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RESEARCH PAPER

Development of iron-fortified chocolate milk for preschool children based on sensory acceptability

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Abstract

Milk is among the most important foods used in the daily diet of children and maybe a suitable option for fortification with minerals such as iron that help reduce iron deficiencies and anemia in children. The objective of the present investigation is to fortify with heme iron from pig blood powder, and pasteurized milk with chocolate flavor and to study its sensory acceptability in a group of children aged 3-5 years. The study variables in milk preparation were iron concentration and sugar content. The compound factorial design and the response optimization allowed for finding an optimal range corresponding to 25% of the recommended dose of iron for children. The formula with the highest acceptability (4.72/5) presented a concentration of 13.65 mg Fe L^{-1}, 693 mg L^{-1} vitamin C and 60 g L^{-1} sugar. According to the physicochemical and microbiological results, the fortified flavored milk complied with regulations and had an approximate shelf life of 5 days. These results showed an alternative for heme iron fortification of chocolate milk, whose content could contribute to the daily requirement of this mineral in children aged 3-5 years (11 mg of iron per day for a consumption of 250 mL).

1. Introduction

From the physiological point of view, iron is considered a mineral of great importance for the normal development of human beings, as well as necessary for the correct functioning of the organism. The lack of iron in the body is due, in most cases, to the fact that the mineral is found in greater proportion in its ferric form, which is more difficult to absorb; in turn, the deficiency of this is also attributed to poor consumption in the daily diet (Serpa *et al*., 2016). On the other hand, heme iron is the biological iron that is easily absorbed by the intestinal mucous membrane cells and can be used not only as a part of iron supplements but also as an enhancer of food nutrition (Man *et al*., 2022).

Dairy products are poor in iron and some other minerals, so fortification with Fe would help avoid the previously mentioned nutritional deficiencies. The concentration of Fe in milk is around 0.2 mg kg^{-1}, therefore adding Fe to this product can favor the daily intake of this nutrient. Parameters for evaluating the effects of iron added to dairy products include fat oxidation, flavor, shelf life and microbial physiology, sensory quality, and general acceptance of the fortified product (Gahruie *et al.,* 2015). Some research details that fat oxidation in chocolate milk fortified with ferric polyphosphate whey protein complex was avoided and showed an acceptable taste (Douglas *et al.,* 1981). However, fortification with ferric chloride or ferrous gluconate was not acceptable and ferric ammonium citrate increases oxidation in milk (Gahruie *et al*., 2015). Due to the above, it is necessary to find a balance between the incorporation of iron (and its forms), as well as sensory acceptability.

Among the various techniques used for sensory analysis, affective tests are used with the purpose of evaluating consumer preference and/or acceptance with respect to products (Navarro *et al.,* 2013). The illustrated face scales show a spectrum of sensory experiences, thus

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eliminating the need for raters to quantify their experience numerically. For example, when these are children (Garra *et al.,* 2010). These sensory and hedonic methods have been applied with the purpose of optimizing food to develop healthier options that are liked by children (Laureati *et al.,* 2015).

Among the investigations of sensory evaluation of fortified milk drinks is a chocolate drink with hydrolyzed lactose (50%) and enriched with amino-chelated iron such as iron bisglycinate at 5% of the RDI (recommended daily intake), which obtained good sensory acceptability in a sensory ordination test that was carried out with 7 trained judges. It was also concluded that iron bisglycinate affected the color property, which limited its use in chocolate milk beverages (Hernández, 2017). On the other hand, Villalpando *et al.* (2006) evaluated the efficacy of whole cow's milk fortified with ferrous gluconate and zinc oxide, along with ascorbic acid, to improve iron status in children 10 to 30 months of age. The prevalence of anemia decreased from 41.4 to 12.1% ($P < 0.001$) after 6 months and the results could lead to expanding a subsidized fortified milk distribution program to 4.2 million beneficiary children aged 1 to 11 years old in Mexico. Recently de Matos *et al.* (2021) developed a fermented drink, replacing milk with whey, adding mangaba pulp (*Hancornia speciosa Gomes*) and iron, to improve the nutritional quality of the products. The work highlighted the contribution of proteins, calcium and iron to the daily value of recommended intake of formulations, 8.4%, 15.2% and 44.3%, respectively, and the increase in serum concentration in the formulation improved acceptability, ranking 91.5% for children and 73.6% for adolescents.

In a previous investigation, heme iron was used, establishing as variables the concentration of iron and chocolate, which influenced the acceptability of fortified milk (García *et al*., 2022). This was appreciated in children between 8 and 11 years old, presenting the highest acceptability from 6.76 mg Fe kg^{-1} (6.4- 12.8 mg Fe kg^{-1}) and 2.0 g kg^{-1} (2- 4 g kg^{-1}) of chocolate. The iron content represented approximately 21% of the recommended dose for children in this age range (8 mg Fe/day, with a consumption of 250 g of milk). For this reason, it is also necessary to evaluate other parameters that can influence sensory acceptability for children in other age ranges and that have a higher iron content that contributes to improving iron deficiencies in the daily diet.

Therefore, this study aimed to identify the ideal composition of heme iron-fortified chocolate flavored milk samples, based on a central composite design by analyzing iron and sugar concentration levels with respect to their sensory acceptability appreciated by a group of children between 3 and 5 years old measured in a hedonic scale facial test and its subsequent physicochemical and microbiological characterization.

2. Materials and methods

2.1 Materials

Homogenized milk from the Milk Pilot Plant of La Molina National Agrarian University. Whole blood powder of pig origin Aprosan™ (188 mg Fe per 100 g of product), alkalized cocoa powder, ascorbic acid, carrageenan, liquid vanilla, cinnamon, aniseed, clove, commercial sucrose and chocolate bit of Frutarom Peru S.A. Food additives were food grade.

2.2 Iron-fortified chocolate milk

Iron-fortified chocolate flavored milk was prepared based on the Peruvian Technical Standard NTP 202.189.2020 (INACAL, 2020), using fresh milk, commercial sucrose, flavorings, ascorbic acid and authorized additives. Aprosan™ powder was completely diluted in cold milk at 10°C, then heated to 30°C, and subsequently cinnamon, cloves and anise were added. Heating continued up to 50°C, where sugar, cocoa, carrageenan and vitamin C were added and after that vanilla and chocolate were added at 60°C. The product was pasteurized under Low Temperature and Long Time (LTLT), at 72°C for ten minutes, using a stainless steel 100 L jacketed pot. Subsequently, the iron-fortified chocolate flavored milk was filtered to retain the remaining clove, cinnamon and anise residues. Finally, milk was bottled in 250 mL plastic bottles and stored at room temperature. Table 1 shows the ingredients used in the formulation of chocolate milk and their respective amounts.

Table 1. Ingredients used in the formulation of fortified chocolate milk.

Ingredients (g/ L milk)	Formulation
Sugar	50.0-60.0
Alkalized cocoa	6.0
Carrageenan	0.7
Vitamin C	0.7
Liquid vanilla	2.0
Chocolate	4.0
Cinnamon, aniseed and clove	1.0
Aprosan*	5.85-6.90

Iron*: 0.011-0.013 g L^{-1}

2.3 Experimental design

The central composite design was used to investigate the effects of heme iron and sugar concentrations to determine the optimum formulations. Three concentration levels (low, medium and high) were evaluated as variables, for nine formulations included in

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the experimental design (Table 2). The choice of concentration levels is related to the recommended daily intake for stage preschool-age children, 3-5 years old (11 mg Fe/per day) who consume 250 g of milk daily (Institute of Medicine (US) 2001). The low level represents 25.0% fortification (11 mg L^{-1}), the intermediate level 27.5% (12 mg L^{-1}) and the high level 30% fortification (13 mg L^{-1}), for 1 L of milk. The sugar levels were chosen based on the concentration of 50 g of sugar per liter provided in Law No. 30021, Law for the Promotion of Healthy Eating (El Peruano, 2017). The low level of sugar represents 50 g L^{-1}, the intermediate level 55 g L^{-1} and the high level 60 mg L^{-1}, for 1 L of milk.

Table 2. Variables and levels used for the experimental design.

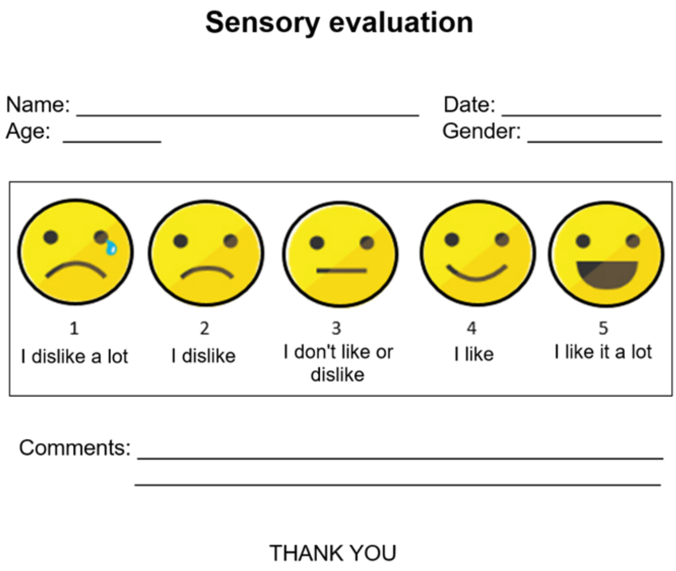
Formulation	Heme iron (mg L^{-1})	Sugar (g L^{-1})
F1	11.0	50
F2	13.0	50
F3	11.0	60
F4	13.0	60
F5	11.0	55
F6	13.0	55
F7	12.0	50
F8	12.0	60
F9	12.0	55

Fortification for 1 L of milk (Recommended dose, 11 mg Fe per day for a portion of 250 g of milk).

2.4 Sensory analysis

The facial hedonic scale test was performed on a total of 120 preschoolers, aged 3-5 years, untrained users, from a public school in the city of Lima, Peru. They were asked to rate their overall acceptability on a 5 -point hedonic scale (1 = I dislike a lot, 2 = I dislike, 3 = I don't like or dislike, 4 = I like, 5 = I like it a lot). Children were asked to mark the facial image that best represented how much they enjoyed each product (Figure 1). A 5-point hedonic facial scale was chosen because it has been reported to be applicable in children of the same age range (Chen *et al*., 1996), furthermore, it is recommended to use hedonic scales with words only with children older than 8 years old (Laureati *et al*., 2015). Some researchers show the advantage of using facial images from a nonverbal approach that conveys meaning and evaluates children's emotional responses to food products, which makes them feel more comfortable with their use (da Cruz *et al.,* 2021).

The overall acceptability value for each formulation was obtained from the sum of the children's acceptability, divided by the number of children surveyed.


Sensory evaluation

Name: ____________ Date: ________
Age: ______ Gender: ________

1 I dislike a lot | 2 I dislike | 3 I don't like or dislike | 4 I like | 5 I like it a lot

Comments: ____________

THANK YOU

Figure 1. Sensory evaluation sheet of fortified chocolate milk used in pre-school children.

2.5 Statistical analysis

The results of the sensory analysis of the formulations obtained by the central composite design were subjected to an analysis of variance (ANOVA) using the software Minitab Version 17 to determine the significance of the coefficients in the model at a confidence level of 95% ($p < 0.05$). The model fit was verified using the R^2 value. The most accepted formulation was then used in the preparation of the fortified flavored milk for its subsequent characterization. Analysis of variance (ANOVA) is an optimal and well-known statistical tool for comparing products, and sensory acceptance data is generally evaluated using this analysis. Sensory acceptance is a parametric test and presents assumptions to be validated, such as the homogeneity of the residual variance (Navarro *et al*., 2013).

2.6 Milk characteristics

Physicochemical characterization was carried out for fresh milk and fortified flavored milk based on Peruvian technical regulations. The protein analysis was carried out by the Kjeldahl total nitrogen determination method (NTP 202.119: 1998-2014) (INACAL, 1998a). The amount of total solids and ash content were obtained based on NTP 202.118:1998-2021 and NTP 202.012:2008-2018, respectively (INACAL 1998b, 1998c). Viscosity was determined using a Brookfield viscometer. The fat analysis was determined by the Weibull–Berntrop gravimetric method (ISO 8262, 2005) and iron was analyzed according to AOAC 985.35 (AOAC, 2016). Vitamin C was analyzed by titration method according to AOAC 985.33 (AOAC, 2016a). The determination of the mesophilic aerobes and coliform was carried out based on ICMSF (2000).

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3. Results and discussion

3.1 Sensory analysis

The acceptability of the fortified flavored milk was evaluated by sensory tests. According to the results (Figure 2), the highest acceptability was attributed to the F3 formulation milk sample and the lowest score was attributed to the F6 sample. In general, all the formulations had a range of appreciation between I like it and I like it very much. The higher score (4.72) was attributed to a lower concentration of iron (11 mg L^{-1}) and a higher amount of sugar (60 g L^{-1}), probably associated with the fact that a less metallic taste and a greater amount of sugar are more appreciated by children. The greater preference for sugar during infancy may be related to the rapid physical growth during this time (Forestell, 2017).

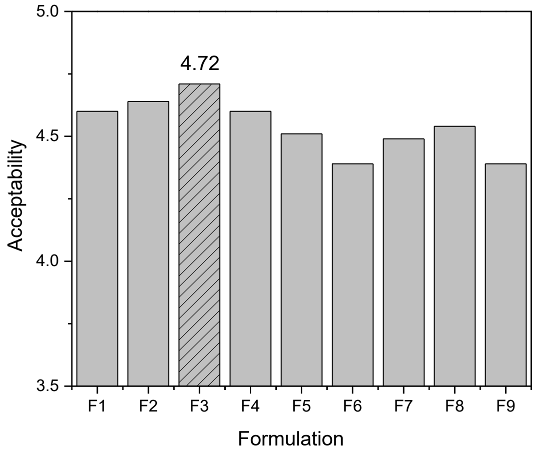

Figure 2. Acceptability score of fortified chocolate milk formulations.

3.2 Statistical analysis

The analysis of variance (ANOVA) for the significance test of the model coefficients is shown in Table 3, where the factors and their combinations were: factor X1: iron concentration and factor X2: sugar concentration. The analysis indicates that the quadratic effect of the concentration of iron (X_1) and sugar (X_2) are the factors with the greatest significance ($p < 0.05$). According to the dimension of these coefficients in the model, the two variables (X_1*X_1 and X_2*X_2) are determinants of the sensory acceptability of fortified flavored milk.

Table 3. ANOVA data for factorial experimental design.

Factor	df	F-value	p-value
Model	5	4.45	0.016*
X_1	1	0.21	0.654^{ns}
X_2	1	5.74	0.211^{ns}
X_1* X_1	1	5.77	0.033*
X_2* X_2	1	14.37	0.003 *
X_1* X_2	1	0.14	0.712^{ns}
Error	12	0.009428	
Total	17		

X_1: iron concentration, X_2: sugar concentration, *significant $p < 0.05$, ^{ns} not significant $p > 0.05$.

The adjustment of the model for sensory acceptability (SA), resulting from the exclusion of non-significant terms ($p > 0.05$) is presented in Equation 1. The R^2 value of 0.650 indicates the efficiency of the model. The response optimization process found optimal values for the factors and sensory acceptability: Fe concentration = 11 mg Fe L^{-1} sample, sugar concentration = 60 g L^{-1} and SA = 4 .72 (scale from 1 to 5), under a confidence level of 95%. These concentrations coincide with the formulation (F3) with the highest score in the sensory test.

$$SA = 4.354 - 0.013 X_1 - 0.037 X_2 + 0.117 X_1^2 + 0.184 X_2^2 - 0.013 X_1 * X_2 \quad (1)$$

Figure 3a shows the development of the response surface, where the optimal combinations of factors, both individual and their interactions, were found. The objective is to maximize the score parameter that represents the desirability function. The desirability

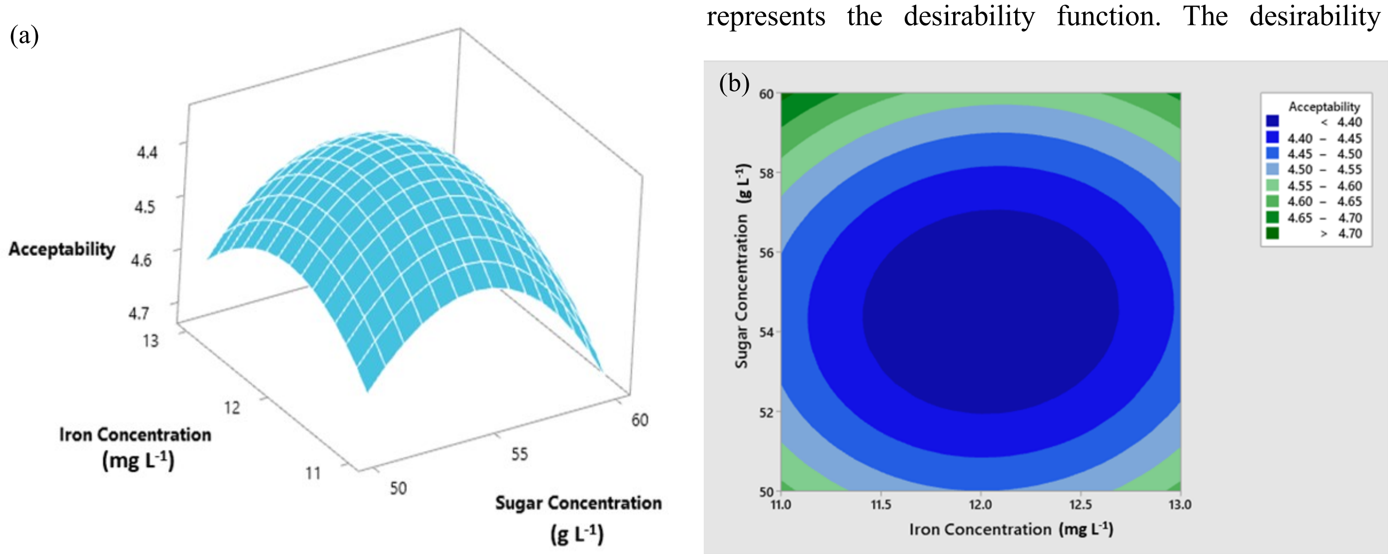

Figure 3. (a) Response surface plot and (b) Contour plot for overall acceptability as function of concentration of iron and sugar.

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function evaluates whether the combination of factors satisfies the goals defined for the responses. In this case, the desirability function (0.8046) is close to 1, indicating that the predicted combination of factors would achieve the optimal score. Therefore, the model indicates that the best score is obtained with an iron concentration of 25% of the RDA (11 mg L^{-1}) and a sugar concentration of 60 g L^{-1} of milk; obtaining an average score of 4.72.

In addition, the contour plot for overall acceptability as a function concentration of iron and sugar is shown in Figure 3b, which also indicates a general acceptability greater than 4.70 for a formulation of 11 mg L^{-1} of iron and 60 g L^{-1} of sugar.

Under this iron concentration, chocolate-flavored fortified milk can supplement a percentage of the recommended levels for children 3 to 5 years old (11 mg of iron/day) if 250 g of milk is consumed (Institute of Medicine (US), 2001) and also represents milk with desirability for children of these ages according to the optimized sugar level.

3.3 Milk characteristics

Flavored milk must meet certain physicochemical and microbiological requirements according to the Peruvian Technical Standard NTP 202.189.2020 (INACAL, 2020), for this its physicochemical parameters were evaluated (Table 4). The protein content of fortified flavored milk was higher than that of fresh milk and on the contrary for fats, the content was lower for fortified milk. The viscosity value 4.43 cP can also be influenced by the addition of carrageenan (Yanes *et al*., 2002).

Table 4. Results of the physiochemical analysis of fresh milk and fortified chocolate flavored milk.

Characteristics	Fresh milk	Fortified flavored milk
Protein (g per 100 g)	3.0	3.6
Fat (g per 100 g)	3.50	2.72
Ash (g per 100 g)	0.70	0.76
Moisture (g per 100 g)	88.0	81.3
Total solids (g per 100 g)	-	18.7
Viscosity (cP)	-	4.43
Vitamin C (mg L^{-1})	10.40	693.0
Iron (mg L^{-1})	< 0.5	13.65

The concentration of Vitamin C (0.693 g L^{-1}) coincides with the amount added for fortified milk (0.70 g L^{-1}). The addition of Vitamin C favors the absorption of heme and non-heme iron in fortified milk formulations and in other foods eaten by children (FAO and WHO, 2002).

The iron concentration of fresh milk and iron-fortified milk is 13.65 mg Fe L^{-1} and <0.5 mg Fe L^{-1}, respectively. This value represents 3.4 mg of iron in 250 mL, which is within the daily requirement parameters for said mineral in preschool children (11 mg of iron/day) if they consume 250 g of milk per day (Institute of Medicine (US), 2001). The content of incorporated iron is also related to the metallic taste, since at very high concentrations this taste can be appreciated (Toldrà *et al.,* 2011). This research worked with formulations with concentrations that contribute to nutritional quality but at the same time have optimal general acceptability.

According to Villalpando *et al.* (2006), the iron content is similar with 5.8 mg/400 mL of iron as ferrous gluconate, 5.28 mg/400 mL of zinc as zinc oxide, and 48 mg/400 mL of ascorbic acid assigned to children between 10-30 months of age. Ferrous gluconate added to whole cow's milk as a fortifier along with ascorbic acid was effective in reducing the prevalence of anemia and improving iron status in children. On the other hand, Gupta *et al.* (2015) prepared microcapsules of iron from salts and incorporated them into a mixture of cow and buffalo milk, obtaining concentrations of 25 mg L^{-1} of iron, which is higher than that obtained in this investigation.

According to NTP 202.189.2020 (INACAL, 2020) regarding the microbiological requirement of flavored milk, the number of coliforms must be below 3 MPN and the number of mesophilic aerobes must not exceed 50,000 CFU mL^{-1}. For this work, after 14 days, the recommendation in the number of coliforms is met, only that the number of mesophilic aerobics exceeds 50,000 CFU mL^{-1} at 5 days (Table 5). Thus, its consumption is recommended before 5 days.

Table 5. Microbiological analysis of fortified flavored milk.

Microbial Agent	NTP 202.189.2020	Day 0	Day 2	Day 5
Aerobic mesophilic (CFU mL^{-1})	50000	6400	6600	53000
Coliforms (MPN mL^{-1})	10	< 3	< 3	< 3

Source: Peruvian Technical Standard NTP 202.189.2020 (INACAL, 2020).

According to the nutritional information of two national commercial chocolate milks, these present a total sugar content of 4.9 and 9.0 g per 100 mL, respectively; additionally, these contain added sugars and sweetener additives. In addition, Nutrition Facts labels omit claims of vitamin C or iron content. The Regulation of Law No. 30021 – "Ley de Promoción de la Alimentación Saludable para niños y adolescentes del gobierno peruano" (El Peruano, 2017), requires the indication of an advertising warning sign in the form of an octagon if the sugar content in beverages is greater than or equal to 5 g per 100 mL. Since the optimal formula exceeded this content, it would be necessary to indicate the advertising label or carry out a study on the incorporation of sweeteners in the future that produces a balance between acceptability and the amount of sugar below the limit according to the Law No. 30021 above mentioned.

These results show a method of fortifying flavored milk that enables the technological use of heme iron as a nutrient extender in the production of dairy products, since it complements the daily requirement of said mineral in children between 3-5 years of age.

4. Conclusion

The concentration of iron and sugar has a significant effect on the sensory acceptability of chocolate-flavored milk intended for children aged 3-5 years, obtaining a greater acceptance for 25% (11 mg L^{-1}) of the recommended dose of iron for children in this age range (11 mg for a portion of 250 mL per day) and a sugar content of 60 g L^{-1}. Fortified flavored milk has physicochemical and microbiological characteristics similar to flavored milk, complying with the range established by the Peruvian technical standard. The useful life is influenced by the number of mesophilic aerobics, which exceeds the values recommended by regulations in a period of 5 days. The results of this study revealed the potential of iron fortification from blood powder in chocolate milk formulation and its use in infant feeding programs.

Conflict of interest

The authors declare no conflict of interest.

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