in polyphenol content determined by in vitro methods and antioxidant capacity compared to literature reports may be due to extraction methods and climatic conditions such as temperature, precipitation, humidity, and environmental conditions. Soil, altitude, etc. that modify the bioactivity of plant products (Granato et al., 2016). This proposes that the stems of Oxalis tuberosa Mol. "oca" and the *Gaultheria glomerata* (Cav.) Sleumer can be used as an enhancer of antioxidant activity in multiple products.

5. Conclusions

The results of the sensory evaluation of the functional drink from the mixture of juice from the stem of Oxalis tuberosa Mol. "oca" and juice of Gaultheria glomerata (Cav.) Sleumer "laqa-laqa", determined treatment B2 as the best treatment, because it had greater acceptance in the attributes of color, smell, flavor and general appearance. In this sense, the physicochemical analysis of the B2 treatment was carried out, the results show that the drink presents 40.13 kcal/100 g of total energy, which is essential for the consumption of people who are in constant physical activity, while the total polyphenols result was 1825.13 mg gallic acid/100 g, this total polyphenol result shows the highest correlation with antioxidant activity for DPPH and FRAP ($r = 0.800$, $r = 0.795$) respectively. The results of the antioxidant activity were 89.56% µmol of trolox/100 g, this shows that the antioxidant activity of the determined drink could be attributed mainly to the phenolic compounds rather than to the vitamin C concentration. Therefore, the production of functional drink from "oca" stem juice and "laqa-laqa" fruit juice could be a good functional product due to its energy content and high antioxidant activity. This study is the potential for future research focused on obtaining functional beverages with specific functional characteristics.

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