Sensory evaluation of dairy supplements enriched with reduced iron, ferrous sulfate or ferrous fumarate

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Abstract

Objective. To determine the degree of liking of the Oportunidades programme dietary supplements (DS) –purees and beverages– added with different iron salts (IS): reduced iron (RI), ferrous sulphate (FS) or ferrous fumarate (FF) during 24 weeks of storage. Materials and methods. The DS were evaluated through a hedonic scale for aroma, flavour and colour attributes; at time zero and every eight weeks, each panel member evaluated three DS with same flavour and presentation but different IS. Seventy women participated as panel members. Results. The chocolate and banana DS exhibited a change in preference by colour and flavour due to storage. DS with FS or RI showed the least preference by flavour and colour in the context of the three IS considered. The chocolate and neutral DS enriched with FS changed their colour and flavour. Conclusion. DS were, in general, well-liked; nonetheless, for purees enriched with FS and for beverages enriched with RI, the less-liked attributes were colour and flavour.

Key words: iron; supplementary feeding; food, fortified; dietary supplements; food analysis; food preferences

Resumen

Objetivo. Determinar el nivel de agrado de los suplementos alimenticios (SA) (papillas y bebidas) del Programa Oportunidades, adicionados con diferentes sales de hierro (SH): hierro reducido (HR), sulfato ferroso (SF) o fumarato ferroso (FF), durante 24 semanas de almacenamiento. Material y métodos. Se evaluaron mediante una escala hedónica los atributos olor, sabor y color; a tiempo cero y cada ocho semanas, cada juez evaluó tres suplementos, mismo sabor, presentación y diferente SH. Participaron 70 mujeres. Resultados. Los SA sabor chocolate y plátano presentaron modificación del agrado por color y sabor durante el almacenamiento. Los SA con SF o HR presentaron el menor agrado para sabor y olor por efecto de las SH. En los SA sabor chocolate y natural adicionados con SF se afectó el color y el sabor. Conclusión. Los SA en general presentaron agrado; sin embargo, en las papillas adicionadas con SF y las bebidas con HR los atributos limitantes fueron color y sabor.

Palabras clave: hierro; alimentación suplementaria; alimentos fortificados; análisis de alimentos; preferencias alimentarias
Anemia and micronutrient deficiencies are major nutritional public health problems in Mexico,\(^1\) the “2012 Mexican National Nutritional Survey” (Ensanut 2012) informs a sustained decrease in the prevalence of anemia in children under five years and women between 1999 to 2012, from 31.6 to 23.3\% and 21.6 to 11.6\%, respectively; but still they recorded high prevalence in children from 1 to 2 years with 38\% and pregnant women with 17.9\%.\(^1\) Iron-deficiency was the most common cause of anemia.

In 1997 the Mexican Government launched the Oportunidades Human Development Program (Oportunidades) to improve the quality of life of rural families living in extreme poverty. This program provides conditional cash transfers and a dietary supplement (DS) fortified with iron and other micronutrients to reduce the prevalence of iron-deficiency anemia, aimed to pregnant or breastfeeding women and children younger than two years, or older if malnutrition is present.\(^8\)

The iron source (IS) added to the DS must be bioavailable, assuring that it does not modify the sensorial properties of food. It is known that water-soluble IS such as ferrous sulfate (FS) and ferrous gluconate promote the oxidation of lipids which cause changes in color and aroma of cereal-based foods. While water-insoluble IS such as reduced iron (RI), ferric pyrophosphate and ferric orthophosphate, and those soluble in weak acids such as ferrous fumarate (FF) and ferrous succinate, are less likely to cause sensory changes. Some researchers reported unacceptable changes in the odor of cereal-based foods for infants added with FS at concentrations of 10 and 50 mg/100 g of product. While a similar food fortified with ferrous succinate or FF reported no change in odor at the same concentrations, even after storage.\(^9,10\) It has also been reported the appearance of green streaks on cereal-based foods fortified with hydrated FS, dry FS alone or encapsulated with either soy oil or amino chelated with mono- or diglycerides resulting in the refusal for consumption.\(^10\)

Due to the reasons described, on iron compounds it is mandatory to include a sensorial analysis to evaluate fortified foods in general. The vast majority of reports about sensorial changes were performed by experienced experts or trained panels, who possess proven discriminative abilities.\(^11\) In a similar study by Morales, et al. 2008 only the degree of liking was assessed using a hedonic scale; thus, the objective of this study was to evaluate, in addition, the degree of liking on the attributes color, aroma, and flavor, of the DS distributed by Oportunidades (fortified with RI, FS or FF), according to a consumer panel and after a 24 weeks storage.

**Materials and methods**

*Characteristics of the dietary supplements.* The DS were produced by Liconsa, based on powdered whole cow milk, sugar, maltodextrins, flavoring and artificial colorants and several micronutrients described in table I. The powder was packed in laminated 240g pouches. It was necessary to add water to provide a mush or beverage consistency. The mush was presented in vanilla, banana or chocolate flavors and the beverage in plain, vanilla or banana flavors. Both DS to be tested were added with RI, FS or FF at the factory.

*Storage of the dietary supplements.* Recently manufactured lots of DS, with expiration date of one year, were used. The pouches were stored in a chamber at room temperature for 24 weeks, the temperature and humidity were recorded weekly. Every eight weeks, the pouches of DS from each presentation, flavor and IS were selected to carry out the sensory evaluation.

*Sensory evaluation.* The color, aroma, and flavor of the DS were evaluated through a 5-point hedonic scale, from 1= “dislike very much” to 5= “like very much”.\(^12-14\)

*Consumer panel.* Adult mothers were invited to part-

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Mush</th>
<th>Beverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein, g</td>
<td>13.18</td>
<td>23.08</td>
</tr>
<tr>
<td>Energy, kcal</td>
<td>440.91</td>
<td>480.77</td>
</tr>
<tr>
<td>Iron*, µg</td>
<td>22.72</td>
<td>28.85</td>
</tr>
<tr>
<td>Zinc, mg</td>
<td>22.72</td>
<td>58.85</td>
</tr>
<tr>
<td>Vitamin A, µg</td>
<td>909.10</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin E, mg</td>
<td>13.64</td>
<td>19.23</td>
</tr>
<tr>
<td>Vitamin C, mg</td>
<td>90.91</td>
<td>134.61</td>
</tr>
<tr>
<td>Vitamin B12, µg</td>
<td>1.59</td>
<td>5.00</td>
</tr>
<tr>
<td>Folic acid, µg</td>
<td>113.63</td>
<td>192.31</td>
</tr>
<tr>
<td>Iodine, µg</td>
<td>0</td>
<td>192.31</td>
</tr>
</tbody>
</table>

For the preparation of one portion of mush or beverage, 44 and 52 g of the respective supplement powders are required, respectively.

* Reduced iron, ferrous sulfate or ferrous fumarate
Preparation and presentation of dietary supplements. The DS were prepared before the evaluation, according to the manufacturer’s instructions; 44g of powdered mush were mixed with 21mL and 52g of powdered milk beverages with 120mL of boiled water. Once hydrated, the DS were presented in disposable cups labeled with random numbers, containing 34g of mush or 40mL of beverage.

Each flavor of mush or beverage was evaluated in different days, with each IS evaluated the same day. The flavors were presented randomly between days and IS per session; the samples were tasted from left to right, between samples the panelists rinsed their mouth with distilled water to eliminate the residual flavor of the previous sample.

Statistical analyses. To determine the effect of storage time on each mush and beverage, the Friedman test was applied. Those tests showing statistical difference were analyzed with a Wilcoxon signed ranks test; for such a comparison each week was compared with the prior.

The effect of the IS on mush and beverages of the same flavor, as well as the effect of flavor on the DS within the same IS were tested using the Kruskal-Wallis method. Data were analyzed using the SPSS 12.01 software. A p<0.05 was considered as significant. The categories “like very much” and “like slightly” are presented graphically to observe the tendencies of the effect of the IS and the supplement flavor on the degree of liking; in the same graphics we inform the p value obtained between comparisons.

To evaluate the tendencies of the effect of storage time on the degree of liking, the frequency obtained in the category of “like slightly” is presented in table II, including p for comparisons.

The attributes not showing statistical differences by iron source, flavor of the DS or storage time were not presented as graphs or in tables. In the text we referenced the category which presented more frequency.

Results

During the 24 weeks, the mean temperature and relative humidity were 16.5 ± 3°C and 62.3 ± 10%, respectively. The evaluation of mush was made by 38 women and of drinks by 32 women.

Effect of IS on the degree of liking for the attributes color, aroma and flavor of mush and beverages

A. Mush

The addition of different IS did not affect overall liking for color in all flavored mushes. The evaluation of flavor show statistical differences (figure 1); for vanilla, scores at wk 0, FF rated for “like slightly” as high as 45% and FS as low as 20% (p=0.039) and maintained the same pattern at wk 8 (50 and 20 %, p=0.036), wk 16 (65 and 10 %, p=0.0001) and wk 24 (60 and 25%, p=0.001). The flavor was rated in banana mushes as high as 60% of “like slightly” category in those with RI and as low as 15, 20 and 25% of “like slightly” category in those added with FS, at wk 0 (p=0.0001), wk 8 (p=0.0001) and wk 16 (p=0.0001). In wk 24 the FF increased to near 60% and FS stayed near 20% (p=0.001) of “like slightly”. Chocolate flavor rated at wk 0 as low as 25% in the category of “like slightly” and 55% in the category “like very much” for the RI mushes (p=0.0001), while the FF was rated 45% in the category of “like slightly”. At wk 8 RI increased to 55% of “like slightly”, and FS and FF were as low as 10 and 35% (p=0.0001); at wks 16 and 24 RI was maintained at 40 and 35% of “like slightly” and FS and FF were within 30 and 40%, respectively (p=0.01 and 0.03).

The aroma attribute did show statistical differences for the vanilla and banana mushes. In both, the mushes TABLE II

<table>
<thead>
<tr>
<th>Taste of food supplement/IS</th>
<th>Category</th>
<th>Weeks of storage (percentage of consumers)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>A. Mush</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chocolate/RI</td>
<td>“like slightly”</td>
<td>40.74</td>
<td>66.67</td>
</tr>
<tr>
<td>Chocolate/FS</td>
<td>“like slightly”</td>
<td>59.26</td>
<td>66.67</td>
</tr>
<tr>
<td>Chocolate/FF</td>
<td></td>
<td>48.15</td>
<td>62.96</td>
</tr>
<tr>
<td>B. Beverage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plain/RI</td>
<td>“like slightly”</td>
<td>39.29</td>
<td>25.00</td>
</tr>
<tr>
<td>Plain/FS</td>
<td>“like slightly”</td>
<td>57.14</td>
<td>35.71</td>
</tr>
<tr>
<td>Plain/FF</td>
<td></td>
<td>57.14</td>
<td>39.29</td>
</tr>
</tbody>
</table>

* Probability of the effect of storage time on the liking degree of the mushes or beverages

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added with FS showed the lowest liking since only 50% scored it in “like slightly” and “like very much” but at wk 0 \( (p=0.039) \) and wk 8 \( (p=0.036) \) for vanilla and at wk 24 for banana mushes (data not shown).

B. Beverages

In beverages, the addition of IS had no effect on the color attribute. Flavor (figure 2) was graded signifi-
cantly higher (50%) for the “like slightly” category by FF, in comparison with around 30% of FS and RI (p=0.000) at wk 0. At wk 8 FS increased to 48% and RI and FF remained in 30% for the “like slightly” category (p=0.013). At wks 16 and 24 all IS were graded below 30% and FF increase to 40 and 35% for the category “like very much” (p=0.003 and 0.035). The flavor for the vanilla flavored drink was rated for RI with 20% of “like slightly” and increased progressively with time up to 40% at wk 24. The flavor of FS was rated 55% of “like slightly” at wk 0 and stayed very near up to wk 24. The flavor of FF was rated 40% for “like slightly” and 40% for like very much at wk 0, then it declined for “like slightly” but increased up to 50% of “like very much”. The flavor for the banana flavored drink increased in RI from 20 to 35% from wk 0 to wk 24, but the ranking of “like very much” was around 35% from wk 0 through wk 16 and at week 24 it declined to 20%. The ranking for FS varied from 55-50% in the “like slightly” category from wks 0-24, except wk 16 in which it fell to 35%. However, in the category “like very much” it increased from 50 to 65% from wks 0-24.

On the aroma attribute statistical differences were found only for vanilla beverages at wk 8 (p=0.029), the beverage added with RI showed the lowest liking, where 46% of the consumers rated it in “like slightly” and “like very much” (data not shown).

**Effect of time of storage on the degree of liking for color, aroma and flavor of mush and beverages of the same flavor and IS**

A. Mush

The color was evaluated as “like slightly” in all flavor along the storage period. For all flavors a significant difference was found in chocolate mush in the three IS at week 8 of storage in which the preference for “like slightly” increased to more than 65% (p<0.05, <0.01 and <0.001), table IIA, as the same change occurred in the banana mush with RI (p<0.0001) in wk 24 (data not shown).

The aroma for all flavors and SI had no significant differences and the rating was generally “like slightly” along the storage period.

The flavor, along with storage time, was rated as “like slightly” for all flavors and SI. However, the banana sample with FS showed a significant change (p=0.014): evaluated as “dislike slightly” at the beginning of the experiment, at wk 8 and 16 around 50% of the panelists changed to “neither like nor dislike” and, at wk 24 to “like very much”.

B. Beverage

The overall degree of liking for color, in all the mush samples was “like slightly”. However, the plain flavor beverage added with RI and FS, table IIB, as well as the banana drink with FS showed significant differences (p<0.05) along storage time. In the case of the difference in plain beverages the panelists changed their scores from “like slightly” at the beginning to “neither like nor dislike” from wk 16 on.

As for the banana sample with FS, the panelists up to wk 16 rated it “like slightly”, and increased the score from “like slightly” to “like very much” (22% data not shown).

No significant differences in the effect of time of storage were found on overall flavor and aroma. Aroma rated “like very much”, while flavor rated “like slightly” (data not shown).

**Effect of DS flavor on the degree of liking for the attributes color, aroma and flavor of mush and beverages with the same IS along storage**

A. Mush

When assessing the effect of mush’s flavor (vanilla, banana or chocolate) on the degree of liking, the three samples added with RI showed statistical differences only for color and aroma. Such differences were observed at wk 0 (p=0.030) and wk 16 (p=0.041), respectively. Vanilla mush scored the highest with 92% of panelists placing it in categories 4, “like slightly” and 5, “like very much”, but never returned to a high spot; banana mush scored the lowest with only 63% of scores in the same categories.

Mush added with FF showed no significant differences in any of the sensory attributes tested.
B. Beverage

Regarding the effect of flavor (natural, banana or vanilla) on the degree of liking, significant differences \( p>0.05 \) were found for beverages added with RI respecting aroma and flavor, at wk 24 for aroma and at wks 0 and 16 for flavor. At those times of storage and attribute, approximately 78% of panelists rated the banana beverage with 4 and 5, which was the highest score, while the lowest was given to plain flavor with 43% of panelists giving the same rates.

Beverages added with FS showed significant difference \( p=0.049 \) for flavor at wk 24, when the banana sample scored the highest level in overall liking with 87% of panelists rating it 4, “like slightly”, and 5, “like very much”. Lowest score was for the plain flavor drink, with 56% of panelists rating it in the same categories. However, for this last flavor the highest frequency was “neither like nor dislike”.

Samples added with FF showed statistical differences for aroma \( p=0.043 \) at week 24 as well, when the highest liking was for banana with 91% of ratings in the top two categories, while vanilla scored only 57% in the same categories.

**Discussion**

**Effect of IS on the degree of liking for the attributes color, aroma and flavor of mush and beverages**

When evaluating the IS related to DS over storage time, it was observed that the overall degree of liking for flavor attribute was mainly affected by the form of iron added. Mush with FS obtained significantly lower ratings along the whole storage time than other IS, which was independent from the particular flavor of the DS, indicating that this compound scores low. Some investigators have suggested to reduce the storage time of the foods added with FS to avoid the chemical and sensorial changes detected\(^{17,18}\), however, they found low disliking levels. Also the purity of the salt used is an important factor and it was suggested to utilize the microencapsulated form, that has the same bioavailability, but it presents stability problems when temperature changes\(^{17,19}\).

Beverages had a different result and the addition of RI was relevant for the plain and vanilla drinks, which showed the lowest scores along storage time for the flavor attribute. The results herein presented are due to the fact that 50% more iron was added to beverages in comparison to mush, even though level of hydration was also higher (1.2 as compared to 2:1), the low overall liking score may be related to the low solubility of RI. Some authors have reported that RI does not produce relevant sensory changes in cereal-based foods, with low moisture (<15%), which has been attributed to its low speed of lipid oxidation\(^{20}\). However, RI may have a different behavior in our beverages that is important to determine, since this tendency was observed until the last storage wk for the banana sample; it is well known that the particle size is important for the bioavailability of this particular form of iron\(^{17,19}\), but it is unknown if this has an influence on the sensorial characteristics of the fortified foods. Nowadays, super-dispersion technologies have been developed for insoluble iron sources using specific emulsifiers\(^{21}\).

Although it is important to mention that it has been reported in studies that measure the effect of repeated hedonic measurements, that the initial hedonic judgments are predictive in the majority of cases\(^{22}\).

**Effect of time of storage on the degree of liking for color, aroma and flavor of mush and beverages of the same flavor and IS**

We found that color is the limiting sensory attribute that may determine the sensory shelf life of DS. However, in this study, changes in the overall liking of color attribute did not show relevant decreases of scores that may place them in the category of dislike. Therefore, it may be considered that DS, both mush and beverages, showed acceptable overall liking levels for the sensory attributes tested along the storage time tested.

Changes in color along storage of mush and beverages with FS may be attributed to the size of the particle of FS. Some reported that big particles of the iron compound can be related to changes in color and appearance of color streaks\(^{23}\). Others reported deterioration and reduction of shelf-life of fortified dry products and cereal-based strained foods due to Maillard reactions and the oxidation of lipids, catalyzed by this form of iron. This originates changes in color and unpleasant odors during storage\(^{24}\). However, sensory changes in color are also attributed to the oxidation of ferrous iron, a 3.08% of which is converted to ferric ion after 42 days of storage, in foods with a moisture of less than 10%, but when the moisture increases between 15-20%, conversion can be up to 3.48%. The same occurs in fortified packed foods with similar content of moisture, but to a lesser extent\(^{17,19}\).
Regarding the FF, adding from 0.5mg affects this attribute showing the appearance of red dashes that are transformed to grey with greater storage time. For RI no reference indicated a similar change; however consumers detected a change that should be studied afterwards.

It is important to mention that there were not color changes by storage time reported by consumers in the mush and beverages flavored with vanilla and banana. This seems to indicate that the color of the DS with these flavors do not allow to perceive changes or that the presence of some compounds do not allow chemical changes; it was reported that reducing agents as ascorbic acid can diminish the oxidation of ferrous ion.

**Effect of DS flavor on the degree of liking for the attributes color, aroma and flavor of mush and beverages with the same IS along storage**

For these DS, it has been reported that pregnant women had the highest liking for the banana drink, while lactating women showed no difference. Nevertheless, the present study did not show any difference in the flavor of DS along storage.

Supplementation with iron is technically more difficult than with other nutrients because bioavailable forms of iron are chemically reactive and often produce undesirable effects when added to a food. Although studies on the topic generalize the effect of iron salts in foods, the results obtained in this study, suggest it is necessary to evaluate each case sensorially, because it would be ideal that it was invisible to the consumers; it is well known that the visual appearance, flavor and odor of any food are the firsts organoleptic senses that a consumer experiences and the consumer must not be able to detect a discernible difference.

**Conclusions**

Both DS (mush and beverages) showed liking ratings in the overall degree of liking hedonic scale along the established storage time for the evaluated attributes.

Mush added with FS and beverages added with RI scored a lower level in the flavor scale from the beginning of the storage, without showing dislike. Such behavior may be due to the physicochemical properties of these particular iron sources.

However, color was the limiting attribute and it may be used to determine the sensory shelf life of these products.

No consistent preference was observed among the different flavors of the DS along the given storage time.

**Declaration of conflict of interests** The authors declare that they have no conflict of interests.

**References**