



RESEARCH ARTICLE

REVISED Testing for sensory threshold in drinking water with added calcium: a first step towards developing a calcium fortified water [version 2; peer review: 2 approved]

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v2 First published: 19 Oct 2021, 5:151
<https://doi.org/10.12688/gatesopenres.13361.1>
 Latest published: 10 Jan 2022, 5:151
<https://doi.org/10.12688/gatesopenres.13361.2>

Abstract

Background: Food fortification is an effective strategy that has been recommended for improving population calcium inadequate intakes. Increasing calcium concentration of water has been proposed as a possible strategy to improve calcium intake. The objective of this study was to determine the sensory threshold of different calcium salts added to drinking water using survival analysis.

Methods: We performed the triangle test methodology for samples of water with added calcium using three different calcium salts: calcium chloride, calcium gluconate and calcium lactate. For each salt, a panel of 54 consumers tested seven batches of three water samples. Data were adjusted for chance and sensory threshold was estimated using the survival methodology and a discrimination of 50%.

Results: The threshold value estimation for calcium gluconate was 587 ± 131 mg/L of water, corresponding to 25% discrimination, for calcium lactate was 676 ± 186 mg/L, corresponding to 50% discrimination, and for calcium chloride was 291 ± 73 mg/L, corresponding to 50% discrimination.

Conclusions: These results show that water with calcium added in different salts and up to a concentration of 500 mg of calcium/L of water is feasible. The calcium salt allowing the highest calcium

Open Peer Review

Reviewer Status

Invited Reviewers

1

2

version 2

(revision)

10 Jan 2022



report

**version 1**

19 Oct 2021



report



report

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concentration with the lowest perceived changes in taste was calcium gluconate. Future studies need to explore stability and acceptability over longer periods of time.

Keywords

Drinking water, calcium salts, survival analysis, triangle test, calcium inadequacy

Any reports and responses or comments on the article can be found at the end of the article.

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Author roles: **Cormick G:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; **Matamoros N:** Conceptualization, Data Curation, Formal Analysis, Methodology, Writing – Review & Editing; **Romero IB:** Data Curation, Supervision, Writing – Review & Editing; **Perez SM:** Data Curation, Writing – Review & Editing; **White C:** Data Curation, Writing – Review & Editing; **Watson DZ:** Conceptualization, Data Curation, Writing – Review & Editing; **Belizán JM:** Supervision, Writing – Review & Editing; **Sosa M:** Conceptualization, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; **Gugole Ottaviano MF:** Data Curation, Formal Analysis, Writing – Original Draft Preparation; **Elizagoyen E:** Data Curation, Formal Analysis, Writing – Review & Editing; **Garitta L:** Data Curation, Formal Analysis, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by the Bill and Melinda Gates Foundation [OPP1190821].

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How to cite this article: Cormick G, Matamoros N, Romero IB *et al.* **Testing for sensory threshold in drinking water with added calcium: a first step towards developing a calcium fortified water [version 2; peer review: 2 approved]** Gates Open Research 2022, 5:151 <https://doi.org/10.12688/gatesopenres.13361.2>

First published: 19 Oct 2021, 5:151 <https://doi.org/10.12688/gatesopenres.13361.1>

REVISED Amendments from Version 1

The main changes in this new version are in the methodology section of the manuscript. We have described with more details the quality of the salts used, the baseline water and more information regarding the steps performed in the triangle tests.

Any further responses from the reviewers can be found at the end of the article

Introduction

Calcium intake is well below recommendations in most low- and middle-income countries, and in many countries calcium availability from foods does not cover the needs of their populations¹⁻⁵. Appropriate calcium intake has shown many health benefits besides the prevention of osteoporosis such as reduction of hypertensive disorders in pregnancy, lower blood pressure, lower cholesterol values, lower blood pressure in children whose mothers were supplemented with calcium during pregnancy and prevention of recurrence of colorectal adenomas⁶⁻¹¹.

Food fortification is an effective strategy that has been successfully used to reduce micronutrient deficiencies¹². Increasing the calcium concentration of water is a possible strategy to improve calcium intake¹³. Although there are natural mineral waters with high calcium contents on the market, calcium concentration in tap water and commercially bottled water seems to be low in most parts of the world¹⁴⁻¹⁷. There are many advantages to using water as a fortification vehicle as it is universally consumed, calcium in water has good bioavailability, similar to that of milk, and it is consumed throughout the day, which also improves absorption^{18,19}. Simulations of the impact of water supplemented with 500 mg of calcium on the calcium intake of populations of different countries with low calcium intake have shown an increase in the percentage of people reaching adequate intakes without exceeding the risk for excess, measured by the recommended upper limit for calcium¹³.

Designing a strategy to increase the calcium concentration of drinking water first requires an exploration of the physicochemical changes and organoleptic properties of water with added calcium^{20,21}. A first step is to define the type of salt and concentration at which the organoleptic characteristics are acceptable for consumers. Thresholds are useful measures for determining an individual's or group's average sensitivity to a tastant or odorant chemical. Sensory thresholds are often collected through ascending forced-choice methods, like three-alternative forced choice (3-AFC)²² or triangle test²³. However, in methods of ascending concentrations a person may guess the correct answer by chance or might detect it at low concentrations, but fail after several steps due to fatigue or adaptation. The use of statistical survival analysis considers the group's results of threshold data collected through the forced-choice method reducing the probability of chance in methods with consecutive correct answers²⁴.

The objective of this study was to determine the sensory detection threshold of different calcium salts added to drinking water using survival analysis.

Methods**Ethics statement**

This study was approved by the ethical committee of the Posadas Hospital (ref: 318 EUPeSe/19). Participants in this study received oral and written explanations of the protocol and signed an informed consent form for participation and use of data.

Selection of salts and concentrations

For this study, we selected three calcium salts suitable for human consumption, commonly used by the food industry and with a reported solubility allowing solutions of at least 500 mg of calcium per liter at room temperature (20°C). The solubility of these three salts theoretically allows solutions to be obtained that widely exceed this value. Calcium chloride dihydrate has solubility in water of 740 g/L at 20°C (up to 200,000 mg Ca/L), calcium gluconate monohydrate has a solubility in water of 32,7 g/L at 20°C (up to 2900 mg Ca/L), and calcium lactate pentahydrate has a solubility in water of 58 g/L at 20°C (7500 mg Ca/L)²⁵⁻²⁷. Calcium chloride dihydrate was purchased from Sigma-Aldrich Corporation, and meets analytical specifications of The European Pharmacopoeia (Ph. Eur.), Pharmaceutical Reference Standards (USP), FCC, E509, whereas calcium lactate pentahydrate and calcium gluconate Monohydrate were purchased from Surfactan, and both meet analytical specifications of USP and European Pharmacopoeia (EP).

The concentrations used to determine the sensory detection threshold of each calcium salt are shown in [Table 1](#). They were defined taking into account the solubility of different calcium salts, previous studies performed in different countries showing that 500 mg of Ca/L increases the percentage of people reaching adequate intake without exceeding the recommended upper limit for calcium intake and a triangle test performed by an expert panel¹². This expert panel of six assessors were selected and trained following the guidelines of ISO 8586-1²⁸, and had a minimum of 100 hours experience in discrimination and descriptive tests.

Table 1. Calcium concentrations for each salt tested in the triangle test.

Samples	mg of calcium/L of water		
	Calcium gluconate monohydrate	Calcium lactate pentahydrate	Calcium chloride dihydrate
1	116	146	104
2	151	195	130
3	231	233	177
4	361	378	260
5	394	474	372
6	692	702	518
7	796	820	755

Using the triangle test, the expert panel compared concentration where no sensory differences were detected and concentrations where marked differences were detected (77 and 800 mg of Ca / L for calcium chloride, 107 and 820 mg of Ca / L for calcium lactate, and 107 and 863 mg of Ca / L for calcium gluconate) against water without added calcium to define the seven samples used in the sensory threshold test. [Table 1](#)

Baseline water

All the concentrations prepared from the different calcium salts were tested against a reference bottled table water that is commonly consumed in the country and complies with the standards of the water cooperative in Argentina (Instituto verificador de elaboración de soda en sifones, IVESS)²⁹. According to the information provided by the cooperative, this water is filtered, dechlorinated, ozone purified tap water. The baseline bottled water had a calcium concentration of 27 mg/L and water hardness of 104 mg/L (CaCO₃). This water also contained 32 mg/L of sodium, 10mg/L of nitrates, 38 mg/L of chlorides, 80 mg/L of alkalinity (CaCO₃) and 270 mg/L total dissolved solids.

Sample preparation

Samples were prepared using the previously described baseline bottled table water. Solutions were prepared registering the weight of the salt added. The calcium concentration was measured in a sample of the final solution by atomic absorption spectroscopy at 422.7 nm (Varian AA 240FS) in an acetylene-air flame, the technique used was based on the Standard methods for the examination of water and wastewater³⁰. [Table 1](#) shows the calcium concentrations tested for each salt.

Consumer panel

The panel was selected from a consumer's database hold by the Departamento de Evaluación Sensorial de Alimentos- Instituto Superior Experimental de Tecnología Alimentaria (DESA-ISETA) from the city of 9 de Julio (Buenos Aires, Argentina). Individuals registered in the database have previously participated in consumer panel tests and agreed to be contacted by the institute for similar tests. Those who were aged 18 or older and reported drinking water every day were invited to participate.

The number of consumers was based on the requirements of the ISO 4120:2004²³, for similarity tests. The parameters considered for this work were: β :5%; α :10%; Pd:30%; leading to a total of 54 consumers. The tests were conducted in the facilities of DESA-ISETA.

Sensory methodology

We perform a triangle test to detect the threshold taste for each salt following the ISO/FDIS- 4120:2004 (E)²³. Before the triangle test the consumer panel received a short training session on the triangle test methodology and the procedures required to taste water samples. As part of the training, participants were asked to detect the odd sample of three 10 ml samples, two containing water and one water supplemented with 10 grams of sugar per liter.

After the training, threshold tests were carried out. In this test, a batch of three samples were presented simultaneously to the

panelists, two samples were from the same concentration, and one is from a different concentration. There were six possible serving orders (AAB, ABA, BAA, BBA, BAB, ABB) which were counterbalanced across all panelists. Consumers were asked to taste each sample in the row (left to right) and to select the odd sample. Between batches, participants were asked to neutralize taste with mineral water and white bread.

Each consumer received seven batches of three solutions of 30 ml each. The tests were performed on different days to avoid tiredness or flavour carryover. For each salt, consumers tested three batches one day and the remaining four batches on a second day.

Samples were presented at room temperature (18–23°C) in similar polystyrene 70 mL cups coded with three-digit random to allow blinding, following sensory analysis protocols, where only participants are blinded to the samples³¹.

Statistical analysis

The statistical model developed by Hough *et al.* (2013) was applied to the data obtained in the triangle test²⁴. A random variable C is the salt concentration at which an assessor correctly discriminates a sample. The discrimination function D(c) can be defined as the probability of an assessor discriminating a sample before concentration c, i.e., $D(c) = P(C \leq c)$. For example, the log-normal distribution is expressed by:

$$\% \text{ Discrimination} = \Phi \left(\frac{\log(\text{concentration}) - \mu}{\sigma} \right) \times 100 \quad (1)$$

and the Weibull distribution is expressed by:

$$\% \text{ Discrimination} = \exp \left(- \exp \left(\frac{\log(\text{concentration}) - \mu}{\sigma} \right) \right) \times 100 \quad (2)$$

Where, in [Equation \(1\)](#), $\Phi (\cdot)$ is the cumulative normal distribution function and μ and σ are the model's parameters.

In [Equation \(2\)](#), $\exp [-\exp (\cdot)]$ is the distribution function of the smallest extreme value distribution and μ and σ are the model's parameters.

Threshold estimations were calculated for 50% discrimination³².

To estimate percent discrimination probability versus concentration distribution, data were performed in R version 4.0.0 Statistical package (The R Foundation for Statistical Computing). The survreg function of the survival package was used.

Results

Calcium gluconate monohydrate

When applying the survival analysis methodology to the calcium gluconate threshold data, the best fitting distribution was the Weibull ([Equation 2](#)). The resulting parameters \pm 95% confidence intervals were $\mu = 6.9 \pm 0.2$ and $\sigma = 0.44 \pm 0.15$ ³³. Percent discrimination versus concentration for this Weibull distribution is plotted in [Figure 1](#).

The threshold value estimation corresponding to 25% discrimination \pm 95% confidence intervals was 587 ± 131 mg of Ca/L, corresponding to a water sample with a calcium gluconate concentration of 6.6 ± 1.4 g/L.

We were not able to estimate the threshold value estimation corresponding to 50% discrimination as the maximum number of successful answers obtained from the consumer panel reached 44%.

Calcium lactate pentahydrate

For the calcium lactate threshold, the Weibull was the best fitting distribution (Equation 2). The resulting parameters \pm 95% confidence intervals were $\mu = 6.8 \pm 0.3$ and $\sigma = 0.76 \pm 0.25$. Percent discrimination versus concentration for this Weibull distribution is plotted in Figure 2. The threshold value estimation corresponding to 50% discrimination \pm 95% confidence intervals was 676 ± 186 mg of Ca/L, corresponding to a water sample with a calcium lactate concentration of 5.2 ± 1.4 g/L.

Calcium chloride dihydrate

When the survival analysis methodology was applied to the calcium chloride threshold data, the best fitting distribution

was the log-normal (Equation 1). The resulting parameters \pm 95% confidence intervals were $\mu = 5.7 \pm 0.3$ and $\sigma = 0.83 \pm 0.20$. Percent discrimination versus concentration for this log-normal distribution is plotted in Figure 3.

The threshold value estimation corresponding to 50% discrimination \pm 95% confidence intervals was 291 ± 73 mg of Ca/L, corresponding to a water sample with a calcium chloride concentration of 1.1 ± 0.3 g/L.

Discussion

This study shows that the sensory detection threshold of water with added calcium salts allows the increase of calcium concentration of water up to a level of 500 mg of calcium /L. The feasibility of using water with added calcium to improve dietary intake will depend on the drinking water distribution system, which will define the type of salt and concentration to be used. Inorganic salts such as calcium chloride could be used to increase the calcium content of bottled or tap water. Further tests should be done in order to determine the maximum level that could be added to tap water while complying with regulations for tap drinking water. On the other hand, organic salts such as calcium

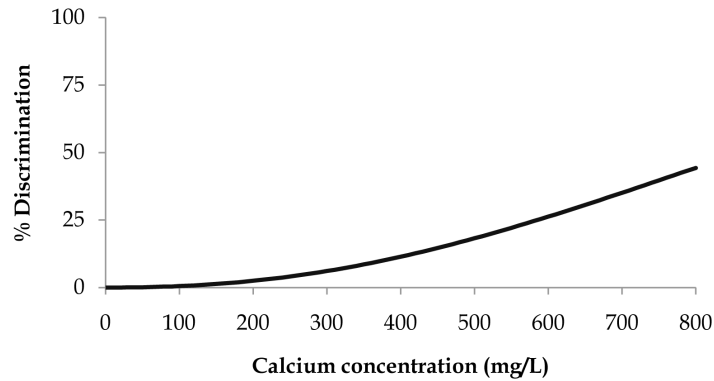


Figure 1. Calcium gluconate monohydrate threshold taste. Percent discrimination versus calcium concentration for the Weibull distribution.

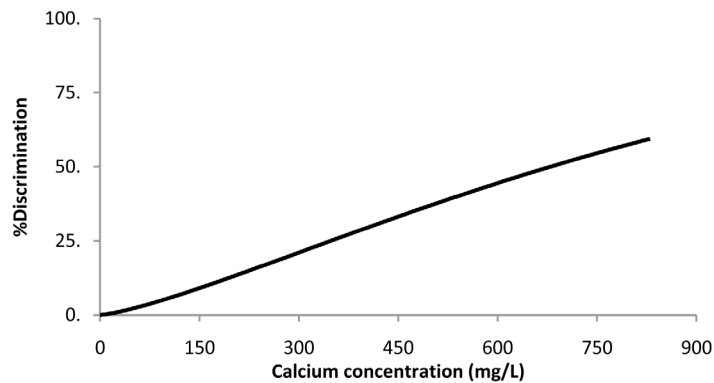


Figure 2. Calcium lactate pentahydrate threshold taste. Percent discrimination versus calcium concentration for the Weibull distribution.

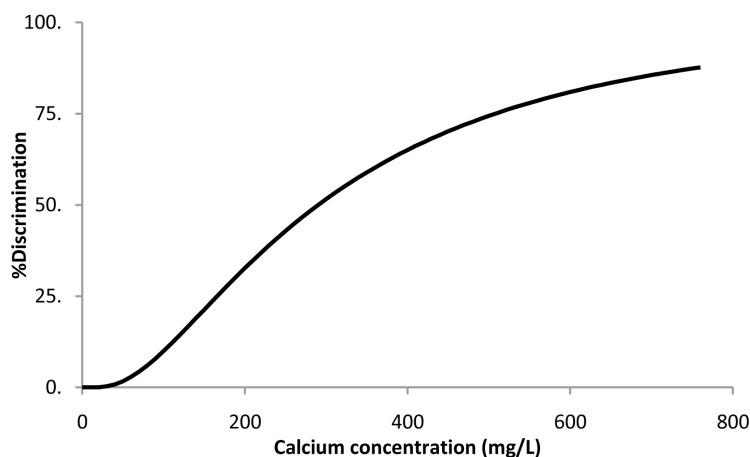


Figure 3. Calcium chloride dihydrate threshold taste. Percent discrimination versus calcium concentration for the log normal distribution.

gluconate and calcium lactate can only be used to increase the calcium concentration of bottled drinks, and their application needs further studies on safety and stability. Further studies should also be performed to establish shelf life.

Considering that most drinking tap and bottled waters have very low calcium concentrations, the level of calcium attained in this study would involve a significant increase to impact calcium intake at population level^{13,17}. In this study, it was possible to define the threshold for taste using the triangle test methodology and survival analysis statistics.

Alcaire *et al.* (2014) applied survival analysis to estimate equivalent sweet concentration of low-calorie sweeteners in orange juice³⁴. They found its main advantage is the consideration of individual differences among assessors, which may lead to more accurate estimations than those obtained with other methodologies.

Reis *et al.* (2016) compared two sensory methodologies (paired comparison and magnitude estimation) and two data analysis approaches (logistic regression and survival analysis) to estimate equivalent sweet concentration of high-intensity sweeteners³⁵. They found paired comparison and magnitude estimation provided similar estimations for the sweeteners, but logistic regression and survival analysis differed in the accuracy of the estimations. Data analysis performed using survival analysis gave more accurate estimations.

The study followed a standardized methodology and analysis of survival for measurements that took into account answers given by chance. The sensory discrimination test is easy to perform and understand for assessors³⁴.

One limitation of this study is that the panel of water consumers was all from a town in Argentina where calcium concentrations in drinking water are below 50 mg/L. Therefore, if this strategy is intended to be applied in populations with different water composition, the same test would need to be replicated. Another limitation is that solutions were prepared the previous day; further studies should test solution stability for longer periods of time to assess if there is any precipitation.

Conclusion

These results show that it is feasible to obtain water with added calcium using different salts and reach a concentration of up to 500 mg of calcium/L of water. The calcium salt allowing the highest calcium concentration with the lowest perceived changes in taste was calcium gluconate. Future studies need to explore stability and acceptability over longer periods of time.

Data availability

Underlying data

Mendeley Data: Water with added calcium. <https://doi.org/10.17632/9fj6fs7kf2.1>³³

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Acknowledgements

We would like to thank Maximo Compagni for his advice on the selection of water and Guillermo Hough for his support in the methodology applied.

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Open Peer Review

Current Peer Review Status:  

Version 2

Reviewer Report 11 January 2022

<https://doi.org/10.21956/gatesopenres.14755.r31602>

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Thank you for the responses to the comments. They have very much helped answer my questions.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Nutrition science, micronutrients, biochemistry.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 22 November 2021

<https://doi.org/10.21956/gatesopenres.14606.r31276>

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The study of widely consumed vehicles (foods, condiments and beverages) to delivery nutrients that are not adequately present in sufficient bioavailable amounts in a population's typical diet is

an important public health measure. Although the importance of calcium by itself to prevent pregnancy induced hypertension, high blood pressure in general, or osteoporosis has not been adequately studied, the low supply of calcium in most of the LMIC is a fact that has been reproduced by different researchers using relatively different methodologies. Drinking water is an attractive potential vehicle for minerals like Ca, Zn and Fe. This is a proof of concept investigation for delivering calcium using three likely calcium salts regarding solubility and organoleptic effects of gluconate, lactate, and chloride).

Regarding applicability of results, it would have been useful to model the cost of this intervention with calcium chloride using a hypothetical water supply system or an organic salt for bottled water (and adjusting results for water utilization for purposes other than drinking/cooking, e.g., wastage). Although survival analysis is more often used for acceptability during shelf life studies, it is adequate for acceptability testing, which uses 9 point Hedonic scales more often. Given the relative simplicity and low cost of the methodology used, it seems important for increasing the external validity of results to conduct similar experiments in other populations (e.g., India or Bangladesh). The long term effects of increased calcium content on delivery systems and on human health should also be discussed. The potential risk of renal load when using calcium levels above 200mg/L for young children and labeling implications for bottled water should be discussed as well.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Human nutrition, micronutrients (iron, zinc, vitamin A, iodine), biofortification, vitamin A fortification of sugar, iodine fortification of salt, food fortification.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 15 November 2021

<https://doi.org/10.21956/gatesopenres.14606.r31280>

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This manuscript determined the sensory threshold of different calcium salts added to drinking water using survival analysis. It provides a new perspective and a mechanism for improving calcium intake through fortification.

Their methodological approach is good, and it is a well-written manuscript. It should be indexed once some minor edits are made.

- Include information on purity of the salts and if they were food-grade.
- An explanation of the judge panel, particularly their experience, would have been helpful. What was the range of concentrations provided to the expert panel?
- The test samples were labeled with a 3-digit code to blind the consumer panel but were the study administrators also blinded?
- An experiment with a case-control design would have provided more experimental reliability. It would help us understand how fortified water differs from regular water in terms of sensory quality.
- A description of the water's quality, such as its natural hardness, is essential. As it is written it is unclear how much calcium is in the water before the experimental calcium is added. It states calcium 27 mg/L, alkalinity (CaCO₃) 80 mg/L, hardness (CaCO₃) 104 mg/L. What is the total amount of calcium already present?
- More information on the tasting order and procedure would be helpful. How many different samples did participants receive? If each batch contained 2 identical and one different sample, and they received a total of 7 batches over 2 days, it seems like they did not get to taste all of the solutions. Was each batch different, did the participants test only one salt form or did they get all three?
- How do these findings compare with the organoleptic and sensory tests done previously for calcium (as mentioned in the introduction)?

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Nutrition science, micronutrients, biochemistry.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 14 Dec 2021

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Response to reviewers

APPROVED WITH RESERVATIONS

This manuscript determined the sensory threshold of different calcium salts added to drinking water using survival analysis. It provides a new perspective and a mechanism for improving calcium intake through fortification.

Their methodological approach is good, and it is a well-written manuscript. It should be indexed once some minor edits are made.

1. Include information on purity of the salts and if they were food-grade.

ANSWER: Thank you for your remark, we have included the specification given by the provider to reinforce that the salts were suitable for human consumption.

Material and Methods: "Calcium chloride dihydrate was purchased from Sigma-Aldrich Corporation and meets analytical specifications of The European Pharmacopoeia (Ph. Eur.), Pharmaceutical Reference Standards (USP), FCC, E509, whereas calcium lactate pentahydrate and calcium gluconate Monohydrate were purchased from Surfactan, both meet analytical specifications of USP and European Pharmacopoeia (EP)."

1. An explanation of the judge panel, particularly their experience, would have been helpful. What was the range of concentrations provided to the expert panel?

ANSWER: Thank you for this comment, we have now included information on the process in the section: "Selection of salt and concentration" (page 3):

Material and Methods: "The expert panel compared different concentrations of each salt against water without added calcium. Using a triangle test the range was set from a concentration where no sensory differences were detected to a concentration where marked differences were detected. After 7 triangular sessions the panel set the range between 77 and 800 mg of Ca / L for calcium chloride, 107 and 820 mg of Ca / L for calcium lactate, and 107 and 863 mg of Ca / L for calcium gluconate (Table 1). "

1. The test samples were labeled with a 3-digit code to blind the consumer panel but were the study administrators also blinded?

ANSWER: We have included the following details of the blinding process.

Material and Methods: "following sensory analysis protocols, where only participants are blinded to the samples.[32] (Lawless et al. 2010)"

Lawless, Harry T. T. 2nd ed. 2010. Sensory Evaluation of Food: Principles and Practices.

1. An experiment with a case-control design would have provided more experimental reliability. It would help us understand how fortified water differs from regular water in terms of sensory quality.

Answer: Thank you for this valuable comment. In this study we only tested the sensory threshold, following your suggestion further studies should be performed to assess the acceptability of the water in the long term.

1. A description of the water's quality, such as its natural hardness, is essential. As it is written it is unclear how much calcium is in the water before the experimental calcium is added. It states calcium 27 mg/L, alkalinity (CaCO₃) 80 mg/L, hardness (CaCO₃) 104 mg/L. What is the total amount of calcium already present?

Material and Methods:

"Baseline water

All the concentrations prepared from the different calcium salts were tested against a reference table bottled water that is commonly consumed in the country and complies with the standards of the water cooperative in Argentina (Instituto verificador de elaboración de soda en sifones, IVESS). [30] According to the information provided by the cooperative, this water is filtered, dechlorinated, ozone purified tap water. The baseline bottled water has a calcium concentration of 27 mg/L and water hardness of 104 mg/L (CaCO₃). This water also contained 32 mg/L of sodium, 10mg/L of nitrates, 38 mg/L of chlorides, 80 mg/L of alkalinity (CaCO₃) and 270 mg/L total dissolved solids.

Samples preparation

Samples were prepared using the previously described baseline table bottled water"

1. More information on the tasting order and procedure would be helpful. How many different samples did participants receive? If each batch contained 2 identical and one different sample, and they received a total of 7 batches over 2 days, it seems like they did not get to taste all of the solutions. Was each batch different, did the participants

test only one salt form or did they get all three?

ANSWER: *Thank you for highlighting this, we have reordered the section and added more information on the methodology.*

"After the training, threshold tests were carried out. In this test, a batch of three samples were presented simultaneously to the panelists, two samples were from the same concentration and one from a different concentration. Each panelist had to indicate which sample was the odd sample. There were six possible serving orders (AAB, ABA, BAA, BBA, BAB, ABB) which were counterbalanced across all panelists. Consumers were asked to taste each sample in the row (left to right) and to select the odd sample. Between batches, participants were asked to neutralize taste with mineral water and white bread.

Each consumer received seven batches of three 30 ml solutions. The tests were performed on different days to avoid tiredness or flavour carryover. For each salt, consumers tested three batches one day and the remaining four batches on a second day."

Samples were presented at room temperature (18–23°C) in similar polystyrene 70 mL cups coded with three-digit random to allow blinding. Following the sensory analysis protocol, participants were blinded to the samples however the panel leader and assistant were not.[32]

1. How do these findings compare with the organoleptic and sensory tests done previously for calcium (as mentioned in the introduction)?

ANSWER:

The studies mentioned in the introduction are based on simulations to assess the effectiveness of calcium fortified water to decrease calcium inadequate intake. However, these simulations did not consider the sensory threshold of water with added calcium. As we mention in the discussion, this study shows that the sensory detection threshold of water with added calcium salts allows the increase of calcium concentration of water up to a level of 500 mg of calcium /L which is the value we have shown it would improve calcium intakes without posing any risk of calcium excess to the population.

Discussion: "This study shows that the sensory detection threshold of water with added calcium salts allows the increase of calcium concentration of water up to a level of 500 mg of calcium /L. "

Second reviewer: Boy Erick

Thank you for these valuable comments that will orient further studies of our group.

Competing Interests: None to declare