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Research article

Effect of fortification with $CaCO_3$ nanoparticles obtained from eggshell on the physical and sensory characteristics of three food matrices

Luz Marina Gómez-Alvarez, José E. Zapata Montoya [*]

Research Group on Nutrition and Food Technology, NUTEC, University of Antioquia, Av. Ferrocarril, 050010, Medellín, Colombia

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A B S T R A C T

Food fortification has attracted interest in recent years, due to the understanding that micronutrient deficiency is one of the causes of the global burden of disease, and that food fortification aims to prevent or correct a demonstrated deficiency of one or more nutrients in a specific population or population groups. Nutritional value is an important concern regarding fortification and new product development. However, people are not willing to sacrifice the organoleptic characteristics of food products. Therefore, the effect of $CaCO_3$ nanoparticles (NPs-$CaCO_3$) and commercial $CaCO_3$ on the physical and sensory properties of three food matrices (cookies, fruit rolls and dairy desserts) was evaluated. A texture analysis was performed on cookies and fruit rolls; a viscosity analysis on dairy desserts; and a color analysis and sensory profile on the three matrices. The results showed that both types of calcium increase hardness in fortified biscuits and fruit rolls but, in the latter case, commercial calcium caused a higher increase in hardness ($p < 0.05$). Viscosity was higher in the desserts with NPs. Color presented significant changes in all the fortified matrices. These findings demonstrated that Ca-NPs are a good strategy for food fortification compared to commercial calcium carbonate, as fortification with high levels of calcium is a challenge for the food industry due to its effects on the product. The results showed that, in the matrices with commercial calcium, the changes were more evident, while the matrices fortified with Ca-NP have a better sensory response than commercial Ca, with a higher level of acceptance by the judges. Therefore Ca-NPs can be considered to be a good source of calcium for food product fortification that causes a slight effect on physical and sensory properties.

1. Introduction

A third of the world population is affected by micronutrient deficiencies, especially deficiencies of vitamins and minerals [1]. These deficiencies represent 7.3 % of the global burden of disease, making them a public health problem, with iron and calcium deficiencies contributing substantially to maternal mortality and low birth weight of babies (<2500 g) [2]. For this reason, most governments and health organizations stress the importance of fortifying food products with calcium. Food fortification has been identified as the most cost-effective strategy to overcome micronutrient deficiencies and has the advantage of not requiring modification of dietary habits, which may lead to a higher level of acceptability compared to other strategies [1]. However, calcium absorption through diet is not

* Corresponding author.
E-mail address: edgar.zapata@udea.edu.co (J.E. Zapata Montoya).

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Abbreviations

NPs	Nanoparticles
CaCO3	Calcium carbonate
TP	Average particle size
Ca-Comm	Commercial calcium
Ca-NPs	Calcium carbonate nanoparticles
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
RVD	Reference Daily Value
ICP-OES	Atomic Emission Spectroscopy with Inductively Coupled Plasma
CIE	Commission Internationale de l'Eclairage

efficient, as the human body absorbs approximately 10 % of calcium ingested, which represents an important limitation [2].

Different calcium salts have been approved for use as food fortifiers, including calcium carbonate. Most calcium salts used in food fortification for human consumption have a bioavailability between 20 % and 40 %. Their solubility, source, interaction with food and bioavailability vary widely [1].

Eggshell is a biomaterial whose structure is mainly made up of a mineral phase and interstitial membranes. Its chemical composition consists of calcium carbonate (94–95 %) in the form of calcite, a 3 % of organic material, which corresponds to the protein structure of the membrane, and the residual material, which contains magnesium carbonate and calcium phosphate [3]. Eggshells have a great application potential, which can be expanded if taken to the nanoscale [4], since particle size (PS) significantly influences the physicochemical characteristics of this biomaterial, to the point that $CaCO_3$ nanoparticles (NPs) derived from eggshells have improved bioaccessibility, bioavailability, and biological activity compared to, microparticles [5]. Therefore, obtaining $CaCO_3$ NPs from eggshells represents an opportunity to increase the value of this by-product that is currently treated as waste [6,7].

When it comes to the interaction between an additive and food, possible organoleptic changes are very important. In this sense, it is known that calcium fortification can change the flavor of food, increasing acidity and bitterness due to the effect of calcium itself or of associated ions. These changes will depend on the type of salt used, the food matrix, and the industrial process [4]. Additionally, calcium carbonate can add an earthy taste and a gritty mouthfeel, especially if added in large amounts. Most calcium salts are white or colorless. Therefore, some insoluble calcium salts such as carbonate may lighten the color of food or even cause the formation of sediments during storage and affect the shelf life of products, chiefly of liquids [8]. Due to these sensory problems, it is necessary to conduct studies to evaluate the acceptability of calcium added to food. For this reason, other sources of calcium are proposed for the food industry, such as eggshells, which are composed of inorganic salts, mainly calcium carbonate (95 %), magnesium carbonate (0.8 %) and tricalcium phosphate (0.8 %) [1,8]. Some studies have reported on the incorporation of eggshell powder into foods, such as fortified pork sausages and yogurt [4], calcium diet cookies [9], roasted and ground coffee [10], chocolate cake, and other homemade food products such as pizza and pasta [8]. The objective of this study, was to evaluate the effect of the addition of $CaCO_3$ nanoparticles (Ca-NPs) on the physical and sensory properties of three food matrices (oatmeal cookies, fruit rolls, and dairy desserts), compared to the commercial $CaCO_3$ (commercial Ca) currently used by the food industry, a high level of calcium (40 %) was added in order to fulfill the fortification criterion necessary to designate it as a "good source of calcium".

2. Materials and methods

2.1. Calcium nanoparticles

The $CaCO_3$ NPs used to fortify the food matrices were obtained from AA red eggshells from chickens (*Gallus gallus domesticus*) according to the methodology described by Gómez-Álvarez et al. [11], by combining high shear scattering and ultrasound. The characteristics of the $CaCO_3$ NPs used were the following: particle size of 281.34 ± 25.83 nm; polydispersity index of 0.51 ± 0.05; zeta potential of -22.4 ± 3.47; 33.48 % Ca; an irregular shape observed by scanning electron microscopy (SEM) with a size between 45 nm and 280 nm; crystals in the range of 10–60 nm observed by transmission electronics (TEM); porous and polycrystalline nature; and a surface area of 11.13 m^2/g and 98.7 % calcite. To evaluate the effect of fortification with NPs, three food matrices supplemented with $CaCO_3$ nanoparticles (Ca-NPs) were formulated, of which physical and sensory characteristics were determined, as well as the calcium content of the bioaccessible fraction of each matrix. These analyses were also performed using commercial Ca as a control.

Biosafety studies were carried out in vitro in the Caco-2 cell line and *in vivo* in Sprague-Dawley mice, verifying that the $CaCO_3$ nanoparticles obtained from eggshells did not cause a cytotoxic effect on cells or acute toxicity in animals (Data not shown).

2.2. Calcium fortification of food matrices

Three food matrices, which were fortified with $CaCO_3$ nanoparticles (Ca-NPs) and commercial $CaCO_3$ (comm-Ca), were formulated. For each treatment, a control sample was prepared (without the addition of calcium). The foods designed were mango fruit rolls, oatmeal cookies, and dairy dessert.

The effect of Ca-NPs and commercial Ca on the texture of oatmeal cookies and fruit rolls and, the viscosity of the dairy dessert, and the color changes in the three food matrices were evaluated. The fortification criterion was the incorporation of 40 % of the daily reference value (DRV) of calcium, bearing in mind three populations susceptible to requiring the addition of this mineral to their diet, the dose (low, medium, high), and the type of matrix. The calcium content added as $CaCO_3$ was adjusted taking into account the calcium contribution of other ingredients in each of the foods designed, in order to be declared as a "good source of calcium" according to Resolution 810 of 2021 issued by the Ministry of Social Protection of the Republic of Colombia [12].

2.3. Determination of Ca^{2+} by ICP-OES

The bioaccessible fractions of each matrix were obtained by in vitro digestion, which was carried out following the procedure described by Minekus et al. [13], with some modifications. To determine the Ca^{2+} content, the bioaccessible fractions were digested in nitric acid (HNO_3 #160317 – Merck, Germany), treated with H_2O_2 (Merck, Germany), and heated at 90 °C for 15 min. Subsequently, they were analyzed by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES).

2.4. Texture analysis

The texture of the fruit rolls and oatmeal cookies was analyzed with the puncture compression method using a texture analyzer (TAXT2 model, Stable Micro System, United Kingdom). For the cookie hardness test, a 4-mm-diameter TA-54 cylindrical probe was used, while a Warner-Bratzler blade TA-7 probe with a guillotine block was used for the fruit rolls. Both used a descent speed of 1 mm/s, a penetration distance of 2 mm and a contact force of 0.5 N. Cookie hardness was the maximum force a cookie could withstand before breaking, as well as the shear force in fruit rolls. For each of the tests, 10 repetitions of each treatment were performed [14].

2.5. Viscosity analysis

The powdered dairy dessert samples were reconstituted 50/50 (w/v) in water. They were brought to 100 °C for 10 min until the desired consistency was achieved. The viscosity test was performed with 100 mL of sample in a rotational viscometer ST-2001-SELECTA (Barcelona, Spain) with an L2 spindle, at a shear rate between 10 and 30 rpm for desserts fortified with Ca-NPs, with commercial Ca, and control samples (no added calcium), for 120s [15].

2.6. Color analysis

Instrumental colorimetric determination enables the objective evaluation of the important physical property of color, as this is a perceptual phenomenon that depends on the observer and on psychological, physiological, and physical conditions [16]. In this case, color was measured directly on the surface of each solid sample, and, in the case of the dairy dessert, on both the powdered and reconstituted product. This variable was determined with a CM-2600d colorimeter (Konica Minolta, Tokyo, Japan), under the conditions established by the "Commission Internationale de l'Eclairage", CIE. A standard D65 illuminant combination and an observation angle of 10° were used, excluding the specular component (SCE). Color was expressed in the CIELAB color space to evaluate the coordinates L* (lightness), a* (red/green) and b* (yellow/blue) of each sample [17]. However, the main objective in this case was to determine the color differences caused by the fortification of the samples with Ca-NPs and commercial Ca. To that effect, color differences (ΔE*) were estimated based on Equation (1) and interpreted according to the scale reported by Popov-Raljic et al. [18], who indicate several categories of color difference: imperceptible difference (0–0.5), barely noticeable difference (0.5–1.5), evident difference (1.5–3), marked difference (3–6), extremely marked difference (6–12), and colors of different shades (greater than 12).

$$\Delta E* = \left\{ (\Delta L*)^2 + (\Delta a*)^2 + (\Delta b^*)^2 \right\}^{0,5} \tag{1}$$

In addition, hue (h^*_{ab}) and chroma (C^*) were calculated with Equations (1) and (2) (Alonso et al., 2005). At least three measurements were taken from each individual sample.

$$C^* = \sqrt{\left(a^{*^2} + b^{*^2}\right)} \tag{2}$$

$$h^*_{ab} = \arctan\left(\frac{b^*}{a^*}\right) \tag{3}$$

2.7. Sensory profile

Sensory quality was evaluated taking into account the sensory characteristics of the fruit rolls, oatmeal cookies, and dairy desserts fortified with $CaCO_3$, using a quantitative descriptive analysis, and a panel of eight judges trained in these types of matrices, who identified and quantified between 10 and 12 descriptors in each matrix. Three batches of each product were analyzed. The overall quality assessment was performed according to the specifications given in the Colombian technical standards 3932 of 1996 and 5328 of 2004 [19]. The analysis considered changes in visual appearance, objectionable characteristics, and acceptability. The descriptors evaluated in the three fortified matrices were: appearance, color, characteristic odor, characteristic flavor, sweet flavor, bitter flavor, earthy flavor, and hardness. For each matrix, the characteristics evaluated were gumminess, fruit flavor, chewiness, and stickiness were determined for fruit rolls; fracturability and chewiness for cookies; and consistency, viscosity, and sandiness for dairy desserts. The sanitary quality of the matrices was analyzed in previous studies, using microbiological tests.

The descriptors were graded on a ten-point structured continuous linear scale, where 10 is very marked (characteristics of the product, appearance, color, aroma and flavor), 8 is marked (loss of appearance, color, flavor, or aroma), 6 is moderate (greater loss of hardness, texture, viscosity, gumminess, flavor, and aroma), 5 is at the limit of consumption (slight objectionable flavor is perceived), 4 means the initial product characteristics are slightly noticeable (flavor, aroma and appearance), 2 means the initial characteristics are barely noticeable (appearance, flavor, aroma, texture, chewiness, stickiness, and consistency), and 0 corresponds to absence (of the characteristic appearance, aroma and flavor, the products is not consumable).

2.8. Statistical analysis

Differences in physical parameters such as texture, viscosity, and color, as well as sensory attributes in the matrices fortified with Ca-NPs and Ca-Ccial were analyzed. All data were expressed as mean ± standard deviation (SD) of at least three independent measurements for each of the samples. The statistical analysis was performed using Statgraphics® Centurion XVI, version 16.2.04 (Statpoint Technologies, Inc., Warranton, VA, USA), using analysis of variance (ANOVA) with Fisher's least significant difference (LSD) to detect significant differences between the data ($p < 0.05$). Results are represented as mean ± SD. Different letters show significant differences.

3. Results and discussion

The bioaccessible fractions were obtained after the simulated gastrointestinal digestion process, after which the bioaccessible calcium content was calculated.

3.1. Ca^{2+} content by ICP-OES

The concentration of Ca^{2+} of the bioaccessible fractions can be seen in Fig. 1. The results indicate that foods fortified with Ca-NPs present a higher content of bioaccessible calcium, most notably in oatmeal cookies and the dairy dessert, which presented significant differences with respect to Ctrl and commercial Ca, in all matrices ($p < 0.05$). However, the levels of bioaccessible Ca^{2+} from Ca-NPs in the fruit rolls were approximately 12 % below those of the cookies and dairy dessert. This can be attributed to the fact that the soluble fibers of mango, such as pectin, can have an effect on the solubility and absorption of calcium, which may be more related to its physical properties than to its chemical capacity to form complexes. While the calcium present in the cookies and dairy dessert was more easily released by enzymatic digestion through the digestive enzyme pepsin, this is probably due to the proteolytic effect of this

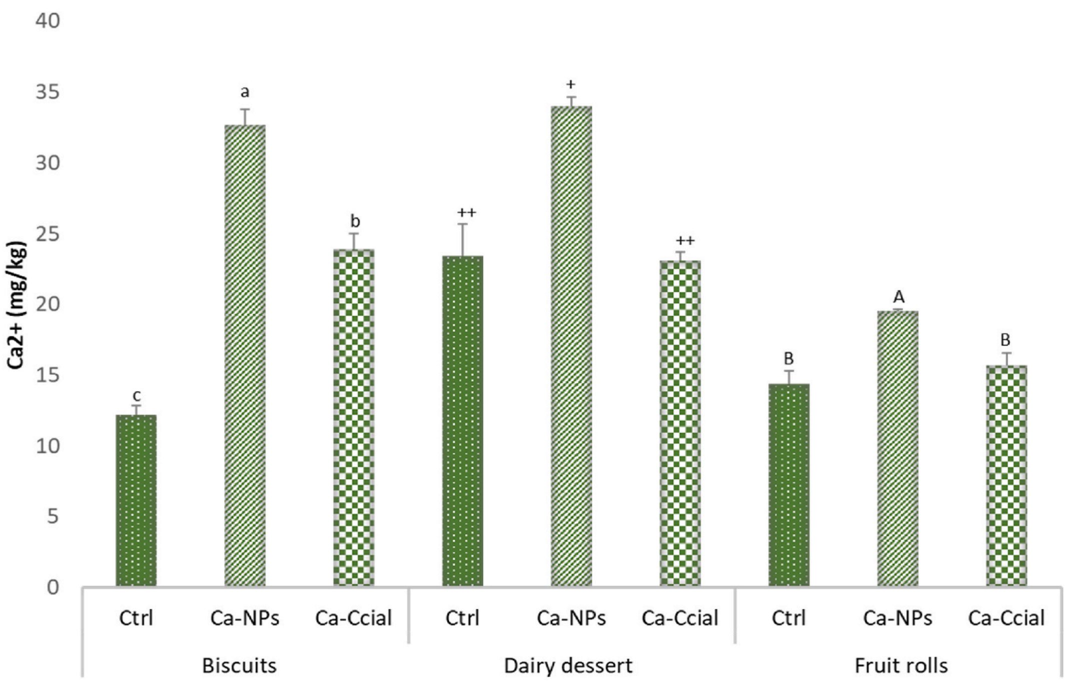

Fig. 1. Ca^{2+} concentration in the bioaccessible fractions. Results are represented as mean ± SD (n = 4). Different letters and symbols show significant differences ($p < 0.05$) for each of the food matrices.

enzyme on the proteins present in the cookies and dairy dessert [20]. The higher bioaccessible Ca^{2+} concentrations in the samples fortified with Ca-NPs demonstrate that the reduction in particle size and the increase in specific surface area of these particles have a positive effect on the solubility and bioaccessibility of this mineral with respect to commercial Ca.

3.2. Physical properties

The physical properties of foods have a direct relationship to product quality and consumer acceptance. Therefore, the following parameters were evaluated.

3.2.1. Texture analysis

The textural quality of the cookies was measured as hardness (N). Fig. 2 shows that fortification with commercial Ca and Ca-NPs significantly influenced the textural properties of this matrix in terms of hardness, coinciding with Therdthai et al. [21], who reported that the use of eggshell powder with 4–6 g/100 g flour significantly increased the hardness of the biscuits. The results indicate that there are significant statistical differences between treatments ($p < 0.05$) with respect to the control (no $CaCO_3$ addition). The increase in the strength required to fracture fortified cookies may be related to the role of $CaCO_3$ as a stabilizing agent in foods, since in weak flours, calcium salts form cross-links with gluten proteins and increase the stiffness of the dough. Likewise, the calcium-starch interactions and the network formed by the interactions between water and matrix components cause the increase in the hardness of Ca-NPs and commercial Ca, with respect to the control [22–24].

As for the comparison between treatments, Fig. 2 shows how the cookies with Ca-NPs presented a higher level of hardness ($p < 0.05$), which may be due to the fact that the smaller the particles are, the greater their cohesion with the other matrix ingredients [25].

The behavior of the texture in the different treatments of the fruit rolls made from mango is represented in Fig. 3, in which it is observed that there are significant differences between the samples at a confidence level of 95 % ($p < 0.05$). The highest values correspond to the samples fortified with commercial Ca (229.29 ± 7.52 N), while those fortified with Ca-NPs presented a mean shear force of 110.45 ± 8.31 N and the control samples presented the lowest, of 72.16 ± 6.30 N. Similar values were reported by Zuluaga et al. [16], who fortified mango with commercial calcium through a direct drying process, obtaining hardness values of 216.26 N. The drying temperature was selected taking into account that, for heat-sensitive products such as fruit, high temperatures degrade the physical, sensory, and nutritional quality of the product [26].

In the fruit rolls fortified with commercial Ca, a higher level of hardness was evidenced, which may be related to a greater loss of water, since the permeability of the mango cell matrix membrane modifies its osmotic response and generates structural changes as a response of the polymeric structure of the cell wall and the interaction of calcium bridges that act on the different transport mechanisms in the fruit tissue [27]. The texture of the samples fortified with Ca-NPs resulted in lower hardness (N). Therefore, it is inferred that the smaller the particle size, the greater the solubility of calcium, which allows for a greater entry of Ca^{2+} into the mango plant cell, which improves the texture of the sample [27].

3.2.2. Viscosity analysis

The viscosity results did not show statistical differences between treatments ($p < 0.05$). Higher viscosity values were obtained for the samples with Ca-NPs (2.96 ± 0.75 Pa s), while the viscosity of commercial $CaCO_3$ and control samples were 2.37 ± 0.75 and 1.91 ± 0.61 Pa s respectively. This is associated with the ability of nanoparticles to form biocrowns with milk proteins, especially colloidal

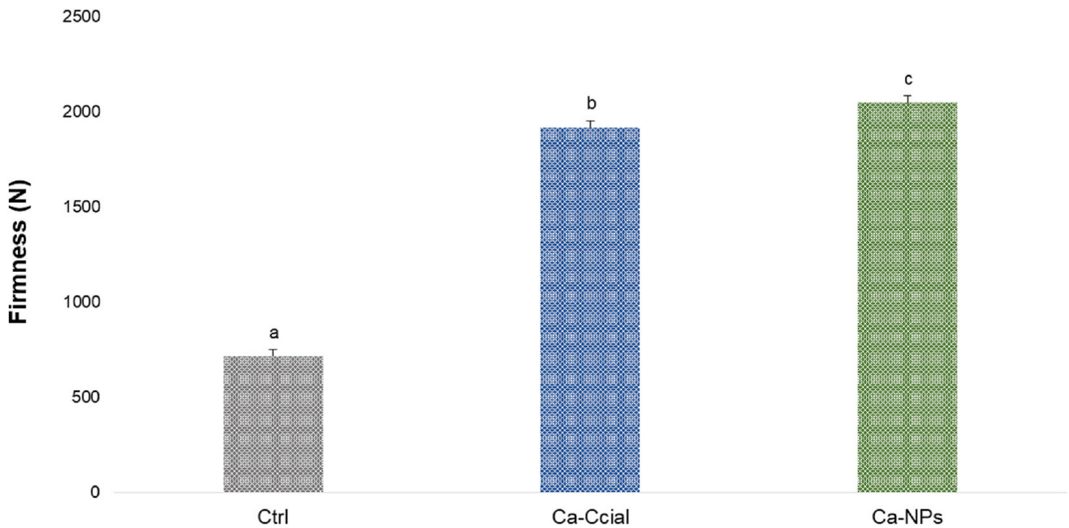

Fig. 2. Texture of calcium-fortified oatmeal cookies. Results are represented as mean ± SD (n = 4). Different letters and symbols show significant differences ($p < 0.05$).

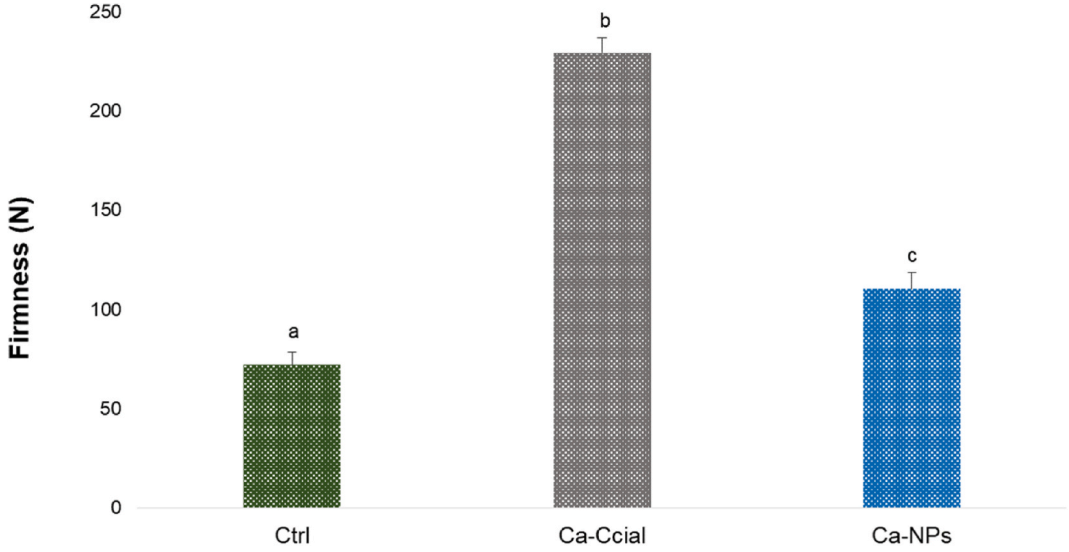

Fig. 3. Texture of calcium-fortified fruit rolls. Results are represented as mean ± SD (n = 4). Different letters and symbols show significant differences ($p < 0.05$).

calcium phosphate (CCP) [28]. Therefore, the firmness of this dessert with Ca-NPs is attributed to the higher degree of crosslinking between calcium and casein micelles, due to the higher content of soluble calcium after the fortification process [29].

The results indicated that the quality of the product in relation to viscosity is positively influenced by the fortification with commercial $CaCO_3$ and on a nanometric scale, since the addition did not affect the product's firmness and viscosity characteristics. This may be because the calcium system in milk is highly buffered, that is; if the concentration of calcium ions in milk is decreased by some treatment, the supply is taken from the pool of soluble and colloidal non-ionic forms. Similarly, when the calcium content of milk increases, this increase is distributed between the ionic and non-ionic soluble and colloidal phases [30].

3.2.3. Color analysis

Table 1 shows the color parameters for each of the treatments applied to the cookies, indicating the statistical differences at a confidence level of 95 % ($p < 0.05$).

The values expressed in Table 1 enabled us to detect, in the case of the variable L*, that the luminosity of samples fortified with commercial Ca showed no significant differences with respect to the Ca-NPs ($p < 0.05$); similar to the study carried out by Fekadu et al. [31], which showed a decrease in the luminosity of the injera as the level of eggshell powder increased, without showing significant differences. Meanwhile, in the variables a* and b*, a trend was observed in the behavior of the Ca-NPs with values that on the chromatic circle are closer to red tones, typical of products such as cookies. This trend in tonality is confirmed by the hab* value. This darker shade is possibly also due to the grayish color, characteristic of Ca-NPs. Additionally, this slightly more intense tonality in the cookies fortified with Ca-NPs is related to the products of the Maillard reaction generated during the baking process, such as melanoidins, which have the capacity to form moderate complexes with Ca^{2+} nanoparticles [17]. The caramelization of sugar and the Maillard reaction, which is the reaction between the free amino group of lysine, peptides, or protein, and carbonyl groups of reducing sugars, might take place during the baking process of bread products, which accelerates the browning reaction [32]. The C* values indicated higher saturation in the samples fortified with Ca-NP, followed by those fortified with commercial Ca and, finally, the control samples. The latter coincided with the results reported by Therdthai et al. [33], where the addition of eggshell powder significantly reduced redness and yellowness, due to the white color of the eggshell powder.

The ΔE* values of the cookies fortified with Ca-NPs (Table 1) place them in the category of "obvious difference", while those fortified with commercial Ca are located in "extremely marked differences", which indicates that this type of carbonate affects the color of the cookies more than the control, and more than the addition of Ca-NPs.

In the fruit rolls with Ca-NPs, the ΔE* values indicate that there were "extremely marked" color changes, while the samples with commercial Ca fall into "colors of different shades" based on ΔE*. In this product, there were significant differences in all the color

Table 1
Cookies color analysis.

	COOKIES					
	L*	a*	b*	C*	hab*	ΔE*
Control	32.33 ± 2.38^{a}	8.63 ± 0.72^{a}	17.01 ± 1.65^{a}	19.07 ± 1.65^{a}	63.03 ± 2.17^{a}	–
Ca-Comm	39.14 ± 3.97^{bA}	8.31 ± 1.26^{aA}	19.54 ± 3.10^{bA}	21.24 ± 3.26^{b}	66.87 ± 2.02^{b}	9.71 ± 1.03^{a}
Ca-NPs	37.61 ± 2.96^{cA}	9.47 ± 0.82^{bB}	20.87 ± 2.85^{cA}	22.93 ± 2.80^{c}	65.41 ± 2.30^{c}	2.70 ± 0.22^{b}

variables (L*, a* and b*). However, in the chromaticity of a* and b* there are two homogeneous groups (Ctrl and Ca-NPs), as shown in Table 2.

The mango fruit rolls were subjected to a drying process for 5 h, during which the color changes became evident until the product reached the required dehydration point. As evidenced in Table 2, the samples fortified with commercial Ca had higher luminosity (L*), a* and b* ($p < 0.05$) than the samples fortified with Ca-NPs and the control samples. The C* value in the samples with commercial Ca indicated high color saturation, which suggests that this source of $CaCO_3$ intensifies the tonality of the product.

The fruit rolls with commercial Ca presented values close to those reported by Zuluaga et al. [16], in their study on the physical characteristics of dehydrated mango ($a^* = 16.19 \pm 1.92$ and $b^* = 41.09 \pm 5.15$). This was attributed to a darkening or browning, where the yellow color (the natural color of the fruit) becomes less evident and the brown becomes more marked, due to the formation of a syrup crust and the caramelization reaction of the sucrose that remains on the surface of the fruit rolls after the drying process [16, 34].

Regarding the dairy dessert, the L* values of the samples fortified with commercial Ca were significantly higher ($p < 0.05$) than those of the samples with Ca-NPs (Table 3), which can be attributed to the gray tonality, typical of eggshell powder, reducing the whiteness of the product. Regarding variable a*, the values are negative and close to 1. Therefore, they do not exhibit any change of tonality, while b* showed a significant tendency ($p < 0.05$) towards yellow tones in the case of commercial Ca and control samples, with respect to Ca-NPs (Table 3).

The white color of milk is caused by fat globules, casein micelles, and colloidal calcium phosphate. In this case, the fortification with Ca-NPs and commercial Ca caused a slight variation in the matrices as indicated by the C* and hab* values, which is confirmed by the result obtained in the estimation of the color difference ΔE* for the samples fortified with Ca-NPs and commercial Ca.

3.3. Sensory profile

The effect of fortification with Ca-NPs and commercial Ca on the sensory properties of the matrices studied, caused some changes to their characteristic attributes. The analysis of variance regarding the oatmeal cookies revealed statistical differences in almost all sensory parameters, except for color ($p = 0.1712$) and chewiness ($p = 0.3195$).

The effect of the treatments used for the fortification of the oatmeal cookies was determined by quantifying the intensities of the predominant sensory attributes in this matrix. Fig. 4 shows that there were significant changes in the characteristic flavor, earthy flavor (in a small proportion), odor, hardness, and fracturability of the cookies ($p < 0.05$). However, although both sources (Ca-NPs, Ca-Comm) generally affect the sensory characteristics of this product, the overall score gave greater favorability to the samples containing $CaCO_3$ nanoparticles. Unlike these results, Zerek et al. [35], in their research with eggshell fortified cookies (ESP), observed significant changes only in the size of the particles ($p < 0.05$), although the acceptability of all amounts of ESP added in the sensory evaluation were similar.

The sensory response for the fruit rolls and the influence of calcium fortification in each of its characteristics presented significant differences in almost all the attributes, except chewiness ($p = 0.1176$) and stickiness ($p = 0.1016$).

Fig. 5 shows the effect caused by the addition of micronized calcium (Ca-Comm) and of nanoparticles (Ca-NPs). Notable changes were observed mainly in attributes such as color, characteristic odor, fruit flavor and appearance, and likewise a reduced gumminess of the product. In the case of hardness, Ca-Comm had a greater effect on this matrix, reported by the sensory panel as a negative characteristic and which correlates with the data obtained in the texture analysis (Fig. 3).

The analysis of variance of the dairy dessert showed statistical differences in all sensory parameters ($p < 0.05$). In the sensory analysis, it was evidenced how the addition of calcium changes the perception of this matrix with respect to the control (Fig. 6). This product, due to its nature, composition and even complexity, since it contains several ingredients, allowed us to detect differences with respect to the control samples, in attributes such as the characteristic smell, milky taste, sweet taste, viscosity, appearance and, in general, the overall score ($p < 0.05$). However, between the two added calcium sources in this matrix, the Ca-NPs showed a more favorable behavior with respect to the Ca-Comm, which showed more marked changes in its attributes.

The fortification of foods with calcium is a challenge for the food industry, due to the different factors involved in achieving an efficient fortification, including the levels of calcium when inorganic salts are used, which mainly affect taste and oral sensation [36]. The insoluble forms, such as carbonate and phosphates, tend to produce a calcareous (sandy) sensation and promote an astringent, bitter taste, even $CaCO_3$ can generate soapy and acid notes [37]. These defects are currently masked with calcium chelating agents to reduce the impact on the sensory characteristics of foods. However, $CaCO_3$ nanoparticles, thanks to their size, are a promising alternative since they can improve solubility, in addition to being one of the calcium sources used in industry with the highest calcium content (40 %). Therefore, the favorable effect of calcium carbonate nanoparticles from eggshells shown in this study represents the possibility of a better response to fortification processes, due to the ability of Ca-NPs to interact with other food ingredients of each

Table 2
Fruit rolls color analysis.

	FRUIT ROLLS					
	L*	a*	b*	C*	hab*	ΔE*
Control	27.90 ± 2.32^{a}	10.91 ± 1.76^{a}	21.53 ± 7.02^{a}	24.19 ± 7.01^{a}	62.13 ± 4.26^{a}	–
Ca-Comm	47.70 ± 2.54^{b}	17.83 ± 0.70^{bA}	40.68 ± 4.38^{bA}	44.43 ± 4.24^{bA}	66.19 ± 1.68^{bA}	32.95 ± 2.54^{a}
Ca-NPs	37.18 ± 2.41^{c}	10.59 ± 0.59^{aB}	25.25 ± 3.70^{aB}	27.39 ± 3.62^{aB}	67.02 ± 1.82^{cA}	11.61 ± 0.71^{b}

Table 3
Dairy dessert color analysis.

	DAIRY DESSERT					
	L*	a*	b*	C*	hab*	ΔE*
Control	73.26 ± 0.37^{a}	-0.93 ± 0.03^{a}	12.38 ± 0.13^{a}	12.41 ± 0.11^{a}	-85.69 ± 1.75^{a}	–
Ca-Comm	74.03 ± 0.35^{b}	-0.71 ± 0.52^{bA}	12.33 ± 0.13^{aA}	12.34 ± 0.32^{aA}	-86.76 ± 0.05^{b}	1.09 ± 0.07^{a}
Ca-NPs	72.61 ± 0.12^{c}	-1.07 ± 0.05^{aB}	10.84 ± 0.09^{bB}	10.89 ± 0.13^{bB}	-84.37 ± 0.02^{c}	1.59 ± 0.03^{a}

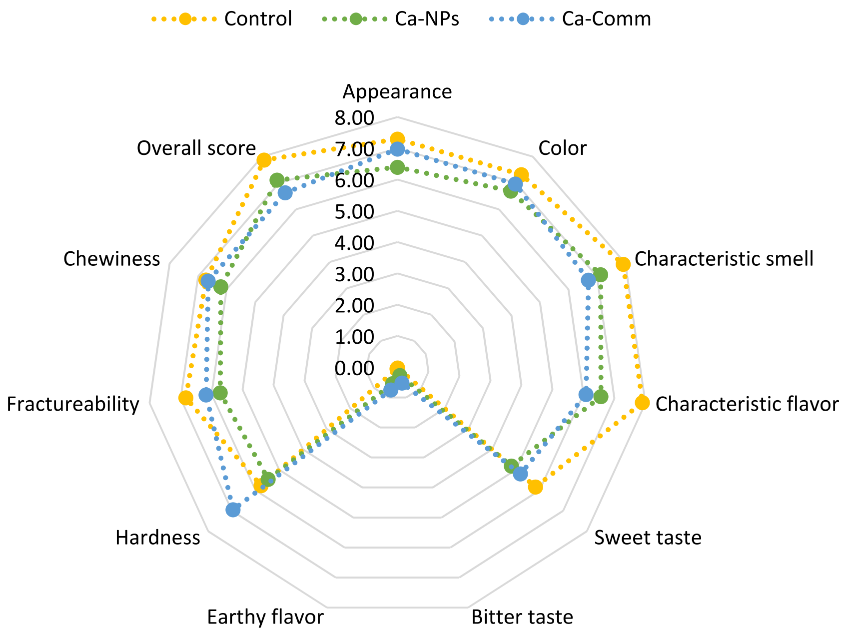

Fig. 4. Intensity of the sensory attributes of oatmeal cookies. Results are represented as mean ± SD (n = 3). Different letters and symbols show significant differences ($p < 0.05$).

matrix and a greater solubility, which results in a more positive perception of the sensory attributes of food [38].

4. Conclusions

According to the results presented in this study, it can be concluded that fortification with calcium carbonate in the form of NPs and micronized (commercial) calcium produces an effect on the physical and sensory properties of food matrices: 1) The textural quality in terms of hardness (N), is affected for cookies and fruit rolls is affected,and the viscosity of dairy desserts increases; 2) color parameters (L*, a*, b*, C* and hab*) are affected differently depending on the matrix; 3) matrices fortified with Ca-NPs have a better sensory response than commercial Ca, with a higher level of acceptance by the judges; and 4) Ca-NPs extracted from eggshells could be a good source of calcium for the enrichment of food products, with a slight effect on physical and sensory properties.

Ethics Statement

The sensory evaluation of the biscuits, fruit rolls and dairy dessert samples were carried out in accordance with established ethical guidelines and informed consent was obtained from the participants. Participants were informed in advance of the purpose and the procedures of the study. Participants were assured of the confidentiality of their data. These experiments were carried out under established protocols and responsibility and commitment to the quality of the results obtained in this study is declared, ensuring that the procedures and methodologies used are in accordance with the relevant standards and regulations.

Data availability statement

Data will be made available on request.

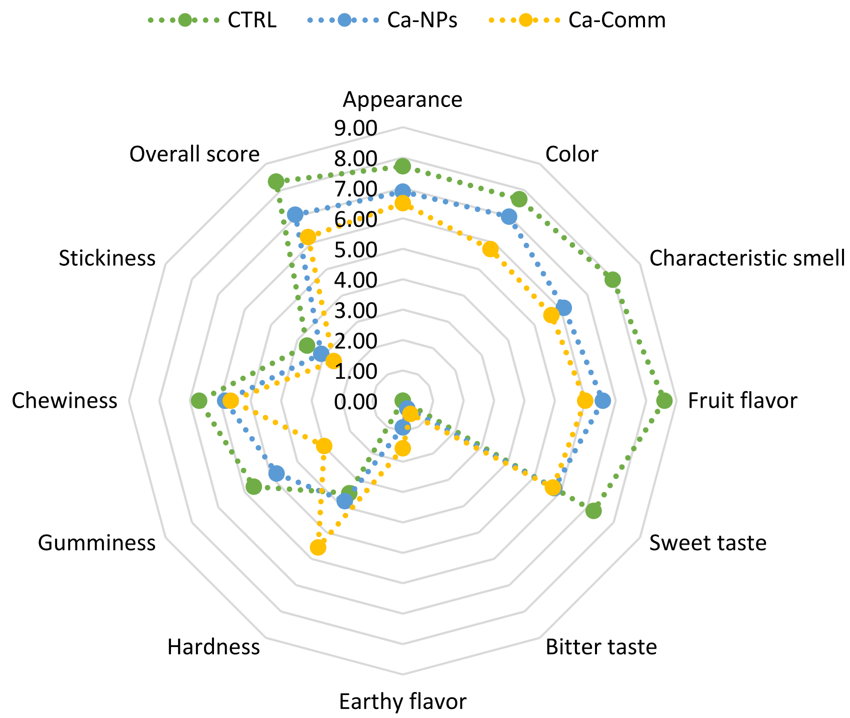

Fig. 5. Intensity of the sensory attributes of fruit roll. Results are represented as mean ± SD (n = 3). Different letters and symbols show significant differences ($p < 0.05$).

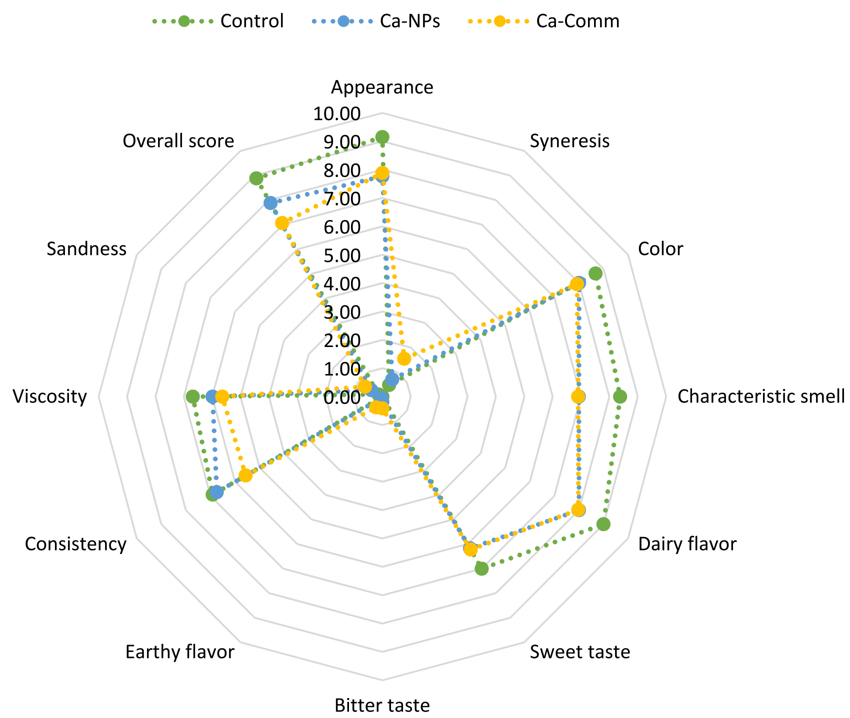

Fig. 6. Intensity of sensory attributes of dairy dessert. Results are represented as mean ± SD (n = 3). Different letters and symbols show significant differences ($p < 0.05$).

CRediT authorship contribution statement

Luz Marina Gómez-Alvarez: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **José E. Zapata Montoya:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

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

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