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Nutritional contents of a traditionally fermented spice: the case of Datta/Qochqocha in Ethiopia

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Abstract

Introduction: *Datta/Qochqocha is a traditionally fermented Ethiopian spice believed to boost appetite and help prevent and treat certain diseases. Its ingredients are rich in micronutrients with anti-inflammatory, immune-boosting, and antioxidant properties. However, Datta/Qochqocha micronutrient content and inhibition capacity have not yet been determined. The study aimed to identify micronutrients that possess antioxidant, anti-inflammatory, and immunity-boosting effects; bioactive compounds; and the inhibition capacity of Datta/Qochqocha in Ethiopia.* ***Methods:*** *in August 2023, 250 grams of fermented Datta/Qochqocha were collected from 43 producers in addition to four prepared standard samples. The samples underwent solid-phase extraction. Datta/Qochqocha's zinc, manganese, and magnesium concentrations were analyzed using a flame atomic absorption spectrometer, vitamins E, D, and A were analyzed using HPLC. Phenol and flavonoid contents were determined using the lambda 950 UV/VIS/NIR spectrometer. Datta/Qochqocha´s inhibition capacity was determined using the DPPH assay and ascorbic acid standard.* ***Results:*** *this study found that 100 grams of traditionally fermented Datta/Qochqocha contains, on average, 0.0022 mg ± 0.0033 mg of vitamin D, 5.72 mg ± 2.96 mg of vitamin A, 16.11 mg ± 4.42 mg of vitamin E, 9.26 mg ± 0.013 mg of magnesium, 3.61 mg ± 0.042 mg of manganese, 1.62 mg ± 0.027 mg of zinc, 47.36 mg ± 21.52 mg age of phenolic acids, and 116.75 ± 52.54 mg QuE of flavonoids, with an average inhibition percentage of 40.67% and an IC50 value below 10 µg/mL.* ***Conclusion:*** *traditionally fermented Datta/Qochqocha contains essential micronutrients and bioactive compounds with notable inhibition capacity, indicating possible health benefits.*

Introduction

Fermentation is a biological process that enhances the nutritional value of food by increasing the content of beneficial nutrients such as vitamins and minerals [1]. Fermentation is a chemical process in which beneficial microorganisms break down sugars and starches in the absence of oxygen [2]. Fermentation enhances nutrient availability in foods by breaking down sugars and starches, making the foods easier to digest. Additionally, this process increases the levels of vitamins and minerals, enhancing the overall nutritional value of fermented foods [1,3]. In many cases, fermentation has been proven to enhance the antioxidant levels and their effectiveness in foods [4]. Fermentation enhances the antioxidant activity of plant-based foods [5].

Datta/Qochqocha is a traditional Ethiopian spice made through natural (anaerobic) fermentation. Its main ingredients include chili pepper, green pepper with seeds, garlic, ginger, cardamom, fresh sweet basil leaves, coriander (seed, fruit, and leaf), and rue seeds [1,6]. Garlic, pepper, ginger, basil, coriander, and cardamom are all rich in one or more of vitamin A, vitamin D, vitamin E [7,8], magnesium, manganese, zinc [9, 10], antioxidants [11,12], anti-inflammatory properties [13-17], and other essential nutrients [18,19].

Studies indicate that naturally fermented foods are a valuable source of essential nutrients such as vitamins A, D, and E; magnesium, manganese, and zinc, which have anti-inflammatory, antioxidant, anti-carcinogenic, and immunity-boosting properties [14,20-23]. A study found that fermentation enhances the quantity and quality of healthy nutrients in food [3]. Additionally, fermentation enhances the concentration of phenols and flavonoids in foods, as well as the free radical scavenging capacity of food [5,24,25].

Datta/Qochqocha is claimed to have an appetizing effect and may be used to prevent and treat certain health conditions. Its ingredients are rich sources of micronutrients that possess anti-inflammatory, immune-enhancing, and antioxidant properties. However, Datta/Qochqocha´s micronutrient content and inhibitory activity levels have not yet been determined. Besides, since fermentation is dynamic by itself, this study aimed to identify micronutrients that have anti-inflammatory, immunity-boosting, and antioxidant effects in Datta/Qochqocha, including bioactive compounds and the inhibition capacity of Datta/Qochqocha.

Methods

Study design, study population, sample size, and data source: nutrients content survey was conducted on 43 samples collected from producers, along with four standard samples prepared based on preliminary findings from a study conducted in this research regarding Datta/Qochqocha processing. Solid-phase extraction was performed using distilled water, ethanol, and methanol, depending on the nutrient to be analyzed. The sample size was calculated using a 0.058% [17] prevalence of vitamin content in Datta/Qochqocha components and assuming a 50% prevalence of vitamin content in processed Datta/Qochqocha at 95% certainty, 5% margin of error, 80% power, and considering a 5% nonresponse rate. The samples were collected from Datta/Qochqocha producers at their homes or selling shops in Hawassa and Nekemte cities.

Sample selection and data collection technique on Datta/Qochqocha: the snowball sampling method was employed to select study participants, Datta/Qochqocha producers. Each producer provided a quarter of a kilogram of traditionally fermented Datta/Qochqocha samples, which were collected in a clean 250-gram container. The samples were labeled with details including production date, quantity, ingredients, production area, and a unique sample code. These samples were then transported to the laboratory at the Centre for Food and Nutritional Science, College of Natural and Computational Science, Addis Ababa University, where they were stored in a refrigerator at -20°C for subsequent analysis. Conventional laboratory methods were employed to test the micronutrient content and scavenging capacity of the Datta/Qochqocha (Figure 1).

Chemicals used for analyses: the concentrations of zinc, manganese, and magnesium in Datta/Qochqocha were measured using atomic absorption spectroscopy (AAS), with standard solutions of zinc, manganese (reagecon), and magnesium, respectively [26]. Gallic acid, sodium carbonate, quercetin, ascorbic acid, methanol, ethanol, and deionized water were used to determine the phenol, flavonoid, and inhibin capacity of Datta/Qochqocha [27-29]. The concentrations of vitamins A, D, and E in Datta/Qochqocha were measured using ethanol, methanol, potassium hydroxide, sodium sulphate anhydrous, hexane, and L-ascorbic acid [30].

Sample extraction method: solid-phase extraction (SPE) was employed to measure the concentrations of vitamin A, vitamin E, vitamin D, zinc, manganese, and magnesium in traditionally fermented Datta/Qochqocha. Furthermore, SPE was used to analyze the phenol content, flavonoid levels, and antioxidant activity of the traditionally fermented Datta/Qochqocha.

Analysis: conventional laboratory analysis was conducted to determine the micronutrient content of Datta/Qochqocha, including vitamins A, D, and E; zinc; manganese; magnesium; as well as phenolic compounds, flavonoids, and scavenging activity. Microsoft Excel software was used to determine the means and standard deviations of these nutrients per 100 grams of Datta/Qochqocha, as well as its inhibitory and scavenging capacities.

Ethical consideration: the study was approved by Addis Ababa University's College of Natural and Computational Science Institutional Ethical Review Board (Ref. No.: CNCSDO/514/15/2023), and we consulted the community administration for consent. Participants were informed about the study's objectives, potential hazards, confidentiality, and the right to withdraw. The respondent's information was kept private and confidential, and number codes were used.

Procedure for determining micronutrient content in Datta/Qochqocha

Determination of vitamin A, vitamin D, and vitamin E content of Datta/Qochqocha: the procedures were carried out in a dark room. Five grams of blended Datta/Qochqocha, 300 mg of L-ascorbic acid, 15 mL of 50% potassium hydroxide solution, and 50 mL of ethanol were added to the flask sequentially. The mixture was heated under a reflux condenser in a water bath at 74°C for 90 minutes to facilitate saponification, then cooled in ice-cold water. The saponified sample was transferred to a separation funnel by washing with 50 mL of ethanol, followed by 100 mL of deionized water from the flask. The extraction was done three times with 50 mL of hexane, followed by three washes using 100 mL of deionized water. Using Whatman filter paper, the extract was filtered dropwise through one gram of anhydrous sodium sulphate. Then it was heated at 50°C until the hexane evaporated completely. Finally, the residue was dissolved using 10 mL of HPLC-grade methanol. The sample was analyzed using an Agilent 1260 HPLC at a flow rate of 1 mL/min and 30°C temperature for 25 minutes, using 325 nm, 265 nm, and 293 nm wavelengths for vitamins A, D, and E, respectively. The HPLC is the product of Agilent Technology in Shanghai, China [30].

Determination of zinc, manganese, and magnesium content of Datta/Qochqocha: the process involved heating 15 grams of Datta/Qochqocha to form charcoal, burning it in a CSF1200 furnace at 550 °C for 5 hours, rinsing the ash with nitric acid, and heating for further digestion sequentially. Then the ash was dissolved in 5 mL of 6-normal hydrochloric acid, heated, and then further dissolved in 10 mL of 3-normal hydrochloric acid, boiled, filtered, and makeup was made to 250 mL using distilled water. Then, 10 mL was prepared for analysis using a test tube [31]. A flame atomic absorption spectrometer (FAAS) was used to measure the concentrations of zinc, manganese, and magnesium in Datta/Qochqocha using Jena NOV350 at 213, 279, and 285 nanometers of wavelength, respectively [26]. The AAS test for the minerals was conducted using zinc standard solutions, manganese standard solutions, and magnesium standard solutions produced by Sigma-Aldrich Chemie GmbH in Switzerland [32-34].

Determining the phenol, flavonoid, and inhibition capacity of Datta/Qochqocha: a 1.5-gram Datta/Qochqocha sample was diluted in 25 mL of 99% methanol, shaken for 3 hours at 150 revolutions per minute, centrifuged for 15 minutes at 14000 revolutions per minute, filtered using Whatman filter paper, and transferred to the test tube [27-29,35] for conventional laboratory analysis.

Procedure to test phenol: the chemicals used to measure total phenol concentration in Datta/Qochqocha were prepared by dissolving 18.75 grams of sodium carbonate in 250 mL of distilled water for 24 hours, one milliliter of Folin-Ciocalteu reagent in 10 mL of distilled water, and 62.5 mg of Gallic acid in 50 mL of methanol [35,36]. Then a milliliter of the dissolved Folin-Ciocalteu reagent was mixed with one milliliter of sodium carbonate and eight milliliters of distilled water to form a calibration curve.

A total phenol concentration was analyzed using a Folin-Ciocalteu reagent assay and Gallic acid as a standard solution. Half milliliter of the extracted, traditionally fermented Datta/Qochqocha sample was taken in triplicate using three different test tubes. Eight minutes after adding 0.5 mL of methanol to each triplicate, one milliliter of Folin-Ciocalteu reagent was added to each triplicate test tube. Then, 1 mL of sodium carbonate was added to each triplicate. This mixture was made up to 10 mL by adding 7 mL of distilled water. After vortexing, the samples were kept in a dark room for 90 minutes before the absorbance test was performed at 760 nm using a Lambda 950 UV/VIS/NIR spectrometer [35,36]. The result was reported in milligram gallic acid equivalents (AGE) per milligram of dry weight and milligram equivalents of Datta/Qochqocha per gram of dry weight.

Procedure to test flavonoid status of Datta/Qochqocha: a total flavonoid concentration was analyzed using a 12.5 milligrams quercetin reagent assay dissolved in 50 mL of methanol and aluminum chloride as a standard. 0.5 mL of the extracted, traditionally fermented Datta/Qochqocha sample was taken in triplicate using three different test tubes. One milliliter of aluminum chloride was added to each triplicate after 0.5 milliliters of methanol had been added to each. The absorbance test was performed at a 760 nanometer wavelength using the Lambda 950 UV/VIS/NIR spectrometer [28,29]. The result was reported in milligram quercetin equivalents (QuE) per milligram of dry weight and milligram equivalents of Datta/Qochqocha per gram of dry weight.

DPPH scavenging activity test: three milligrams of ascorbic acid were dissolved in ten milliliters of distilled water. A solution of 0.02 grams of DPPH (2, 2-diphenyl-1-picrylhydrazyl) was prepared by dissolving it in 500 milliliters of methanol and kept in the dark for 30 minutes. Next, different volumes, 50, 100, 150, 200, 250, and 300 microliters of the extracted, traditionally fermented Datta/Qochqocha samples were taken and placed into separate test tubes to create a series of solutions. To each, we added enough 99% methanol to bring the total volume up to one milliliter, using 950, 900, 850, 800, 750, and 700 microliters of methanol, respectively. Finally, each tube was filled with four milliliters of the DPPH solution in methanol. Then the analyses were done using a DPPH assay and ascorbic acid as a standard and a Lambda 950 UV/VIS/NIR spectrometer [27]. The result was reported in milligrams equivalent of Datta/Qochqocha per gram of dry weight.

Results

Vitamin A, vitamin D, and vitamin E content of Datta/Qochqocha: this study found that 100 grams of traditionally fermented Datta/Qochqocha contains, on average, 0.0022 mg ± 0.0033 mg of vitamin D, 5.72 mg ± 2.96 mg of vitamin A, and 16.11 mg ± 4.42 mg of vitamin E.

Zinc, manganese, and magnesium content of Datta/Qochqocha: this study found that 100 grams of traditionally fermented Datta/Qochqocha contains, on average, 9.26 mg ± 0.013 mg of magnesium, 3.61 mg ± 0.042 mg of manganese, and 1.62 mg ± 0.027 mg of zinc.

Phenol, flavonoid, and antioxidant activity of Datta/Qochqocha

Phenol content of Datta/Qochqocha: the study found that the mean phenol concentration is 47.36 ± 21.52 mg AGE per 100 grams of traditionally fermented Datta/Qochqocha.

Flavonoid status of Datta/Qochqocha: the study found that the mean flavonoid concentration is 116.75 ± 52.54 mg QuE per 100 grams dry weight of traditionally fermented Datta/Qochqocha.

DPPH scavenging activity of Datta/Qochqocha: the study revealed that the average inhibition percentage of traditionally fermented Datta/Qochqocha is approximately 40.67% ± 10.01%. Additionally, it´s IC50 (half-maximal inhibitory concentration) was found to be less than 10 µg/mL. The IC50 was calculated by subtracting the intercept value from the sample concentration and then dividing the result by the slope (Table 1).

Discussion

The study aimed to identify the micronutrients that have antioxidant, anti-inflammatory, and immunity-boosting effects and the inhibition capacity of an Ethiopian traditionally fermented Datta/Qochqocha. This study determined that

100 grams of traditionally fermented Datta/Qochqocha contains approximately 0.0022 mg ± 0.0033 mg of vitamin D, 5.72 mg ± 2.96 mg of vitamin A, 16.11 mg ± 4.42 mg of vitamin E, 9.26 mg ± 0.013 mg of magnesium, 3.61 mg ± 0.042 mg of manganese, and 1.62 mg ± 0.027 mg of zinc. Additionally, the average concentrations of phenols and flavonoids were 47.36 ± 21.52 mg GAE and 116.75 ± 52.54 mg QuE per 100 grams of Datta/Qochqocha, respectively. The study also found that the mean inhibition percentage of the fermented Datta/Qochqocha was 40.67% ± 10.01%, with its IC50 value determined to be less than 10 µg/mL. Datta/Qochqocha´s micronutrient and inhibition properties might be due to the fermentation effect, which increases or makes foods more nutritious in antioxidant, anti-inflammatory, and immune-boosting micronutrients [3,21,23]. Besides, this might be attributed to Datta/Qochqocha´s ingredients micronutrients content that have antioxidant, anti-inflammatory, and immune-boosting properties [37-39].

This study found that the traditionally fermented Datta/Qochqocha can also be classified as a good source of vitamin A, vitamin D, vitamin E, magnesium, manganese, and zinc, compared to different good sources of respective micronutrients and antioxidant foods. Among the good sources of the respective micronutrients and antioxidants, eggs contain about 0.0013-0.0029 mg of vitamin D [40], carrots contain 0.0011 mg [41] of vitamin A, papaya and avocado contain 1.57 mg [42] and 3.2 mg [43] of vitamin E, chicken meat contains 1.5 gm-3.1 mg of zinc [44], tropical fruit contains 1.5 mg of manganese [45], leafy vegetables contain 32 mg (ranging 12 mg- 74 mg) of flavonoid [45], and papaya contains 14.532 mg of phenols [46] per 100 grams dry weight.

This study found that traditionally fermented Datta/Qochqocha contains a lower amount of vitamin D compared to mushrooms and fish, which contain 0.020-0.057 mg [47] and 0.025 mg [40] of vitamin D per 100 grams of dry weight, respectively. It also found that the vitamin A contained in Datta/Qochqocha is lower than in eggs, which is one gram of vitamin A per 100 grams [48] of egg dry weight. The magnesium and zinc content of Datta/Qochqocha is lower than that of pumpkin seed flour, which contains approximately 693 mg of magnesium and 11.5 mg of zinc per 100 grams [49]. Datta/Qochqocha has a lower flavonoid content compared to lingonberries or blueberries (1100 mg/100 g dry weight) and strawberries (500 mg/100 g dry weight) [50].

Fruits and vegetables exhibit anti-inflammatory and immune-enhancing effects due to their natural content of vitamins, minerals, antioxidants, and polyphenols [51-55]. Similarly, Datta/Qochqocha contains vitamins (A, D, and E), minerals (magnesium, manganese, and zinc), and inhibition capacity, which confer anti-inflammatory, immune-boosting, and free radical scavenging properties. Because it is known that these micronutrients have anti-inflammatory, immunity-boosting, and free radical scavenger properties [14,20-23]. The levels of vitamin D, vitamin A, vitamin E, zinc, and manganese in 100 grams of Datta/Qochqocha are within the scientifically recommended daily intake ranges for individuals, which are 0.005-0.025 mg for vitamin D [56], 0.9 mg for vitamin A [57], 15 mg for vitamin E [58], 11 mg for zinc [59], and 2.3 mg for manganese [60]. However, the magnesium levels in Datta/Qochqocha are below the recommended daily intake of 165 to 240 mg (55). The total flavonoid content of Datta/Qochqocha falls within the recommended daily allowance (RDA) for total flavonoids, which is estimated to be 0.05 to 1 gram per day [61]. But, the phenol content of Datta/Qochqocha is lower than the RDA of total phenol, which is estimated to be 314 mg/day.

It was found that the IC50 value of Datta/Qochqocha is in the range of a very strong classification of free radical scavenging capacity. Other studies' findings confirm that the value of IC50 less than 10 µg/mL shows powerful free radical scavenging capacity [62,63]. Datta/Qochqocha could promote health, prevent

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diseases, and enhance treatment effectiveness among therapy-taking patients by inhibiting free radicals/oxidations [64] and overcoming side effects like oxidants [11,12] and inflammatory problems [13-17].

Limitations: it would be beneficial to conduct comprehensive analyses of all other micronutrient and macronutrient contents in traditionally fermented Datta/Qochqocha.

Conclusion

This study found that Datta/Qochqocha contains vitamins A, D, and E; magnesium; manganese; zinc; phenols; flavonoids; and exhibits free radical inhibition capacity. Further research is recommended to explore additional nutritional components, microbial characteristics, and Datta/Qochqocha ´s potential applications in disease prevention and therapy through clinical trials.

What is known about this topic

- *Previously, only the fermentation process and ingredients of Datta/Qochqocha had been studied to some extent.*

What this study adds

- *The study found that Datta/Qochqocha contains vitamins A, D, and E; magnesium; manganese; zinc; phenols; flavonoids; and exhibits free radical inhibition capacity.*

Competing interests

The authors declare no competing interests.

Authors' contributions

Legesse Tesfaye analyzed and interpreted the findings and was the writer of the manuscript. Ali Solomon, Hailemariam Aynadis, Tigeneh Wondemagegnhu, and Debebe Zelalem served as supervisors of the work. The supervisors participated in formulating the study methodology, analysis, and editing of the manuscript. All the authors have read and agreed to the final manuscript.

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Table and figure

Table 1: percent of radical scavenging activity and IC50 of Datta/Qochqocha from DPPH assay in 2023

Figure 1: traditionally fermented Datta/Qochqocha samples in 2023

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Table 1: percent of radical scavenging activity and IC50 of Datta/Qochqocha from DPPH assay in 2023

Absorbance measurement				
Sample concentration (µg/ml)	**Control**	**Sample**	**Percentage of radical scavenging activity (%RSA)**	**IC50 micromolar (µM)**
50	1.121	0.8058	28.1206	0.2627
100	1.121	0.7543	32.7087	0.7332
150	1.121	0.6938	38.1081	1.2037
200	1.121	0.6514	41.8918	1.6742
250	1.121	0.5875	47.5920	2.1447
300	1.121	0.4974	55.6278	2.6152

Figure 1: traditionally fermented Datta/Qochqocha samples in 2023