Research Paper

Future of Food: Journal on Food, Agriculture and Society
12 (1) January 2024

Study of Physicochemical and Functional Characteristics of Calcium and Magnesium Enriched Apple Juice

Asma Liaquat[1], Arjumand Iqbal Durrani[2], Amjid Iqbal[3]*

[1]Department of Chemistry, University of Engineering and Technology Lahore 54890, Pakistan.
Email: liaquat_asma@yahoo.com
[2]Department of Chemistry, University of Engineering and Technology Lahore 54890, Pakistan.
Email: arjumand@uet.edu.pk
[3]Department of Food Sciences and Human Nutrition, College of Agriculture and Food, Qassim University, PO Box 6622, Buraidah, 51452, Qassim, Saudi Arabia.
Email: a.mansha@qu.edu.sa

*Correspondence: a.mansha@qu.edu.sa

Data of the Article

First Received: 21 March 2024 | Last Revision Received: 11 May 2024
Accepted: 29 May 2024 | Published Online: 20 July 2024
DOI: https://doi.org/10.5281/zenodo.15013471

Keywords

Biofortification, Apple Juice, Micronutrient Deficiencies, Eggshell Calcium, Almond Magnesium, Antioxidant Activity

This study investigates the biofortification of apple juice with calcium and magnesium using eggshells and almonds as natural sources. Addressing the global issue of micronutrient deficiencies, our research offers a sustainable and economical solution. We fortified apple juice with essential minerals and conducted physicochemical analyses, confirming significant increases in calcium and magnesium content. The fortified juice showed enhanced antimicrobial activity and increased antioxidant capacity. Sensory evaluation indicated no significant difference in consumer acceptability between fortified and non-fortified juice. This approach not only improves nutritional value but also utilizes eggshell waste, aligning with sustainable development goals. The findings suggest that biofortified apple juice could be an effective strategy for combating micronutrient malnutrition and promoting public health. Further research is needed to explore long-term effects and consumer acceptance on a larger scale.

1. Introduction

Hunger and malnutrition are among the most emerging problems in global health, and micronutrient deficiencies are observed in billions of people (Janssens et al., 2020). Such dietary inadequacies often referred to as “hidden hunger” lead to many illnesses including cognitive developmental issues in young children besides worsening of chronic diseases in grown individuals (Collado-Mateo et al., 2021). Solving these deficiencies calls for creative strategies towards increasing micronutrient density of carnvore foods (Hamzah Saleem et al., 2022). Proper micronutrient types and levels for addition were reported as ideal for the following reasons: Maintaining proper micronutrient addition levels and ensuring that calcium and magnesium are added as they are beneficial micronutrients that contribute to the growth of bones besides their contribution to metabolic procedures and the prevention of continual diseases such as hypertension, cancer, and type two diabetes as reported by Mosca, Leheup, & Dreumont (2019). As a biofortification strategy, the use of egg shell powder for calcium and almond powder for magnesium is budget friendly, environmentally sensitive and very efficient (Ligas et al., 2021). Essential nutrients that are required to enhance the nutritional worth of these crops are supplied by these biofortificants, apart from which they also help in waste management and sustainability in crop production (Dutta et al., 2022; Saini et al., 2021).

Micronutrient fortification of apple juice is a phenomenon of great interest because of its consumption pattern and nutritional balance (Appleton et al., 2016). This makes it possible to consummate across all ages hence it can reach the targeted demographic approaching a varied population segment with nutrient-deficiency disorders (Bossi Fedrigotti & Fischer, 2020). In addition, due to the natural nutritional values of apple juice and its appealing sensory attributes, it is ideal for fortifying various nutrients and do not impact its taste or the consumers' acceptance (Ribeiro et al., 2022; Zhang et al., 2021). The potential benefits of using apple juice as a fortification vehicle include the ability to introduce biofortificants, the product's popularity among consumers and the potential to reach different communities (Budke et al., 2021; Garg et al., 2021). Also, the use of apple juice as the base matrix for the biofortificants afforded the added advantage as it could dispose numerous biofortificants without changing its attributes about taste and texture to ensure consumers can retain their intake preference (Fernandes et al., 2022). This strategic approach not only opens the possibilities to develop new approaches to nutrition interventions through fortificants but also promotes a circular economy in the food and beverage industry that may use biofortificants from by-products (Bouis et al., 2020; Okello et al., 2022).

Our research contributes novelty by employing biofortification using a natural plant source to counteract micronutrient deficiencies instead of conventional chemical or salt-based fortification. Only on a small scale, micronutrient micro-nutrition deficiency (micronutrients) is also regarded as "hidden hunger", which has become one of the major public health problems in developing countries. Rather, these defects feature as part of a syndrome which is associated with defective growth and immunity leading to early mortality. Empirical studies that beamed the strong light of observation on this problem have moved rapidly to complete a picture, with ideas both simple and complicated for how society might address it. One of the potential remedies is bio-fortification, where foods are fortified with Nutrients extracted from natural means. Eggshells offers calcium as a major source and Magnesium as well contributed by the almonds which are both essential for human health. This study aims to improve mineral intake among the general population by preparing apple juice fortified with bio-sourced calcium and magnesium. Our primary objective is to demonstrate the feasibility of this biofortification strategy. We characterized the fortified juice's nutritional, microbiological, and sensory attributes using standard analytical methods. This approach not only contributes to nutritional science by offering a sustainable fortification strategy but also aligns with global health objectives by providing a practical solution to micronutrient deficiencies. Given the widespread consumption of apple juice, fortifying it with essential minerals could have a substantial public health impact. By utilizing bio-sourced fortificants, this study also supports the valorization of waste products like eggshells, contributing to environmental sustainability. The current state of research indicates a gap in practical applications of bio-sourced fortification in everyday beverages, which this study seeks to address. Here are the objectives of this research: To evaluate the feasibility of fortifying apple juice with bio-sourced calcium and magnesium. To analyze the nutritional, microbiological, and sensory attributes of the fortified juice.

2. Materials and Methods

2.1. Sample Collection and Preparation

In the summer of 2014, a total of 5 kilograms of apples were procured from a local market in Lahore, Pakistan, and shifted to our laboratory of Department of Chemistry, University of Engineering and Technology Lahore 54890, Pakistan. Upon arrival, the apples were thoroughly cleansed first with tap water and then with distilled water to remove any contaminants. Subsequently, the apples were quartered and processed through a juicer to extract the juice. This process was carried out under controlled laboratory conditions, maintaining a temperature range of 25–30 °C. A strainer was employed to filter out the seeds, ensuring that only the pure juice was collected. This filtered juice was then stored in a sealed jar at 4 °C to preserve its freshness until the fortification process commenced, one hour later.

2.2. Preparation of Biofortificants

The preparation of the eggshell powder involved a meticulous cleaning process where empty eggshells were first washed and then boiled in water for 30 mins to ensure all impurities were removed. After boiling, the eggshells were air-dried, finely crushed into a powder, and stored in a covered jar in a dry location for later use. Approximately one teaspoon of this eggshell powder, containing an estimated 750-800 mg of calcium along with other minerals, was produced from each eggshell. Considering the daily calcium recommendation, half a teaspoon of this powder provides around 400 mg of calcium. To determine the optimal solubility, half a teaspoon of the eggshell powder was mixed in two separate solutions: one with 10 ml of 5% malic acid and the other with 10 ml of 5% citric acid. Both solutions were left to stand at room temperature overnight with periodic stirring. The next day, the eggshell powder dissolved in the 5% malic acid solution was found to be more soluble and was thus chosen for the fortification process (Hoekenga, 2014).

Almond preparation began with soaking 10 grams of almonds in lukewarm water for an hour. After soaking, the almonds were peeled, dried, and then ground into a fine powder using a crusher. This almond powder, intended as the magnesium source, was stored in an airtight jar for subsequent use in the fortification process (Lowe et al., 2020).

2.3. Preparation of Fortified Apple Juice

To begin the fortification process, 10 ml of the previously prepared acid solution was placed into a shaking tank along with 200 g of sucrose and 10 g of the almond powder. To the above premixture, few drops of aroma concentrate was added to improve the organoleptic qualities of the juice. After that, the prepared apple juice concentrate was also added to this mixture as well also. The shaking was done vigorously for 3 mins which helped in distributing the contents in the shaking tank evenly. Due to this process the fortified apple juice concentrate was manufactured. The concentrate was then pipetted into 15 individual ampoules and the samples placed on wet ice then stored at −20 °C. This storage condition was kept in order to ensure that the quality of the juice was not interfered with before the time the various characterization tests were carried out. The details of the fortification process of the apple juice are shown below the Figure 1: The fortification process of apple juice involves several steps which include the making of an acid solution to the bottling of the fortified apple juice.

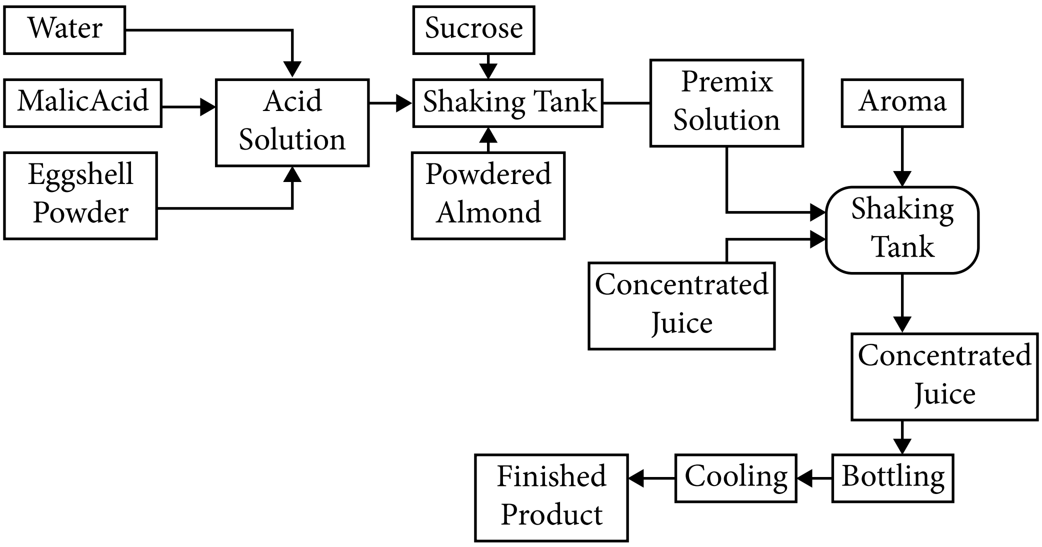

Figure 1: Flowchart of Fortified Apple Juice Production: This Diagram Outlines the Step-by-step Process of Producing Fortified Apple Juice. Starting with the Preparation of the Acid Solution Consisting of water and Malic Acid with Eggshell Powder, the Process Follows Through the Addition of Sucrose, Powdered Almond, and Aroma to the Shaking Tank, Resulting in the Premix Solution. This is then Combined with Concentrated Juice in the Shaking Tank to Produce the Juice Concentrate, which after Cooling and Bottling, Yields the Finished Fortified Apple Juice Product.

2.4. Quality Parameters

2.4.1. Acidity and pH

The acidity of the juice was quantitatively assessed through titration against 0.10N NaOH, using phenolphthalein as an indicator, while the pH levels were measured at ambient room temperature utilizing a pH meter. Titratable acidity, indicative of the juice's total proton availability, was expressed in grams per liter. The procedure involved titrating a 10 ml juice sample with NaOH, where the quantity of base required was calculated as the acid content per gram of the sample (Jaudzems et al., 2019), according to the formula:

Titratable Acidity (%) = $N \times V_1 \times Eq.wt / V2 \times 100$

2.4.2. Total Dissolved Solids

Determination of TDS To obtain the TDS, a filtrated was made with using an adequate mixed sample of juice soaked in standard glass-fiber filter paper. It is an indicative of TDS (Scannell & Jacobs, 2001) compared with the formula: It is an indicative of TDS as follow:

TDS (mg L^{-1})= (mg L^{-1})=$A-B/C$

2.4.3. Refractive Index

The juice's refractive index, correlating to its brix value, was measured using a refractometer at room temperature (Mock, Smith, & Schultz, 2003). This parameter helps infer the juice's sugar content and overall solute concentration.

2.4.4. Antimicrobial Activity

The juice was tested for its antibacterial properties against bacterial strains of S. aureus, B. subtilis and E. coli based on their growth assessment A Muller Hinton Agar medium which was prepared and autoclaved by the agar bore method is streaked with the other bacterium culture. Once the wells were dried, agar was punched through making depressions in which juice sample was added to it. The plates were incubated at 37ºC for 24 hour before being read and inhibition zones measured as previously described (Vlachos, Critchley, & Von Holy, 1996).

2.4.5. Quantitative Determination of Trace Elements

Calcium and magnesium levels of the juice were analyzed by complexometric titration with EDTA. The diluted apple juice sample was titrated using 0.01M EDTA solution, and the endpoint (Postscript file) was determined based on optical color change to measured total calcium concentration content in apples wines compared to standards of known calcium levels. Perchlorate free hexaneseparated the sample components and magnesium was then determined by titrating against 0.1M EDTA to end point color using autoanalyser a Autoanalyzer (SEAC model SP41). It determined the fortified mineral content in juice using these procedures (Alfassi, 2008).

2.4.6. Antioxidant Activity

A variety of tests in the assessment of the juice included total phenolic content (TPC), metal chelating activity, total flavonoid content (TFC), trolox equivalent antioxidant capacity (TEAC), and ferric reducing antioxidant power (FRAP) assays (Makkar et al., 1993). These tests checked the antioxidant effect and the capacity to sequester metal ions of the juice and let us understand some effects on the health benefits.

2.4.7. Sensory Evaluation

The fortified apple juice was subjected to organoleptic evaluation by a sensory panel of ten untrained persons, evaluating the factors color, odor, sweetness (Brix), acidity (% w/v) and flavor as well as overall acceptability. The characteristic was scored on a scale of 1 (minimum) to 5 (maximum), in order to investigate the consumer acceptability and palatability of the product. It is both a nutrient of reference as well represents the better example of quality control for intervention in micronutrient deficiency: given that this range includes apple juice concentrated with ready-to-drink apple juice enriched, which are able to relieve 40-50 % and up to about 60% respectively, it should ensure nutritional enhancement aims large enjoyed by apples (Elortondo et al., 2007).

2.5. Statistical Analysis

Statistical analysis was employed so as to minimize error and enhance the validity of results that are gotten from the quality parameters of the fortified apple juice. To determine the significance of the mean of various tests such as acidity, pH, total dissolved solids, antimicrobial activity and antioxidant capacity, a one-way ANOVA test was used. These experiments were done in triplicate to give adequate data for analysis of the results obtaining. Following the use of the ANOVA, Tukey's Honest Significant Difference (inconducting post hoc comparisons, which highlight specific groups, which vary significantly. In this research, data compilation and some templates of the preliminary analyses were done on spreadsheet program of Microsoft Excel, with the capability of generating simple graphic presentations that were useful in presenting trends and results of the study.

3. Results

3.1. Acidity, pH, Refractive Index, Brix and Density

The investigation into the acidity and pH of apple juices reveals critical insights into their impact on the juice's sensory qualities and stability (Table 1). Our study found that the total acidity of the fortified apple juice settled at an intermediate value of 0.54% (5.4 g L^{-1}), positioning it optimally between the extremes of overly acidic and insufficiently acidic juices. This balanced acidity level is crucial for maintaining the freshness of the juice without compromising its palatability. Further, the pH of the fortified apple juice was measured, reflecting its slightly acidic nature, which aligns with the desirable taste profile and stability against microbial spoilage. The pH value obtained from our fortified apple juice indicates a suitable acidity level that enhances the organoleptic properties of the juice while ensuring its safety and shelf-life.

The refractive index, brix percentage, and density of the samples were meticulously measured and are also presented in Table 1. Distilled water, with no dissolved solids, served as a control and displayed a refractive index of 1.33, a brix of 0%, and a density of 1. Pure apple juice exhibited a refractive index of 1.35, which correlates with a brix of 15% and a density of 1.06104, denoting its natural sugar content and dissolved solid constituents. The fortified apple juice showed a higher refractive index of 1.36, a corresponding brix of 19.8%, and an increased density of 1.08287, indicative of additional solids—presumably from the fortifying agents—which resulted in a higher sugar concentration as reflected by the brix value.

Table 1: Comparative Analysis of Physicochemical Parameters between Pure and Fortified Apple Juice.

Parameters	Pure Apple Juice	Fortified Apple Juice
pH	3.35	4.05
Titratable acidity	0.65	0.54
Refractive Index	1.35	1.36
Brix (%)	15	19.8
Density	1.06104	1.08287

This table displays the measured values of pH, titratable acidity, refractive index, Brix percentage, and density for both pure and fortified apple juice. The data illustrates the physicochemical changes that occur as a result of the fortification process.

3.2. Antimicrobial Activity

The fortified apple juice demonstrated notable antimicrobial properties. When assessed for inhibitory effects against *Escherichia coli*, *Streptococcus aureus*, and *Bacillus subtilis*, the fortified juice exhibited zones of inhibition measuring 8 mm, 6 mm, and 10 mm, respectively, as shown in Figure 2. Comparatively, *Bacillus subtilis* was most susceptible to the antimicrobial effects of the fortified juice, with a zone of inhibition measuring 10 mm, which suggests a potential for higher antimicrobial efficacy against this particular microorganism. The observed antimicrobial activity of the fortified apple juice is significant when contrasted with the standard antibiotic, penicillin, indicating that the fortification process with eggshell-derived calcium and almond-derived magnesium enhances the juice's ability to inhibit the growth of pathogenic bacteria.

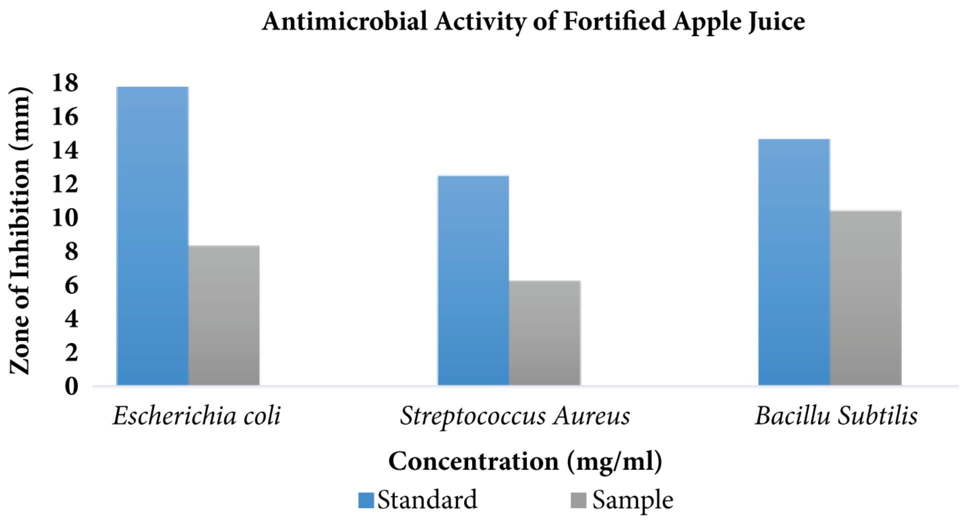

Figure 2: Bar Graph Illustrating the Zone of Inhibition Produced by Fortified Apple Juice on Various Bacterial Strains. The Bars Represent the Diameter of the Inhibition Zone Measured in Millimeters for Streptococcus Aureus, *Bacillus Subtilis, and Escherichia Coli*, Indicating the Antimicrobial Efficacy of the Juice Against these Pathogens.

3.3. Quantitative Determination of Trace Elements

The complexometric titration method revealed significant incorporation of essential minerals in the fortified apple juice (Table 2). The assay determined that the fortified apple juice contained 40.08 mg of calcium per 25 ml serving, achieved by titrating 100 ml of EDTA. This level corresponds to 61% of the RDA for calcium. Similarly, the magnesium content measured 2.67 mg per 25 ml serving, attained by titrating 1.1 ml of EDTA, equating to 34% of the RDA for magnesium. These results confirm that the targeted fortification strategy effectively enriched the apple juice with calcium and magnesium, key minerals for the growth and development of children aged 1-3 years. The study's approach suggests that increasing the serving size could potentially double the intake of these minerals, thereby significantly reducing the risk of deficiency-related diseases within this vulnerable age group. The data supports the premise that fortified apple juice can serve as a substantial dietary source of calcium and magnesium.

ISSN-Internet 2197-411x OLCL 862804632

Table 2: Quantitative levels of Calcium and Magnesium in Fortified Apple Juice.

	Fortified Apple Juice	
	ml of EDTA	mg of Fortification/25ml
Calcium	100	40.08
Magnesium	1.1	2.67

The table presents the results of complexometric titration, detailing the volume of EDTA required and the corresponding milligram content of calcium and magnesium fortificants per 25ml serving of the fortified apple juice.

3.4. Antioxidant Activities

The total phenolic content of the juice was measured at 48.65 mg/L of Gallic Acid Equivalents, indicating a substantial presence of phenolic compounds (Figure 3). The metal chelating activity showed a significant percentage of bound iron at 29.5%, suggesting the juice's capability to stabilize metal ions and potentially reduce oxidative stress. For the total flavonoid content, the fortified juice exhibited an impressive 226 mg/L of quercetin equivalents, which highlights a rich flavonoid content with antioxidant potential (Figure 3). The Trolox equivalent antioxidant capacity value was determined to be 17.4 mmol of Trolox Equivalents, underscoring the juice's ability to scavenge free radicals effectively (Figure 3). Furthermore, the ferric reducing antioxidant power assay gave a value of 3.43, indicating a robust reducing power in the juice's composition (Figure 3). This comprehensive set of results, graphically presented, underscores the fortified apple juice's enhanced antioxidant capacity, derived from the fortification process with naturally sourced minerals and the inherent antioxidant properties of the base juice.

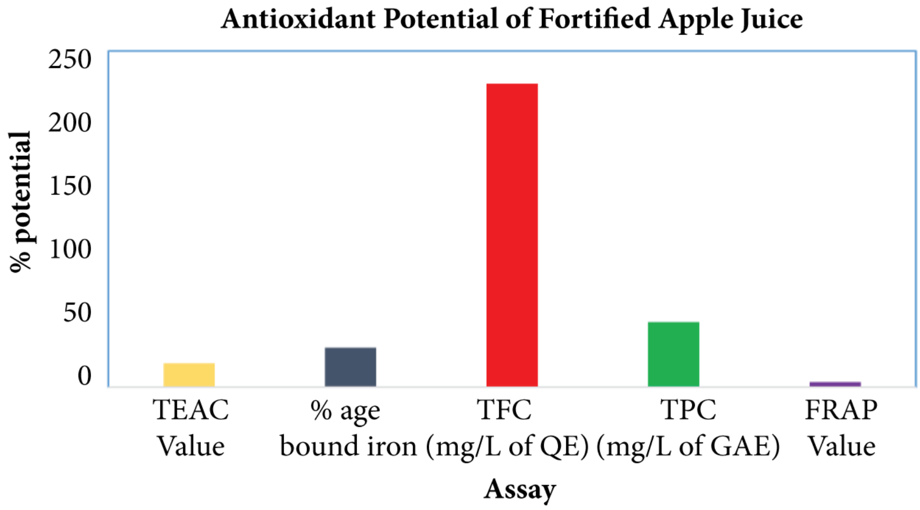

Figure 3: Graphical Representation of Antioxidant Properties of Fortified Apple Juice: The Bar Chart Demonstrates the Results of Various Antioxidant Assays, Including Trolox Equivalent Antioxidant Capacity, Percentage of Iron Binding, Total Flavonoid Content, Total Phenolic Content, and Ferric Reducing Antioxidant Power Measured in the Fortified Apple Juice. Each bar Represents the Quantified Value Obtained from the Respective Assay, Highlighting the Enhanced Antioxidant Profile of the Fortified Beverage.

3.5. Sensory Evaluation

The sensory evaluation of the fortified apple juice, when compared to pure apple juice, revealed nuanced differences in organoleptic properties (Figure 4). Panelists evaluated both juices across several parameters—mouthfeel, color, odor, sweetness, acidity, flavor, and overall acceptability. The fortified juice exhibited a marginally higher opacity, which could be attributed to the addition of powdered fortificants, particularly the finely ground magnesium. This turbidity, however, did not significantly detract from the sensory appeal of the product. In terms of odor and flavor, the fortified juice was noted to have a pleasant aroma and enhanced sweetness, likely due to the addition of aroma concentrate and extra sucrose. These characteristics made the fortified juice more appealing in taste to some panelists. Despite the slight increase in acidity from the added minerals, it did not adversely affect the juice's flavor profile. There was a negligible decrease in overall acceptability for the fortified juice, possibly due to the increased turbidity. Nevertheless, the positive sensory features largely balanced out any potential drawbacks from the fortification process.

 ISSN-Internet 2197-411x OLCL 862804632 January 2024

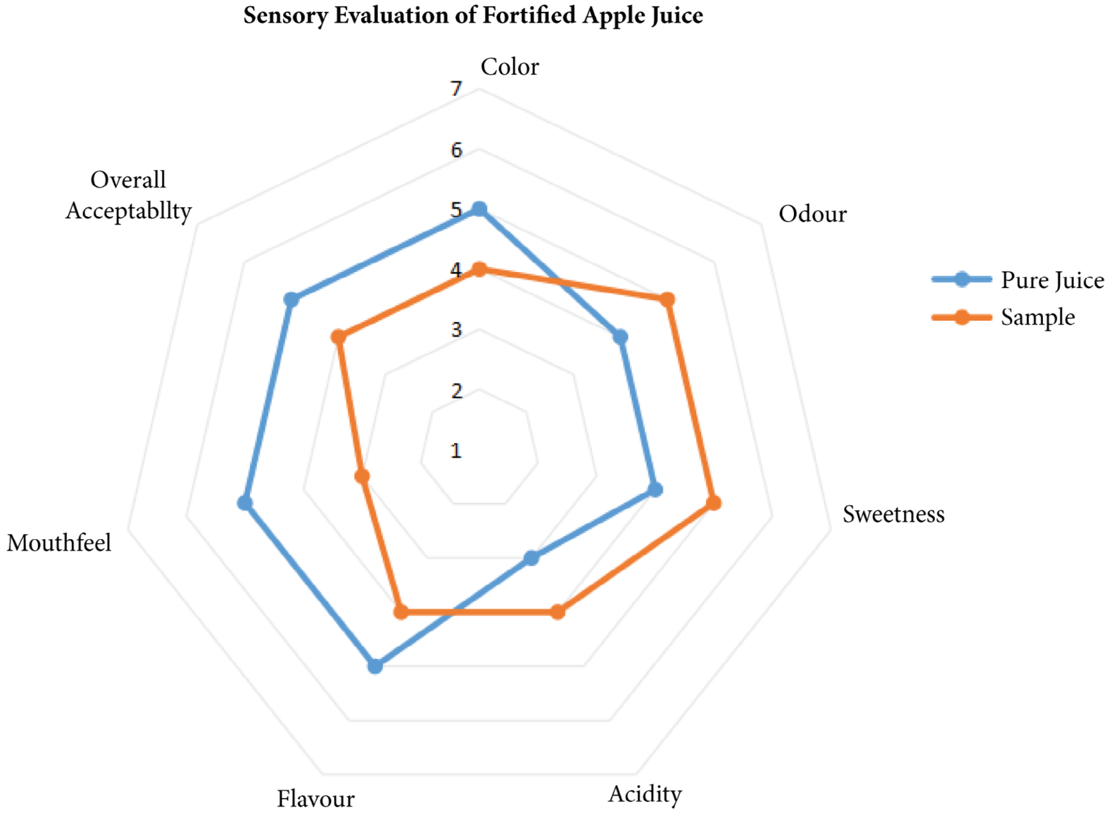

Figure 4: Radar Chart Comparing Sensory Attributes of Pure and Fortified Apple Juice. The Radar Chart Depicts the sensory evaluation scores for both pure and Fortified Apple Juice Across Multiple Attributes Including Color, Odor, Sweetness, Acidity, Mouthfeel, Flavor, and Overall Acceptability. Each Axis Represents a Different Sensory Attribute, with Outer Points Indicating Higher Scores. The Chart Visually Contrasts the Perceptual Differences between the Two Samples as Assessed by a Panel of Untrained Tasters.

4. Discussion

The fortification of apple juice with calcium and magnesium presents a novel strategy to not only enrich the latter not only in terms of nutrients, but also in terms of functional characteristics of one of the favorite drinks (Dutta et al., 2022; Stangoulis & Knez, 2022). From our findings on the changes in quality, antimicrobial efficacy, mineral content and sensory acceptability of the fortified juice, this study affords a holistic view of the possible effect on public health. Subsequent to fortification, there was a moderated increase in the samples' acidity as the apple juice reached a pH 4. 05. This change of pH plays a role in improving microbial stability this is in line with literature suggesting the antimicrobial benefits from mildly acidic solutions without detriment to taste and smell (Sindhu et al., 2017). At the same time, the increase in the refractive index from 1 Three-dimensional geometry and an increase in refractive values characterize the new material. 35 to 1. 36 along with brix value which increases from 15 % to 19 %. 8% and it is underlined their important raise in soluble solids, mainly due to added mineral supplements (Younas et al., 2021). Consequently, we have a denser matrix, which is indicated by increase from a density of 1. 06104 to 1. The results concerning the increase of alkaline-earth metals in the juice substrate 08287 support the researched data about the successful addition of calcium and magnesium into the fortified products, which is exhibited in other data on the modifications of stiffened drinks (Gharibzahedi & Jafari, 2017). This interaction between the fortification process and the physicochemical characteristic of the juice presents a complex improvement, thus supporting the function of fortificants not only for the nutrients' reinforcement but also for the possibility of increasing the shelf life and safety of the beverage. [SIZE=18] This is consistent with Cardoso et al. (2019) and D'Amato et al. (2020).

The fortified juice showcased augmented antimicrobial properties, presenting a notable suppression of pathogenic strains such as *Escherichia coli*, Streptococcus *aureus*, and *Bacillus subtilis*. This enhanced microbial inhibition can be attributed to the added biofortificants, which possibly introduce compounds that destabilize microbial membranes or inhibit enzyme activity crucial for

ISSN-Internet 2197-411x OLCL 862804632

bacterial growth (Pinhal et al., 2019). The introduction of biofortificants appears to have introduced additional antimicrobial mechanisms, thereby increasing the juice's resistance to microbial spoilage (Jagadeesan et al., 2024). Concurrently, the quantification of trace elements revealed that fortification significantly elevated the micronutrient content; a 25 ml serving of juice provided 40.08 mg of calcium and 2.67 mg of magnesium. These concentrations are in line with the nutritional targets for children, thereby bolstering the juice's role in mitigating nutrient deficiencies—a pivotal finding that aligns with research emphasizing the importance of fortified foods in pediatric diets (Gupta, Brazier, & Lowe, 2020). This strategic enhancement of the juice's composition through natural fortificants not only improves its nutritional value but also its functional attributes, lending support to its use as a healthful inclusion in children's diets (Chawla, Sivakumar, & Mishra, 2018).

Asset to the juice has already been substantiated by antioxidant analysis, which showed that fortification approach was successful in terms of nutritional enhancement and improved phenolic and flavonoid level (Mfarrej et al., 2023; Saleem et al., 2024). Whereas higher total phenolic content and total flavonoid content reveals an increase in compounds that are widely recognized for their free-radical scavenging properties (Ghani et al., 2021). Similar to the results described above, TEAC and FRAP measures of oxidative activity have been used as a foundation for why diets rich in antioxidants are beneficial targets for health maintenance (Amamcharla & Metzger, 2014). This likely played a role in the sensory evaluation being high of acceptance for fortified juice (Woods et al., 2020). This suggests that, despite minor changes in mouthfeel and diminished clarity as a result of additional biofortificants; most aspects from an overall sensory profile perspective remained unchanged. Therefore, the high consumer acceptability indicates that an overall goal of food fortification is also being fulfilled by not diminishing the inherent organoleptic characteristics of apple juice during its fortified state (Piochi et al., 2021). Differences were minor and had no effects on consumer perception, supporting the marketability of this fortified juice (Rosero et al., 2022). Our findings are in line with recent literature highlighting the possibility of increasing health-promoting properties of beverages without sacrificing sensory attributes, offering fortified apple juice as a realistic alternative for consumers interested in maintaining their well-being while tasting good.

5. Conclusion

The fortification of apple juice with calcium and magnesium may represent an innovative effort to enhance the nutritional position and functional properties of this frequently consumed drink. Through our detailed investigation, we also proved that this enrichment not just enhances the physicochemical and antimicrobial qualities but increases crucial mineral content as well — without largely compromising on sensory features consumers appreciate. Fortification also achieved a good modulation to the level of acidity on juice. This results in a value-added product with higher nutritional quality even during storage life along increase of soluble solids, indicated by refractive index and brix values. But the very explanation that enriching of a food does not by itself makes it healthy. But too much sugar, fat, and sodium in the fortified food can lead to health problems like high blood pressure obesity and there is also a risk of vitamin overdose. Accordingly, though our enriched apple juice has a believed promise of pros, in order to eliminate the set cannabis threat it must be used as part and parcel about an overall diet. Antimicrobial assays confirmed that the fortified juice possessed superior ability to inhibit bacterial pathogens when compared with control, attributed largely to bioactive compounds imparted by fortificants. The amounts of calcium and magnesium measured in apple juice were shown to supply a large portion, though not all, the daily needs%age for children; this underscores potential benefits from providing fortified juices as means to fill certain nutritional voids within younger populations. Assessment of antioxidant properties by various assays clearly indicated the presence of high levels of polyphenolics and flavonols in fortified juice, which have been reported to possess enormous potential for promoting human health due primarily to their strong natural antioxidant activities. Sensory evaluation revealed that the juice remained highly acceptable to consumers despite fortification; there were few sensory differences between fortified and non-fortified juices. Conclusion: Fortifying apple juice is an effective way to increase dietary nutrients and at the same time retain consumer acceptability. Future studies could further refine fortified juices to deliver the same level of clarity and mouthfeel as expected by consumers. In addition, for a more comprehensive evaluation of the fortified minerals bioavailability and health outcomes further long-term studies need to be conducted as they could have policy implications on nutritional enrichment in everyday foods/drinks.

6. Funding

The authors declare that no funding was received for conducting this study.

6.1. Ethical Approval

Not applicable.

6.2. Consent to Participate

Not applicable.

6.3. Consent to Publish

Not applicable.

6.4. Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

6.5. Availability of Data and Materials

The paper reflects the authors' own research and analysis in a truthful and complete manner. The paper is not currently being considered for publication elsewhere.

References

Alfassi, Z. B. (2008). *Determination of Trace Elements*. John Wiley & Sons. Retrieved from https://www.wiley.com/en-us/Determination+of+Trace+Elements-p-9783527615766

Amamcharla, J. K., & Metzger, L. E. (2014). Modification of the ferric reducing antioxidant power (FRAP) assay to determine the susceptibility of raw milk to oxidation. *International Dairy Journal, 34*(2), 177-179. doi: https://doi.org/10.1016/j.idairyj.2013.09.004

Appleton, K. M., Hemingway, A., Saulais, L., Dinnella, C., Monteleone, E., Depezay, L., et al. (2016). Increasing vegetable intakes: rationale and systematic review of published interventions. *European Journal of Nutrition, 55*(3), 869-896. doi: https://doi.org/10.1007/s00394-015-1130-8

Bossi Fedrigotti, V., & Fischer, C. (2020). Why Per Capita Apple Consumption Is Falling: Insights from the Literature and Case Evidence from South Tyrol. *Horticulturae, 6*(4), 79. doi: https://doi.org/10.3390/horticulturae6040079

Bouis, H., Birol, E., Boy, E., Gannon, B. M., Haas, J. D., Low, J., et al. (2020). Food biofortification: reaping the benefits of science to overcome hidden hunger. In *October webinar on The Need for Agricultural Innovation to Sustainably Feed the World by 2050*. Council for Agricultural Science and Technology (CAST). Retrieved from http://hdl.handle.net/1854/LU-8681290

Budke, C., Dierend, W., Schön, H.-G., Hora, K., Mühling, K. H., & Daum, D. (2021). Iodine Biofortification of Apples and Pears in an Orchard Using Foliar Sprays of Different Composition. *Frontiers in Plant Science, 12*, 638671. doi: https://doi.org/10.3389/fpls.2021.638671

Cardoso, R. V. C., Fernandes, Â., Gonzaléz-Paramás, A. M., Barros, L., & Ferreira, I. C. F. R. (2019). Flour fortification for nutritional and health improvement: A review. *Food Research International, 125*, 108576. doi: https://doi.org/10.1016/j.foodres.2019.108576

Chawla, R., Sivakumar, S., & Mishra, S. K. (2018). Fortification of lassi with vitamin A using natural vegetable powders. *International Journal of Chemical Studies, 6*(2), 2631-2635. Retrieved from https://www.chemijournal.com/archives/2018/vol6issue2/PartAK/6-2-109-965.pdf

Collado-Mateo, D., Lavín-Pérez, A. M., Peñacoba, C., Del Coso, J., Leyton-Román, M., Luque-Casado, A., et al. (2021). Key Factors Associated with Adherence to Physical Exercise in Patients with Chronic Diseases and Older Adults: An Umbrella Review. *International Journal of Environmental Research and Public Health, 18*(4), 2023. doi: https://doi.org/10.3390/ijerph18042023

D'Amato, R., Regni, L., Falcinelli, B., Mattioli, S., Benincasa, P., Dal Bosco, A., et al. (2020). Current Knowledge on Selenium Biofortification to Improve the Nutraceutical Profile of Food: A Comprehensive Review. *Journal of Agricultural and Food Chemistry, 68*(14), 4075-4097. doi: https://doi.org/10.1021/acs.jafc.0c00172

Dutta, S., Pal, S., Panwar, P., Sharma, R. K., & Bhutia, P. L. (2022). Biopolymeric Nanocarriers for Nutrient Delivery and Crop Biofortification. *ACS Omega, 7*(30), 25909-25920. doi: https://doi.org/10.1021/acsomega.2c02494

Elortondo, F. J. P., Ojeda, M., Albisu, M., Salmerón, J., Etayo, I., & Molina, M. (2007). Food quality certification: An approach for the development of accredited sensory evaluation methods. *Food Quality and Preference, 18*(2), 425-439. doi: https://doi.org/10.1016/j.foodqual.2006.05.002

Fernandes, P. A. R., Wessel, D. F., Coimbra, M. A., & Cardoso, S. M. (2022). Apple (Malus domestica) By-products: Chemistry, Functionality and Industrial Applications. In M. F. Ramadan & M. A. Farag (Eds.), *Mediterranean Fruits Bio-wastes: Chemistry, Functionality and Technological Applications* (pp. 349-373). Springer International Publishing. doi: https://doi.org/10.1007/978-3-030-84436-3_14

Garg, M., Sharma, A., Vats, S., Tiwari, V., Kumari, A., Mishra, V., et al. (2021). Vitamins in Cereals: A Critical Review of Content, Health Effects, Processing Losses, Bioaccessibility, Fortification, and Biofortification Strategies for Their Improvement. *Frontiers in Nutrition, 8*, 586815. doi: https://doi.org/10.3389/fnut.2021.586815

Ghani, M. A., Abbas, M. M., Ali, B., Aziz, R., Qadri, R. W. K., Noor, A., et al. (2021). Alleviating Role of Gibberellic Acid in Enhancing Plant Growth and Stimulating Phenolic Compounds in Carrot (Daucus carota L.) under Lead Stress. *Sustainability, 13*(21), 12329. doi: https://doi.org/10.3390/su132112329

Gharibzahedi, S. M. T., & Jafari, S. M. (2017). The importance of minerals in human nutrition: Bioavailability, food fortification, processing effects and nanoencapsulation. *Trends in Food Science & Technology, 62*, 119-132. doi: https://doi.org/10.1016/j.tifs.2017.02.017

Gupta, S., Brazier, A. K. M., & Lowe, N. M. (2020). Zinc deficiency in low- and middle-income countries: prevalence and approaches for mitigation. *Journal of Human Nutrition and Dietetics, 33*(5), 624-643. doi: https://doi.org/10.1111/jhn.12791

Hamzah Saleem, M., Usman, K., Rizwan, M., Al Jabri, H., & Alsafran, M. (2022). Functions and strategies for enhancing zinc availability in plants for sustainable agriculture. *Frontiers in Plant Science, 13*, 1033092. doi: https://doi.org/10.3389/fpls.2022.1033092

Hoekenga, O. A. (2014). Genomics of Mineral Nutrient Biofortification: Calcium, Iron and Zinc. In R. Tuberosa, A. Graner, & E. Frison (Eds.), *Genomics of Plant Genetic Resources: Volume 2. Crop productivity, food security and nutritional quality* (pp. 431-454). Springer Netherlands. doi: https://doi.org/10.1007/978-94-007-7575-6_18

Jagadeesan, Y., Meenakshisundaram, S., Pichaimuthu, S., & Balaiah, A. (2024). A scientific version of understanding "Why did the chickens cross the road"? – A guided journey through Bacillus spp. towards sustainable agriculture, circular economy and biofortification. *Environmental Research, 244*, 117907. doi: https://doi.org/10.1016/j.envres.2023.117907

Janssens, C., Havlík, P., Krisztin, T., Baker, J., Frank, S., Hasegawa, T., et al. (2020). Global Hunger and Climate Change Adaptation Through International Trade. *Nature Climate Change, 10*(9), 829-835. doi: https://doi.org/10.1038/s41558-020-0847-4

Jaudzems, G., Zhang, F., Bolong, W., Bao, L., & Xiao, J. (2019). Chloride in Milk, Milk Powder, Whey Powder, Infant Formula, and Adult Nutritionals Potentiometric Titration: Collaborative Study, Final Action 2016.03. *Journal of AOAC INTERNATIONAL, 102*(2), 564-569. doi: https://doi.org/10.5740/jaoacint.18-0244

Ligas, B., Izydorczyk, G., Mikula, K., Skrzypczak, D., Konkol, D., Korczyński, M., et al. (2021). Valorization of postextraction residues—analysis of the influence of new feed additives with micronutrients on eggs quality parameters. *Poultry Science, 100*(11), 101416. doi: https://doi.org/10.1016/j.psj.2021.101416

Lowe, N. M., Zaman, M., Moran, V. H., Ohly, H., Sinclair, J., Fatima, S., et al. (2020). Biofortification of wheat with zinc for eliminating deficiency in Pakistan: study protocol for a cluster-randomised, double-blind, controlled effectiveness study (BIZIFED2). *BMJ Open, 10*(11), e039231. doi: https://doi.org/10.1136/bmjopen-2020-039231

Makkar, H. P. S., Blümmel, M., Borowy, N. K., & Becker, K. (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *Journal of the Science of Food and Agriculture, 61*(2), 161-165. doi: https://doi.org/10.1002/jsfa.2740610205

Mfarrej, M. F. B., Wang, X., Fahid, M., Saleem, M. H., Alatawi, A., Ali, S., et al. (2023). Floating Treatment Wetlands (FTWs) is an Innovative Approach for the Remediation of Petroleum Hydrocarbons-Contaminated Water. *Journal of Plant Growth Regulation, 42*(3), 1402-1420. doi: https://doi.org/10.1007/s00344-022-10674-6

Mock, J. J., Smith, D. R., & Schultz, S. (2003). Local Refractive Index Dependence of Plasmon Resonance Spectra from Individual Nanoparticles. *Nano Letters, 3*(4), 485-491. doi: https://doi.org/10.1021/nl0340475

Mosca, P., Leheup, B., & Dreumont, N. (2019). Nutrigenomics and RNA methylation: Role of micronutrients. *Biochimie, 164*, 53-59. doi: https://doi.org/10.1016/j.biochi.2019.07.008

Okello, J. J., Just, D. R., Jogo, W., Kwikiriza, N., & Tesfaye, H. (2022). Do Behavioral Interventions Increase the Intake of Biofortified Foods in School Lunch Meals? Evidence from a Field Experiment with Elementary School Children in Ethiopia. *Current Developments in Nutrition, 6*(2), nzac008. doi: https://doi.org/10.1093/cdn/nzac008

Pinhal, S., Ropers, D., Geiselmann, J., & Jong, H. d. (2019). Acetate Metabolism and the Inhibition of Bacterial Growth by Acetate. *Journal of Bacteriology, 201*(13), e00147-00119. doi: https://doi.org/10.1128/jb.00147-19

Piochi, M., Chiavaro, E., Cichelli, A., Torri, L., & Cerretani, L. (2021). Sensory properties of iodine-biofortified potatoes. *Italian Journal of Food Science, 33*(1), 52-60. doi: https://doi.org/10.15586/ijfs.v33i1.1951

Ribeiro, J. A., dos Santos Pereira, E., de Oliveira Raphaelli, C., Radünz, M., Camargo, T. M., da Rocha Concenço, F. I. G., et al. (2022). Application of prebiotics in apple products and potential health benefits. *Journal of Food Science and Technology, 59*(4), 1249-1262. doi: https://doi.org/10.1007/s13197-021-05062-z

Rosero, A., Pastrana, I., Martínez, R., Perez, J.-L., Espitia, L., Araujo, H., et al. (2022). Nutritional value and consumer perception of biofortified sweet potato varieties. *Annals of Agricultural Sciences, 67*(1), 79-89. doi: https://doi.org/10.1016/j.aoas.2022.05.004

Saini, S., Saxena, S., Samtiya, M., Puniya, M., & Dhewa, T. (2021). Potential of underutilized millets as Nutri-cereal: an overview. *Journal of Food Science and Technology, 58*(12), 4465-4477. doi: https://doi.org/10.1007/s13197-021-04985-x

Saleem, M. H., Mfarrej, M. F. B., Khan, K. A., & Alharthy, S. A. (2024). Emerging trends in wastewater treatment: Addressing microorganic pollutants and environmental impacts. *Science of The Total Environment, 913*, 169755. doi: https://doi.org/10.1016/j.scitotenv.2023.169755

Scannell, P. W., & Jacobs, L. L. (2001). *Effects of Total Dissolved Solids on Aquatic Organisms* (Technical Report No. 01-06). Alaska Department of Fish and Game: Division of Habitat and Restoration. Retrieved from https://www.adfg.alaska.gov/static/home/library/pdfs/habitat/01_06.pdf

Sindhu, R., Binod, P., Madhavan, A., Beevi, U. S., Mathew, A. K., Abraham, A., et al. (2017). Molecular improvements in microbial α-amylases for enhanced stability and catalytic efficiency. *Bioresource Technology, 245*, 1740-1748. doi: https://doi.org/10.1016/j.biortech.2017.04.098

Stangoulis, J. C. R., & Knez, M. (2022). Biofortification of major crop plants with iron and zinc - achievements and future directions. *Plant and Soil, 474*(1), 57-76. doi: https://doi.org/10.1007/s11104-022-05330-7

Vlachos, V., Critchley, A. T., & Von Holy, A. (1996). Establishment of a Protocol for Testing Antimicrobial Activity in Southern African Macroalgae. *Microbios, 88*(355), 115-123. Retrieved from https://europepmc.org/article/med/9131809

Woods, B.-J., Gallego-Castillo, S., Talsma, E. F., & Álvarez, D. (2020). The acceptance of zinc biofortified rice in Latin America: A consumer sensory study and grain quality characterization. *PloS One, 15*(11), e0242202. doi: https://doi.org/10.1371/journal.pone.0242202

Younas, N., Durrani, A. I., Rubab, S., Munawar, A., Batool, M., & Sheikh, A. (2021). Formulation and Characterization of Calcium-Fortified Jelly and Its Proximate Composition and Sensory Analysis. *Journal of Oleo Science, 70*(6), 849-854. doi: https://doi.org/10.5650/jos.ess21051

Zhang, S., Hu, C., Guo, Y., Wang, X., & Meng, Y. (2021). Polyphenols in fermented apple juice: Beneficial effects on human health. *Journal of Functional Foods, 76*, 104294. doi: https://doi.org/10.1016/j.jff.2020.104294