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## RESEARCH ARTICLE

# Development and acceptability of a cereal bar with *Atta sexdens* ant flour

[version 1; peer review: 2 approved with reservations]

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## Abstract

In Peru, insect consumption, as a nutritional complement or as the main source in the diet, is limited to the regions of our the central jungle where *Atta sexdens* ants are consumed. An energy bar based on Andean grains with *Atta sexdens* ant flour was formulated. The ants were obtained from the department of San Martin, district of Rioja, province of Rioja. Four different formulations were prepared with different *Atta sexdens* ant flour concentrations: 13%, 17%, 20%, and 23%. Moisture, total fat, ash, proteins, fiber, carbohydrates, instrumental texture, and organoleptic analysis (taste, texture, and color) were performed. The developed cereal energy bar presented a 10 g protein content in 100 g of the final product. Acceptability was evaluated in adolescents and young adult populations. The developed cereal bars presented a high protein content, adequate organoleptic properties and high acceptability.

## Keywords




Entomophagy; energy bar; *Atta sexdens*; protein; ant; insect edible.




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## Open Peer Review

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## Introduction

The Food and Agriculture Organization of the United Nations (FAO) recommends the consumption of edible insects because it is a sustainable alternative that replaces animal meat, such as beef, poultry, and fish.<sup>1</sup> In Africa, Latin America, and Asia, these insects are commonly used to feed their populations.<sup>2</sup> In Mexico alone, 549 species of ants have been reported to be consumed by diverse ethnic groups of this country.<sup>3</sup>

It has been reported that in Thailand their diet has been diversified with the consumption of sago worms<sup>4</sup> and *Acheta domesticus* grasshoppers.<sup>5</sup> Similarly, ants and bees have been reported to be consumed in Cameroon.<sup>6</sup> In Peru, the consumption of Suri, a species of *Rhynchophorus palmarum* worm, and ants known as Mamako or Siqui Sapa (*Atta* sp.) has been reported to be widely consumed because of their pleasant taste.<sup>7</sup>

Insects have high nutritional value because of their high content of fats, proteins, vitamins, fiber and minerals, which is a great opportunity for the development of healthy foods.<sup>8</sup> Mealworm has a high content of unsaturated omega-3 fatty acids and healthy fats in similar quantities than fish, and their content of protein, vitamin and micronutrients is similar to fish and meat.<sup>9</sup> In addition, protein from insects has been reported to produce a lower impact in the environment than obtaining protein from cattle or poultry.<sup>2</sup>

Many insect species are consumed alone or have been included in other preparations. In the Netherlands, pasta has been made with durum wheat with the addition of cricket powder.<sup>10</sup> Cereal bars are in this study, an accessible and an easily consumed product by adolescents and children. Consuming them in the morning and mid-morning can be beneficial to humor and memory behavioral aspects.<sup>11</sup> According to the *Codex Alimentarius*, a cereal bar is mainly prepared with one or more ground cereals.<sup>12</sup> Sales of nutrition bars increased almost tenfold in the last decade, and may be a way to utilize processed insect flour,<sup>13</sup> because of the easiness of consumption. To the best of our knowledge there are no studies that have included ant flour in cereal bars. Therefore, the objective of this study was to develop a cereal bar with Andean cereals and the addition of ant flour, evaluate its nutritional value, fracturability, compression. A microbiological analysis was performed, as well as acceptability in a population of adolescents and young people.

## Methods

### Obtaining *Atta sexdens* ants (Siqui sapa) and Ant flour preparation

*Atta sexdens* ants have been taxonomically assessed based on their morphological characteristics.<sup>14</sup> These ants are brown or dark brown in color and the adult ants present three pairs of spines of approximately 6 to 14 mm, build their nests in underground tunnels of up to eight meters deep and can cover an area of 50 to 100 m<sup>2</sup>, and cut pieces of leaves, which they transport to their nest following visible paths.<sup>15</sup>

The siqui sapa ants were brought from the department of San Martín, province and district of Rioja, and were obtained through a distributor. The ants were transported to Lima and delivered in vacuum packaging to maximize the conservation of the product.

For the preparation of ant flour, the ants were placed in an oven at 65°C for 220 minutes. Of a sample of 100 g, we obtained 72.1 g of our final product. This was taken to an electric grinder until the flour was obtained. To avoid lumps and large particles, the flour passed through a stainless-steel sieve to obtain a homogeneous product.

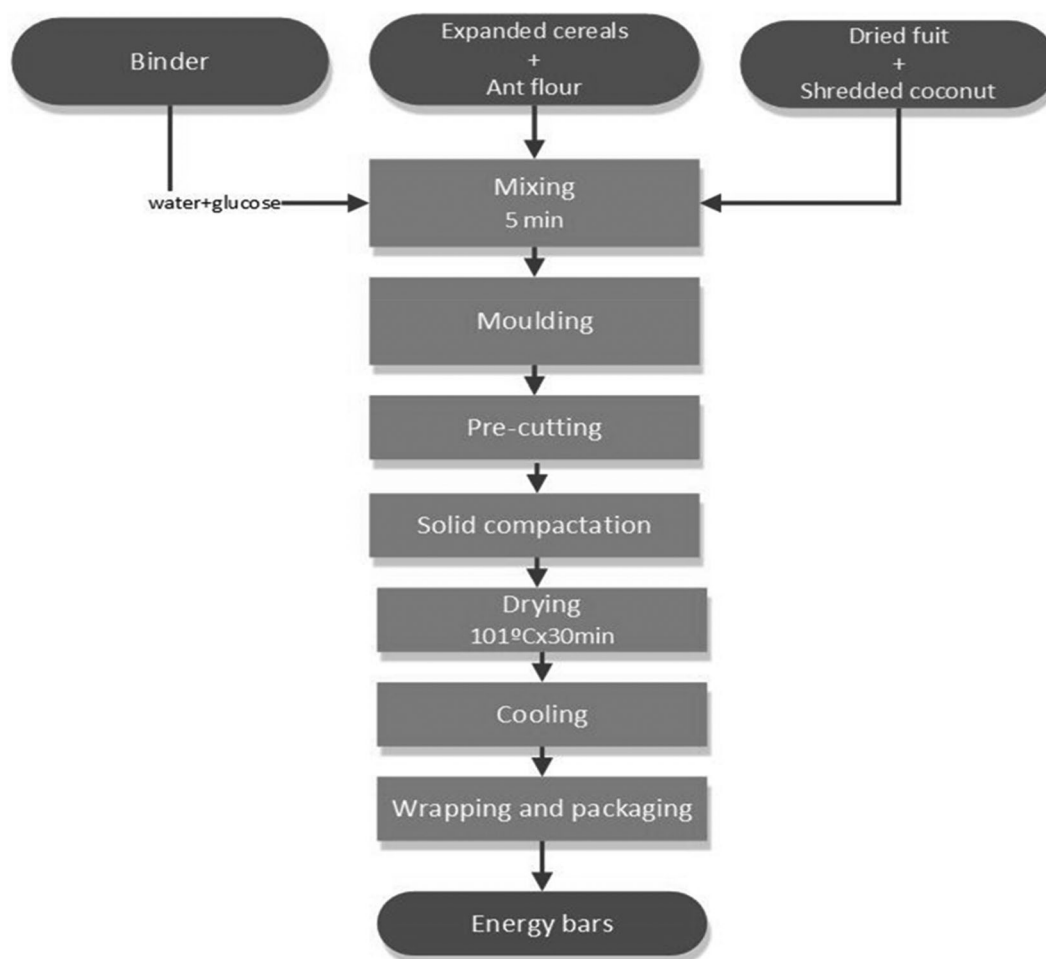
### Materials for the preparation of the energy bar

The ingredients used were expanded kiwicha (100 g: 24.6 g protein, 7 g carbohydrates) and quinoa (100 g, 8 g protein, 85 g carbohydrates), also called expanded cereals. The dried fruits used were pecans (100 g: 9.1 g protein, 73.8 g fat, 11.7 g carbohydrate), peanuts (100 g: 27.1 g protein, 51 g fat, 16 g carbohydrate), almonds (100 g: 23.4 g protein, 54.1 g fat, 14.3 g carbohydrate), raisins (100 g: 2.4 g protein, 63.8 g carbohydrates), and coconut (100 g: 12 g de protein, 23 g total fat, 26 g carbohydrates). Shredded coconut and unflavored gelatin were purchased from a supermarket in the city of Lima. The process began with the preparation of inverted sugar syrup at 60° Brix, H<sub>2</sub>O/Sugar (50%-50%), which was used to compact the energy bars.

The expanded quinoa, kiwicha, dried fruits and ant flour were mixed together with the inverted syrup and glucose. The product was compacted with the help of molds to obtain cylindrical shapes. [Figure 1](#) shows the procedure for the cereal bar formulation.

### Nutritional composition and texture evaluation of the final product

We measured the nutritional composition (fat, moisture, protein, carbohydrates, ash) in the four types of energy bars with ant flour in different concentrations and in the control bar, determined by AOAC standard methods. Moisture was



**Figure 1.** Cereal bar production process.

determined according to AOAC 925.09,<sup>16</sup> fat according to AOAC 922.06,<sup>17</sup> ash according to AOAC 923.03,<sup>18</sup> protein according to AOAC 984.13<sup>19</sup> using 6.25 as the nitrogen to protein conversion factor for all samples. Carbohydrates were calculated by subtracting the percentages of fat, moisture, protein, carbohydrates, and ash from 100%.<sup>20</sup>

Two sensory quality attributes of the energy bars were evaluated. The analysis was performed using the TVT 6700 texture analyzer - Perten Instruments and TexCalc software. Fracturability is defined as the force required to fracture the sample significantly.<sup>21</sup> The three-point bending method was used with the following parameters: initial speed of 1 mm/s, speed of 0.5 mm/s during the test, 0.1961 N tensile strength, 40 mm distance between support points.<sup>22</sup> For the initial speed of compression test we used 2 mm/s; speed during the test, 2 mm/s; recoil speed, 10 mm/s; tensile strength, 0.049 N. The values of the strength required (N) to compress the specimen to 20% of its original thickness were recorded.<sup>23</sup>

#### Microbiological analysis

The detection method for *Salmonella* was ICMSF (International Commission Microbiological Specifications for Foods). For *Escherichia coli* the ICMSF for the determination of fecal coliform organisms was used.<sup>24</sup> For yeast<sup>25</sup> and mold<sup>26</sup> count the ICMSF method was used through seeding plates. Mesophilic aerobic microorganisms were counted using the ICMSF methods.<sup>27</sup>

#### Organoleptic acceptability of samples

The study was conducted by two nutritionists. A total of 100 participants were recruited, including adolescents and young people from two secondary schools, who were chosen as a population that frequently consumes bars. They received an invitation via WhatsApp to participate in this study. Few participants (n=13) were contacted by calling them on their cell phones by the nutritionist in charge of administering the taste test, defined as a preference test. The inclusion criteria were adolescents and young adults who agreed to try the energy bars.

The acceptability was determined using a hedonic test, in which the recruited population was not trained. The samples had different three-digit random codes for each type of bar, a bottle of water was previously given to each participant in order to drink after each test, and strict cleanliness conditions were maintained at all times.

The report of the hedonic test results was recorded in an Excel table, which was subsequently analyzed using the SPSS statistical package. The results of the physical organoleptic analysis, the proximate analysis, and the microbiological analysis were shown in tables, indicating frequencies and percentages. The data was expressed in percentages and the experimental data was analyzed using the Kruskal-Wallis test. Normality was previously measured with Kolmogorov-Smirnov. The IBM SPSS Statistics version 25 statistical package was used.

### Ethical aspects

All participants signed the informed consent form, before participating, all participants were informed that the cereal bar was made with Andean cereals and ant flour. The study received the approval of the ethics committee Exp. N°054-2020 of the Universidad Norbert Wiener.

## Results

### Energy bar formulation

The composition of the four types of energy bars and the amount of ant flour are shown in [Table 1](#). The data are expressed in 100 g of product.

### Evaluation of nutritional composition

The proximate analysis showed the increase in fat and proteins as the percentage of ant flour increases in the four different concentrations ([Table 2](#)).

**Table 1. Composition of the four energy bar formulations.**

Ingredients	F1 (%)	F2 (%)	F3 (%)	F4 (%)
Expanded Kiwicha	43	42	40	38
Expanded Quinoa	26	25	24	23
Shredded coconut	2	2	2	2
Dried fruit				
Raisins	3.25	3.25	3	3
Almonds	3.25	3.25	3	3
Pecans	3.25	3.25	3	3
Roasted peanuts	3.25	3.25	3	3
Unflavored gelatin	1	1	1	1
Glucose	2	2	2	2
Ant flour	13	17	20	23

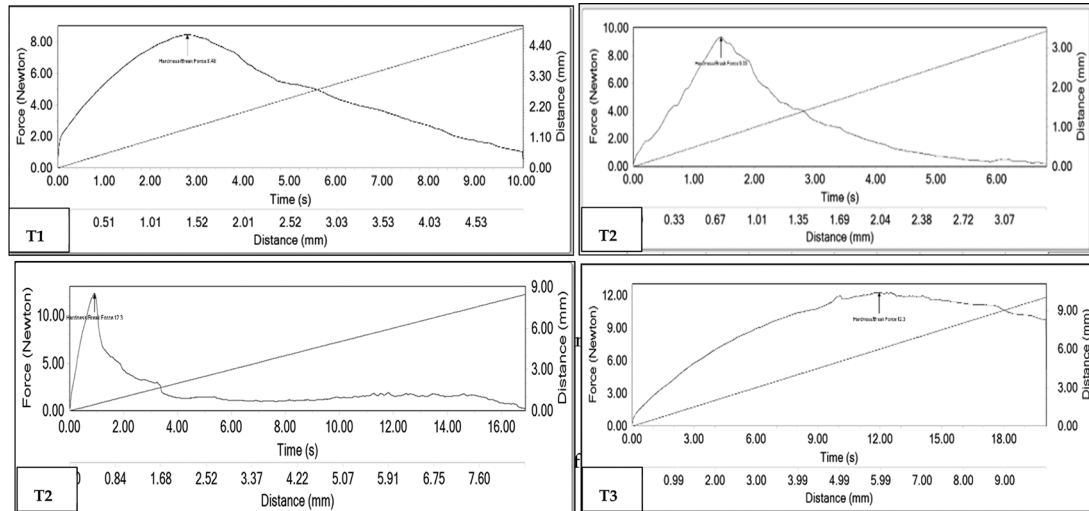
**Table 2. Comparison among the proximate analysis of the four preparations of energy bars and a bar without ant flour (%).**

	Energy bars with different concentrations				
	F0	F1	F2	F3	F4
Fat	5.58	8.61	8.49	6.27	9.62
Moisture	17.60	19.29	19.39	18.89	18
Protein (Nx6.25)	8.68	8.06	8.15	10.04	10.5
Carbohydrates	66.28	62.64	62.45	62.99	60.65
Ash	1.86	1.4	1.52	1.81	1.23

Fo: sample without ant flour; F1(13%); F2(17%); F3(20%); F4(23%).

**Table 3.** Fracturability and compressibility analysis of the four preparations of the energy bar.

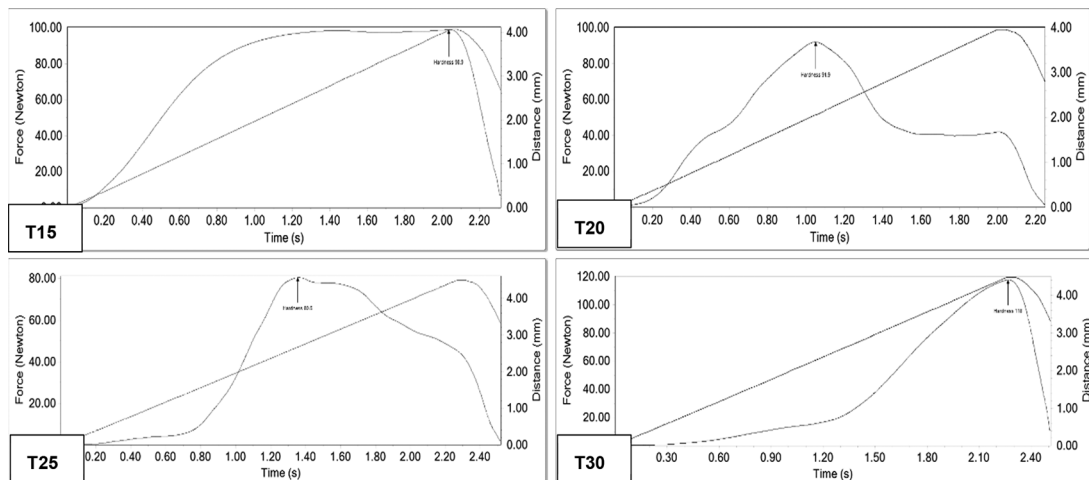
Treatment	Fracturability (N)	Compressibility (N)
F1	8.48	98.9
F2	9.33	91.9
F3	12.33	80.5
F4	12.33	117.6

**Figure 2.** Fracturability curve in the four formulations of the protein.

### Evaluation of fracturability and compressibility

The analysis of compressibility assessed hardness. The method fitted the consistency of the sample due to the fact that, in order to delimit the assays' parameters, the greatest force exerted was within 20% of compression, and values between 80.5 N and 117.6 N were obtained (Table 3, Figure 2).

Figure 2 shows the fracture curves of the treatments with 15%, 20%, 25% and 30% of ant meal T15, T20, T25 and T30 respectively. In general we can observe 3 axes that are force (N), time (s) and distance (mm). The arrow of the indicated point (Hardness) expresses the maximum force exerted to fracture the samples, for example in graph T15, the maximum force exerted to fracture the sample is observed 98.9 N, with a time of 2.9 s and a distance of 1.41 mm. Figure 3 shows the

**Figure 3.** Compressibility curve in the four formulations of the protein.

**Table 4. Mean scores attributed to the energy bar formulations.**

	Taste*		Texture*		Color*	
	Mean $\pm$ SD	AI (%)	Mean $\pm$ SD	AI (%)	Mean $\pm$ SD	AI (%)
T1	4.03 $\pm$ 0.18	95.7	3.84 $\pm$ 0.19	95.3	4.07 $\pm$ 0.17	95.8
T2	3.66 $\pm$ 0.23	94.1	3.74 $\pm$ 0.21	94.5	4.10 $\pm$ 0.17	96.1
T3	3.69 $\pm$ 0.24	93.7	3.84 $\pm$ 0.22	94.5	4.08 $\pm$ 0.19	95.5
T4	4.04 $\pm$ 0.18	95.6	3.84 $\pm$ 0.19	95.3	4.09 $\pm$ 0.18	95.8
Total	3.86 $\pm$ 0.13	97.3	3.81 $\pm$ 0.12	97.4	3.81 $\pm$ 0.12	97.9

Results are the mean of 100 evaluators. Acceptability Index (AI) = mean/upper limit\*100.

\*According to Kruskal-Wallis, p-values >0.05; therefore, the level of taste, texture, and color is the same in each of the formulations.

**Table 5. Microorganism count in the energy bar formulations.**

Microorganism	Permissible count*	Count	Unit
Mold	$10^5 \times \text{gr}$	$1.0 \times 10^1$	CFU/g
Salmonella	Absence	Absence	A-P/25g
Aerobic mesophilic microorganisms	$1.0 \times 10^4 \text{ CFU/g}$	$4.0 \times 10^1$	CFU/g
Escherichia coli	10 MPN/g	<3	MPN/g
Yeast	10 CFU/g	$1.0 \times 10^1$	CFU/g

ICMSF Microbiological analysis.

\*Based on 071-MINSA-DIGESA-V01.<sup>28</sup>

compressibility curves in the same way we can observe 3 axes that are force (N), time (s) and distance (mm), an arrow of the indicated point (Hardness) expresses the maximum force exerted to compress by 20% each of the bars, from which the parameters of distance and time used are obtained, all of these under the same initial conditions mentioned in the methodology of both tests. For example, in the T30 graph, it is observed that to compress the sample by 20%, a force of 118 N is required with a distance of 4 mm in 2.3 s.

### Organoleptic acceptability of the bars

The median age of the tasters was 20.5, with a range of 15 to 25 years old. The four types of bars with different concentrations of ant flour were evaluated. Taste, texture, and color were assessed, and they were expressed through a hedonic scale. The four types showed high acceptability, the percentage of the acceptability index was also high (Table 4).

### Microbiological results

The analyzed sample was T4 for being the one with the highest amount of protein. The obtained values were within the limits allowed according to 071-MINSA-DIGESA-V01 (the sanitary technical norm in Peru) (Table 5).<sup>28</sup>

### Discussion

The four types of bars with different percentages of ant flour were assessed (F1: 13%; F2: 17%; F3: 20%; F4: 23%). We used cereal such as quinoa and kiwicha, dried fruit (chestnuts, nuts, peanuts, almonds, and raisins). The proximate analysis showed that fat and proteins increased as the amount of ant flour increased as well, in comparison with the control bar made without ant flour (F0). Compressibility values ranged from 80.5 N to 117.6 N, and fracturability ranged from 8.48 to 12.33. We did not find any differences among the cereal formulations. All of them showed an acceptability index higher than 95%.

The fat and proteins increase with the addition of the ant flour is noticeable. A study with ant meal from the Rioja area in Peru showed 35.40% fat, 35.5% protein, with a pepsin digestibility of 99.77%.<sup>29</sup> It is known that the ants' abdomen is fatty and that roasted ants are mixed with rice and cassava flour in Brazil and consumed regularly.<sup>30</sup> The protein value of insects has a composition similar to vertebrates' such as pork, chicken, and fish.<sup>31</sup> Cephalotes ants have 42.59% protein, chicken 23%, and beef 20%. In addition, insects are high in sodium, potassium, zinc, magnesium, iron, copper, and calcium.<sup>14,32</sup> Other cereal bars made with similar products provide a lower amount of protein per serving.

In our study we obtained compressibility values ranging from 98.9 N to 117.6 N, which were obtained in the sample with the highest amount of protein (10.5 g). Alvarez obtained values from 130 N to 167 N in his cricket flour bars' formulation, which was higher compared to our results. Alvarez attributed the difference to the protein content of the flour studied. However, it was concluded that the trend does not show significant differences due to the variability of the bars' pieces, in addition to the structure of the bar because of the random distribution of dried fruit fragments.<sup>33</sup> A study with cereal bars with quinoa flakes reported values between 112 N and 216 N,<sup>18</sup> in which the sample with the highest hardness (216 N) that did not have quinoa in its composition was rated in the sensory analysis with "I slightly dislike it" and was associated with the hardness of the product as one of its characteristics. Some products with high fiber content are denser and harder; this does not imply a lower acceptance of the product.<sup>22</sup> but a bar easy to crumble and with excessively firmness would not be attractive to the consumer. At sensory level, this analysis correlates with the maximum force exerted between the molars of each panelist.<sup>34</sup>

The fracturability test imitate chewing with the incisors. One study compared instrumental sensory texture to the maximum force exerted when biting (shear force), that research concluded that a bar with a higher breaking force might be crumbly but a bar that is easy to crumble does not imply that it requires a higher breaking force.<sup>34</sup> In our study, we observed a higher force required by the 25% and 30% treatments, and a lower force by the 15% and 20% treatments. The values obtained varied between 8.48 N to 12.33 N of compression. Marquez L obtained data between 23.9 to 33.8 N, which shows the wide range of firmness in these products when comparing this data with previous research.<sup>22</sup> According to a study on the addition of chontaduro flour to cereal bars,<sup>35</sup> it is expected to observe different resistance characteristics given by the addition of different concentrations of the ingredients during the elaboration of the bars. Texture properties are linked to the composition of each ingredient in the product such as flour granulometry, structure, and nutritional value, which are manifested in the results obtained by the shear or compressive strength of the sample, which give a particular attribute to the product.

The four types of bars with different concentrations of ant flour were evaluated by 100 students. Taste, texture and color were evaluated and expressed by a hedonic scale. The acceptability index had values above 95 %. Although no other bars with *Atta sexdens* flour addition have been found to compare with, information has been collected from several cereal bars with different cereals in their composition. One study revealed that appearance is the limiting factor for consumer acceptability, essentially for cereal bars with dried fruits as ingredients.<sup>36</sup> They found higher acceptability when the bars had medium sugar concentration and high dietary fiber and  $\beta$ -glucan content.<sup>37</sup> In a cereal bar made with textured soy protein, wheat germ, and oats, enriched with ascorbic acid and  $\alpha$ -tocopherol acetate. The formulation de Freitas evaluated was high in protein (15.31%), vitamin E (118.0 mg/100 g). The formulation with 1.1 g/100 g of ascorbic acid added obtained a higher significant sensory preference.<sup>38</sup>

The results in this study showed absence of *Salmonella*, the count of molds and yeasts was  $1.0 \times 10^1$  CFU/g,  $4.0 \times 10^1$  CFU/g for aerobic mesophiles, and for *Escherichia coli* the result was  $<3$  NMP/g. In general, the results were favorable and within the allowed limits. The cereal bars have low water activity, comply with sanitary specifications, and can be stored for 60 days. The yeasts, *Bacillus cereus* and fecal coliforms' microbiological validation, performed by Gutkoski was acceptable for the Brazilian standards.<sup>37</sup>

## Conclusions

The energy bars with ant flour were successfully developed and the sensory result of taste, texture and color attributes showed great acceptance, and any of them can be used for industrial scaling, especially the formulation containing 23% of ant flour that reached a high amount of protein. This product can be a good source of protein, fats and minerals and an alternative for consumers in general. The microbiological results of the energy bars were within the allowed parameters, making this product safe and suitable for consumption.

## Data availability

Figshare: Risk perception. DOI: [10.6084/m9.figshare.23157566](https://doi.org/10.6084/m9.figshare.23157566)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/) (CC-BY 4.0).



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[Publisher Full Text](#)

# Open Peer Review

Current Peer Review Status: ? ?

Version 1

Reviewer Report 13 September 2024

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**Zabentungwa Hlongwane**

University of KwaZulu-Natal, Durban, South Africa

Title: The title needs to be improved and show that the nutritional composition of cereal bars enriched with ant meal was investigated

Abstract: The abstract is good but it lacks a concluding statement what are the implications of the study findings?

Keywords: "insect edible" confusing rephrase to edible insects

Introduction

Paragraph 1 First sentence: Change recommends to recommended

Paragraph 1 second sentence: remove capital letter on It

Paragraph 1 second sentence: No edible insects do not replace meat, they are sustainable, cheap, and rich in nutrients and can be used to combat malnutrition

Paragraph 1 third sentence: "these insects" Which insects are you referring to because no insects were listed before this statement. You must mention the insects that are commonly consumed in the regions that you mentioned because they differ

Paragraph 2 last sentence: Atta should be in italics

Paragraph 3 second sentence: remove capital letter on In

Paragraph 3 last sentence: It would be beneficial to explain why microbiological analysis and consumer acceptability were performed

Materials and methods

The nutrient analysis is not very descriptive, were the samples measured in triplicates or duplicates? Which nitrogen analyzer did the use, and which procedure was used to determine the fat content?

Consumer acceptability test

The methods used are brief and not descriptive for example when were the panelists recruited, where was the study conducted, and where were the panelists seated? How much was the spacing between them? How was the food product presented which labels were used to differentiate between the treatments? Were the panelists selected randomly? What was the ratio of males and

females?

Did the author use 9-point hedonic or 5-point hedonic scales and why?

How did you ensure that the panelists were not allergic to edible ants?

The panelists only focused on taste. The texture, aroma, and colour were not tested and why?

How was the test environment and how did you ensure objectivity in the sensory evaluation method?

## Results

The results are not well explained for example the description of results presented in table 2 only focused on protein and fat. What about Ash and carbohydrates? The author needs to say which treatment had the highest nutrients.

## Discussion

In general, the author did not discuss the implications of his/her findings and hardly compared them to the work done by other scholars. In addition, the author needs to explain the cause and effect of his/her findings. The grammar can be improved in the discussion.

Paragraph 1: "The proximate analysis showed that fat and proteins increased as the amount of ant flour increased as well, in comparison with the control bar made without ant flour (F0)" compare your findings with the work of other scholars and explain the factors that might have led to the increase in protein and fat content of energy bars.

Paragraph 1: "Compressibility values ranged from 80.5 N to 117.6 N, and fracturability ranged from 8.48 to 12.33. We did not find any differences among the cereal formulations. All of them showed an acceptability index higher than 95%" compare your findings with the findings of other scholars and explain cause and effect

Paragraph 2: "A study with ant meal from the Rioja area in Peru showed 35.40% fat, 35.5% protein" It is not clear what was investigated by this mentioned study, in addition, did the fat and protein content increase with the increasing percentage of ant meal added?

Paragraph 2: "It is known that the ants' abdomen is fatty and that roasted ants are mixed with rice and cassava flour in Brazil and consumed regularly" how is this relevant in this study?

Paragraph 2: "The protein value of insects has a composition similar to vertebrates' such as pork, chicken, and fish.<sup>31</sup> Cephalotes ants have 42.59% protein, chicken 23%, and beef 20%. In addition, insects are high in sodium, potassium, zinc, magnesium, iron, copper, and calcium" How is this relevant to the findings of this study?

Paragraph 2: Other cereal bars made with similar products provide a lower amount of protein per serving." Which similar products? Why did you observe higher protein content while other studies observed lower protein content?

Paragraph 4: "One study compared instrumental sensory texture to the maximum force exerted when biting (shear force), that research concluded that a bar with a higher breaking force might be crumbly but a bar that is easy to crumble does not imply that it requires a higher breaking force" rephrase this sentence and find a better way of reporting this

Paragraph 4: "Marquez L" who is this? Is this supposed to be a citation?

Paragraph 4: "According to a study on the addition of chontaduro flour to cereal bars" Chontaduro is not an ant or insect I don't think it is relevant to compare cereal bars fortified with fruit flour to cereal bars fortified with insect flour as plants and animals have different nutrients and digestability.

Paragraph 4: "It is expected to observe different resistance characteristics given by the addition of different concentrations of the ingredients during the elaboration of the bars" I don't think so

Paragraph 5: "One study revealed" this is does not sound right. I suggest you say According to Bchir et al. (2018)..... or Bchir et al. (2018) reported that.....

Paragraph 5: "They found higher acceptability when the bars had medium sugar concentration and high dietary fiber and  $\beta$ -glucan content" This does not make sense, in the previous sentence you said a study by Bschr et al., 2018 revealed however here you are saying they found They found higher acceptability when the bars had medium sugar concentration and high dietary fiber and  $\beta$ -glucan content.....but you are citing a different author (37).

Paragraph 5: "The formulation de Freitas evaluated was high in protein (15.31%), vitamin E (118.0 mg/100 g). The formulation with 1.1 g/100 g of ascorbic acid added obtained a higher significant sensory preference" this is difficult to follow

Paragraph 6: "The results in this study showed absence of Salmonella, the count of molds and yeasts was  $1.0 \times 10^1$  CFU/g,  $4.0 \times 10^1$  CFU/g for aerobic mesophiles, and for Escherichia coli the result was  $<3$  NMP/g. You need to explain what your findings mean and what are the implications of this.

Conclusion

The conclusion is too brief

Any recommendations from the study? What was the significance of this study?

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

Partly

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

Partly

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

No

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

Partly

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

Partly

**Are the conclusions drawn adequately supported by the results?**

No

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** entomophagy, and entomology.

**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, however I have significant reservations, as outlined above.**

Author Response 18 Oct 2024

**Michelle Lozada-Urbano**

Dear Reviewer

Zabentungwa Hlongwane

We appreciate all the comments, we feel that they have helped to improve the paper.

**Title: The title needs improvement and should indicate that the nutritional composition of cereal bars enriched with ant flour was investigated.** The title could be: "Nutritional Composition and Acceptability Evaluation of Cereal Bars Enriched with Ant Flour (*Atta sexdens*).\" I have included this in the document.

**Response:** The change was made.

**Abstract: The abstract is good, but it lacks a concluding statement. What are the implications of the study's findings?**

**Keywords:** \"Edible insect\" is confusing; it should be rephrased as \"edible insects.\"

**Introduction**

**Paragraph 1:** First sentence: Change \"recommends\" to \"recommended.\"

**Response:** The word was changed to recommended.

**Paragraph 1, second sentence:** Remove the capitalization in \"It.\"

**Response:** The capitalization was removed.

**Paragraph 1, second sentence:** Edible insects do not replace meat; they are sustainable, economical, nutrient-rich, and can be used to combat malnutrition.

**Response:** This sentence was modified.

**Paragraph 1, third sentence:** \"These insects\" - which insects are you referring to? Because insects were not mentioned prior to this statement. You should mention the insects commonly consumed in the regions you referred to, as they differ.

**Paragraph 2, last sentence:** \"Atta\" should be in italics.

**Response:** The word Atta was italicized.

**Paragraph 3, second sentence:** Remove the capitalization in \"In.\"

**Response:** The capitalization was removed.

**Paragraph 3, last sentence:** It would be beneficial to explain why microbiological analyses and consumer acceptability assessments were conducted.

**Response:** This paragraph was improved.

### **Materials and Methods**

The nutrient analysis is not very descriptive. Were the samples measured in triplicate or duplicate? Which nitrogen analyzer was used, and what procedure was employed to determine the fat content?

**Response:** Moisture was determined according to AOAC 922.06. Fat was determined according to AOAC 925.09, and extraction was performed using Soxhlet. Ash was determined according to AOAC 923.03. Protein was determined according to AOAC 984.13, using 6.25 as the nitrogen-to-protein conversion factor for all samples; this determination was based on Kjeldahl methodology using conventional analysis. Carbohydrates were calculated by subtracting the percentages of fat, moisture, protein, ash, and carbohydrates from 100%. Analyses were performed in triplicate.

### **Consumer Acceptability Test**

The methods used are brief and not descriptive. For example, when were the panelists recruited? Where was the study conducted, and where did the panelists sit? What was the spacing between them? How was the food product presented? What labels were used to differentiate between the treatments? Were the panelists randomly selected? What was the gender ratio?

**Response:** The recruitment for the analysis took place in August 2020, in a school environment, and the participants were organized in groups of 10 students who entered according to their arrival at the venue. The study was conducted in a ventilated area, with folders set up containing the samples. The samples were served at room temperature in disposable containers, and each participant was provided with a personal bottle of water. The distance between chairs was one meter, and the labels had three-digit codes assigned to each sample. The percentage of women was 46%.

### **Did the author use 9-point or 5-point hedonic scales, and why?**

**Response:** We consider that both scales are essential for measuring consumer preferences and product acceptability. Some advantages of using the 9-point scale are that it allows for capturing subtle differences in consumer preferences compared to the 5-point scale (Wichchukit and O'Mahony, 2015). The 9-point scale also supports more complex statistical analyses that can yield deeper insights into consumer behavior (Wichchukit and O'Mahony, 2015). However, we believe that the number of options can overwhelm respondents, resulting in less reliable data ("How rating scales influence the reliability of responses, the extreme points, the midpoint, and the preferences of respondents").

While the 9-point scales provide detailed information, the number of points can be complex and may hinder responses, suggesting that simpler scales could be more effective in certain contexts. Therefore, we decided to use the 5-point scale, as it may be more effective due to its simplicity.

**Reference:** Wichchukit S, O'Mahony M. The 9-point hedonic scale and hedonic ranking in food science: some reappraisals and alternatives. *Journal of the Science of Food and Agriculture* (2015). doi: [10.1002/JSFA.6993](https://doi.org/10.1002/JSFA.6993).



**How did you ensure that the panelists were not allergic to edible ants?**

**Response:** They were asked, and none of them had previously consumed any products containing ants.

The panelists only focused on the flavor. The color were not evaluated. Why?

**Response:** All samples had exactly the same color.

What was the testing environment like, and how was objectivity ensured in the sensory evaluation method?

**Response:** The study was conducted in a ventilated area, with folders set up containing the samples. The samples were served at room temperature in disposable containers, and each participant was provided with a personal bottle of water. The distance between chairs was one meter, and the labels had three-digit codes assigned to each sample.

**Results**

The results are not well explained. For example, the description of the results presented in Table 2 only focused on protein and fat. What about ash and carbohydrates? The author needs to indicate which treatment had the highest nutrient levels.

**Response:** Regarding moisture content, no significant difference was found between the formulations. Moisture levels ranged from 17.6 g in formulation F0 to 19.39 g in F2. For carbohydrate content, values varied from 60.65 g in formulation F4 to 66.28 g in F0, with no significant difference found. Although variations in moisture and carbohydrate content were observed in both, these were not statistically significant, suggesting that the variation in ant flour content does not impact these values.

The ash analysis revealed significant differences between the formulations. Ash levels ranged from 1.23 g in formulation F4 to 1.86 g in F0. This variability suggests that the mineral composition of the different formulations decreases as the percentage of ant flour increases, which could be associated with the reduction of inputs such as quinoa and kiwicha.

**Discussion**

In general, the author did not discuss the implications of their findings and barely compared them to the work of other scholars. Additionally, the author needs to explain the cause-and-effect relationships of their findings. The grammar in the discussion could be improved.

**Paragraph 1:** "The proximal analysis showed that fat and protein increased as the amount of ant flour increased, compared to the control bar made without ant flour (F0)." Compare your findings with the work of other scholars and explain the factors that may have contributed to the increase in protein and fat content in the energy bars.

**Response:** According to authors such as Guan et al. and Kowalski et al., the incorporation of insect flours, including crickets and mealworms, in bread and cakes showed an increase in protein, minerals, and healthy fatty acids (Guan et al., 2024; Kowalski et al., 2022). This result is similar in our cereal bars, where the additional protein allows for greater moisture, making them softer.

Q.H., Guan., S., Qian., L., Chen., X.C., Feng. 1. Enhancing the nutritional value of bread by the addition of insect powder: a novel class of food protein additives. Journal of insects as food



and feed, (2024). doi: [10.1163/23524588-20230123](https://doi.org/10.1163/23524588-20230123)

Stanisław, Kowalski., Anna, Mikulec., Magdalena, Skotnicka., Barbara, Mickowska., Małgorzata, Makarewicz., Renata, Sabat., Anna, Wywrocka-Gurgul., Aleksandra, Mazurek. 2. Effect of the Addition of Edible Insect Flour from Yellow Mealworm (*Tenebrio molitor*) on the Sensory Acceptance, and the Physicochemical and Textural Properties of Sponge Cake. Polish Journal of Food and Nutrition Sciences, (2022). doi: [10.31883/pjfn/155405](https://doi.org/10.31883/pjfn/155405)

**Paragraph 1:** "The compressibility values ranged from 80.5 N to 117.6 N, and the fracturability varied from 8.48 to 12.33. We found no differences between the cereal formulations. All showed an acceptability index above 95%." Compare your findings with those of other scholars and explain the cause-and-effect relationships.

**Response:** The comparison was made in paragraphs 3 and 4.

**Paragraph 2:** "A study with ant flour from the Rioja region in Peru showed 35.40% fat and 35.5% protein." It is unclear what was investigated in this mentioned study. Additionally, did the fat and protein content increase with the increasing percentage of added ant flour?

**Response:** The meaning of the sentence was clarified in the complete paragraph.

**Paragraph 2:** "It is known that the abdomen of ants is fatty and that toasted ants are mixed with rice and cassava flour in Brazil and are regularly consumed." What relevance does this have in this study?

**Response:** This line was removed from the text due to its lack of relevance.

**Paragraph 2:** "The protein value of insects has a composition similar to that of vertebrates, such as pork, chicken, and fish. Cephalotes ants have 42.59% protein, chicken has 23%, and beef has 20%. Additionally, insects are rich in sodium, potassium, zinc, magnesium, iron, copper, and calcium." What relevance does this have for the findings of this study?

**Response:** It is relevant as it presents a comparison between the protein content of ants and the animal meats we typically consume, such as beef or chicken.

**Paragraph 2:** "Other cereal bars made with similar products provide a lower amount of protein per serving." What similar products? Why did you observe a higher protein content while other studies reported lower protein content?

**Response:** The comparison is made with the study by Guan, who also finds a high protein and fat content.

**Paragraph 4:** "A study compared instrumental sensory texture with the maximum force exerted when biting (cutting force); that research concluded that a bar with greater breaking force could be brittle, but a bar that is easy to crumble does not imply it requires a greater breaking force." Reformulate this sentence and find a better way to convey this information.

**Response:** The idea in this paragraph was reformulated.

**Paragraph 4:** "Marquez L" – who is this? Is this supposed to be a citation?

**Response:** This author was correctly cited.

**Paragraph 4:** "According to a study on the addition of chontaduro flour to cereal bars." Chontaduro is neither an ant nor an insect. I don't believe it is relevant to compare cereal bars fortified with fruit flour to cereal bars fortified with insect flour, as plants and animals have different nutrients and digestibility.

**Response:** The chontaduro is the larva of the black palm weevil (*Rhynchophorus palmarum*). The scientific name has been included in the text.

**Paragraph 4:** "Different resistance characteristics are expected to be observed due to the addition of different concentrations of ingredients during the preparation of the bars." I don't think this is accurate.

**Response:** The resistances were observed according to the percentage of ant flour used.

**Paragraph 5:** "A study revealed" does not sound correct. I suggest saying "According to Bchir et al. (2018)..." or "Bchir et al. (2018) reported that..."

**Response:** This citation was improved as follows: A study by Bchir et al. (2018) revealed that appearance is the main limiting factor for consumer acceptability, especially in cereal bars that include dried fruits as ingredients.

**Paragraph 5:** "They found higher acceptability when the bars had a medium sugar concentration and high dietary fiber and  $\beta$ -glucan content." This does not make sense. In the previous sentence, you said that a study by Bchir et al. (2018) revealed this, but here you are saying that they found higher acceptability with medium sugar concentration and high dietary fiber and  $\beta$ -glucan content, citing a different author (37).

**Response:** The author Gutkoski found higher acceptability when the bars had medium sugar concentration and high dietary fiber and  $\beta$ -glucan content.

**Paragraph 5:** "The formulation evaluated by Freitas was high in protein (15.31%) and vitamin E (118.0 mg/100 g). The formulation with 1.1 g/100 g of added ascorbic acid achieved a significantly higher sensory preference." This is difficult to follow.

**Response:** In a study by Gumul, nut bars with the addition of edible insect flours were evaluated, and this addition reduced the perception of most sensory attributes of the bars compared to the standard bar.

**Paragraph 6:** "The results in this study showed the absence of Salmonella, the count of molds and yeasts was  $1.0 \times 10^1$  CFU/g,  $4.0 \times 10^1$  CFU/g for aerobic mesophiles, and for Escherichia coli the result was  $<3$  NMP/g." You need to explain what your findings mean and what their implications are.

**Response:** The explanation of the findings and their implications was improved.

#### **Conclusion:**

The conclusion is too brief.

**Response:** More conclusions were added to the text.

Are there recommendations from the study? What was the importance of this study?

**Response:** A section with some recommendations was added.

**Competing Interests:** No competing interests were disclosed.

Reviewer Report 01 July 2024

<https://doi.org/10.5256/f1000research.148639.r288948>

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**Samuel Adelani Babarinde**

Ladoke Akintola University of Technology, Ogbomosho, Oyo, Nigeria

## REVIEWER'S COMMENTS TO AUTHORS

**MANUSCRIPT TITLE:** Development and acceptability of a cereal bar with *Atta sexdens* ant flour

### Title

#### Modification is suggested

Modify as follows:

Development and acceptability of a cereal bar with leaf cutter ant, *Atta sexdens*, flour

### Abstract

#### Ln 2-3

Recast as follows:....the regions the central jungle where a leaf cutter ant, *Atta sexdens*

#### Ln 4-5

Recast as Department of... District of... Province of .....

#### Ln 7-8

13%, 17%, 20% and 23% w/w or v/v or what?

#### Ln 8-9

Recast as follows:... Analyses on moisture, total fat.... and organoleptic indices (taste, texture, and color) were performed.

### Introduction

#### Pg 3, Paragraph 2, Ln 2

Change '*Acheta domestica*' to 'crickets'. The insect is not a grasshopper.

Change 'Cameroon' to 'sub-Saharan Africa' and add the following reference and 6 (Tamesse et al.):

Babarinde SA, Mvumi BM, Babarinde GO, Manditsera FA, Akande TO, Adepoju, AA. Insects in food and feed systems in sub-Saharan Africa: the untapped potentials. *International Journal of Tropical Insect Science*. 2021 41 (3): 1923-1951. <https://doi.org/10.1007/s42690-020-00305-6>

**Pg. 3, Paragraph 3, Ln 3**

... 'similar quantities as fish' or ... 'higher quantities than fish'

**Pg. 3, Paragraph 3, Ln 4**

Change 'protein from fish' to 'relying on insect protein.'

**Pg. 3, Paragraph 4, Ln 2**

Recast as follows: Cereals bars are in.....

**Pg 3, Paragraph 4, Ln 2**

Insert 'and' before 'compression'

**Methods**

**Pg. 3, Paragraph 1**

'Obtaining *Atta sexdens* ant' should be changed to 'Obtaining leaf cutter ant, *Atta sexdens*'

The first paragraph is not part of what you did. It only provides information about the studied organism (*Atta sexdens*). Therefore, the content is better placed in the appropriate section of your Introduction.

**Pg. 3, Paragraph 2**

Recast as Department of... District of... Province of .....

'This was taken to an electric grinder .....' should change to 'This was milled with an electric grinder .....

**Pg 3, Paragraph 5**

Merge the paragraph with the preceding one.

**General Comments**

You have to indicate how you formulated your Ant bar mix flour.

**Pg. 3, Paragraph 6, Ln 3**

..in different concentrations... You need to provide appropriate references.

**Figure 1**

Change 'Ceral' to 'Cereal' in the figure legend: Figure 1: Cereal bar.....

**Pg. 5 Paragraph 1**

Which hedonic scale did you use 1-9 or which? The, provide a brief description of what each number stands for.

**Pg. 5 Paragraph 2**

Change 'data was' to 'data were'. Data is plural, while datum is singular

**Results****Table 1**

Why did you not compare the energy bar with ant flour as FO in Table 1? I feel this is necessary.

**Table 2**

The methodology that led to your Table 2 is not quite explicit. You would need to improve the appropriate section of your methodology to allow your readers to understand what you did that led to the collection of the data in Table 2.

**Table 3**

Indicate what F1-F4 stand for as footnote.

**Figure 2**

Indicate what T1-T2 stand for as figure legend.

**Pg. 6, Paragraph 2**

Did you use 13, 17, 20 and 23% or 15, 20, 25 and 30% ant meal?

**Figure 3**

Indicate what T15-T30 stand for as figure legend.

I am of the opinion that you should compare your treatments (T1-T4) with the cereal bar without and flour (F0).

**References**

Add this to your references:

(Babarinde et al., 2021)(Ref-1)

**Abstract**

Abstract has minor corrections highlighted in my Reviewer's comments to the Authors.

**Introduction**

The Introduction also has minor editorial corrections and the need to add more recent references.

**Methods**

There are few inconsistencies in the methods: for instance, the proportions of ant meal used for the experiment should be clarified.

**Discussion**

I feel the cereal bar without ant meal should be used as a standard to establish the results of the experiment.

**References**

1. Babarinde S, Mvumi B, Babarinde G, Manditsera F, et al.: Insects in food and feed systems in sub-Saharan Africa: the untapped potentials. *International Journal of Tropical Insect Science*. 2021; **41** (3): 1923-1951 [Publisher Full Text](#)

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

Partly

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

Partly

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

Yes

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

Yes

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

Yes

**Are the conclusions drawn adequately supported by the results?**

Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Entomology and entomophagy

**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, however I have significant reservations, as outlined above.**

Author Response 14 Oct 2024

**Michelle Lozada-Urbano**

Dear Reviewer, we appreciate your recommendations for change, each of them has been consistent and the changes have been made in the new document, including the new bibliography.

**Competing Interests:** No competing interests were disclosed.

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