A partial supplementation of pasteurized milk with vitamin C, iron and zinc

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After supplementation trials, vitamin C, iron and zinc levels were increased by 1789%, 59% and 30%, respectively. Partially supplemented pasteurized milk could be a new alternative with its high nutritive value, good sensory properties and low cost.

1 Introduction

In recent years nutrition-health surveys of unbalanced and deficient diet have increased [1–4]. It is known that along with genetic potential nutrient deficiencies cause many diseases and delay healthy growth. Nutrition is one of the most important external factors that limit the growth rate of individuals. In Turkey [5] according to weight and height percentile norms, a large percentage of children are found to be retarded in growth and development (54% of boys and 58% of girls were below the 25th percentile in weight, 62% of boys and 60% of girls were below the 25th percentile in height). In the same study it was pointed out that serum iron levels were found to be moderately low in 1.3% of children and seriously low in 0.5% of children; haemoglobin levels of 31.8% and 20.9% of children were moderately and seriously low respectively; hematocrit levels were marginally and seriously low in 41.9% and 12.4% of children; alkaline phosphataes levels in 1.9% of children were found to be high; serum cholesterol and zinc levels were found to fall within normal ranges. All of the results were found to be related to children's socio-economic conditions [5]. In the study of Wetherilt *et al.* [6], high percentages of subjects were at risk for deficiencies of zinc (72.3%) during late pregnancy and bone loss was indicated in 55.0% and 80.0% of the subjects in late pregnancy and *post partum*, respectively. Another study in Turkey reported vitamin C deficiency (10.3–68.1%) and anemia related with iron deficiencies [7]. In order to improve and maintain community health in the long term and to decrease the incidence of nutrient deficiencies supplementation of staple foods such as bread and milk are generally recommended [8, 9]. From this point of view, dairy products are good sources of some vitamins and minerals. It was concluded that calcium intake through milk and dairy products in childhood and adolescence is decisive for obtaining the maximum bone mineral density and content. This prevents risk of osteoporosis in the elderly [10]. However, milk is a poor source of iron and vitamin C. In long-term feeding, milk consumption related anemia could occur. It has been reported that this risk is insignificant with human milk [11]. However, according to the study of Fuchs *et al.* [12], infants fed on whole milk and iron-containing foods were at risk of developing depleted iron stores, not as a result of inadequate intake of iron or vitamin C, but probably because of the poor bioavailability of iron in infant cereal. Bioavailabilty of non-haem iron is low in foods from plant sources. However, adding vitamin C to the diets increases the bioavailability. Therefore, the purpose of this study is to improve a formulation for pasteurized milk by taking into consideration the deficiencies of vitamin C [13, 6], iron and zinc [5, 6] found in our country.

2 Materials and methods

2.1 Materials

Raw milk for supplementation was obtained from a dairy located in Gebze, Kocaeli. During transportation to TUBITAK MRC FSTRI's Laboratories, it was poured into brown glass bottles and protected in an ice box. In the laboratory it was stored at +4 °C in a refrigerator for about half an hour.

2.2 Method

Raw milk was collected from the same dairy at 4 different times in the same month and 4 replicates were prepared for supplemented pasteurised milk (Spm) and control samples. Moisture, ash, protein, fat, vitamin C, iron, zinc and sensory analyses were carried out. According to the Food and Nutrition Board 1989 [14], the average daily iron intakes of medium active men and women in the age group 19–50 years were 10 mg and 15 mg, respectively. In both groups, average daily zinc and vitamin C intakes were 10 mg and 60 mg, respectively. On the supplementation trials iron sulphate ($Fe_2(SO_4)_3$), zinc sulphate ($ZnSO_4$) and vitamin C in the L-(+)-ascorbic acid form were used. The whole procedure was carried out in a darkened laboratory. In the study, the nutrient deficiencies in Turkey were taken into consideration. Consequently, the additions of iron and zinc to raw milk's original ingredients were 59% and 30%, respectively, in Spm samples. To avoid weighing mistakes, stock solutions of the minerals were prepared. 100 mL of raw milk was fortified with 0.12 mg of zinc as zinc sulphate and 0.03 mg of iron as iron sulphate and mixed 5 min continuously. 30 mg/100 g vitamin C was added and mixed continuously for a further 15 min. The sample was pasteurised for 16 s at 75 °C by mixing gently in a water bath [15, 16]. Prior to the study, Spm samples were poured into dark glass bottles, which were capped, then sterilised for 15 min at 121 °C, cooled rapidly to +4 °C in an ice-filled container and

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stored at +4 °C in a refrigerator. In the control samples the same pasteurisation process and same storage condition were applied to a replicate raw milk sample (Fig. 1).

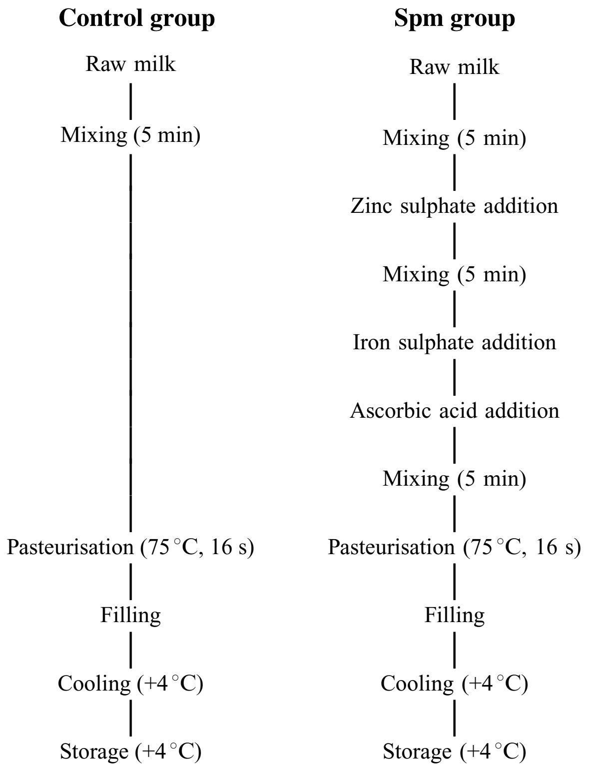

Figure 1. Process description of control and Spm groups production.

Table 1. Chemical analyses results of raw milk (100 mL)

Analyses (*n* = 4)	Raw milk	Range of literature values [22, 31, 32]
Moisture (g)	88.80	87.70–88.40
Ash (g)	0.55	0.81–0.67
Protein (g)	3.37	3.08–3.70
Fat (g)	3.20	3.50–3.62
Carbohydrate (g)	4.08	4.50–4.92
Energy (kcal)	58.00	63.00
Vitamin C (mg)	1.52	1.00–2.40
Iron (mg)	0.060	0.030–0.070
Zinc (mg)	0.360	0.210–0.550
pH	6.55	6.30–6.60

Table 2. Chemical analysis results of control and Spm groups (100 mL)

Analysis (*n* = 4)	Control group	Spm group	Difference[a)] (%)
Moisture (g)	87.95 ± 0.02	89.17 ± 0.05	1.39
Ash (g)	0.62 ± 0.18	0.67 ± 0.02	8.06
Protein (g)	3.41 ± 0.01	3.03 ± 0.02	(–) 11.14
Fat (g)	3.23 ± 0.06	3.06 ± 0.06	(–) 5.26
Carbohydrate (g)	4.79 ± 0.03	4.07 ± 0.09	(–) 15.03
Energy (kcal)	61 ± 0.26	56 ± 0.21	(–) 8.20
Vitamin C (mg)	1.44 ± 0.01	27.20 ± 0.03	1789
Iron (mg)	0.061 ± 0.01	0.097 ± 0.02	59
Zinc (mg)	0.362 ± 0.01	0.472 ± 0.01	30
pH	6.54 ± 0.01	6.54 ± 0.01	0

a) Difference values are mean of four determinations ± standard deviations.

2.3 Analysis

Solid dry matter, ash, protein, fat and vitamin C levels were determined with the methods of the AOAC [17]. Mineral analyses were carried out with an Atomic Absorption Spectrophotometer (Hitachi 180-50) according to the standard method of the AOAC [17]. Total carbohydrate and total energy levels were determined with the Atwater method [18]. Measurements of pH values were carried out with a glass electrode pH meter at room temperature. Sensory analyses were carried out with the Hedonic Scale Method with a panel of ten people [19]. One-way analysis of variance (ANOVA) and the tukey test were carried out with a statistical package program (SSPS Version 10.0) for $P < 0.05$ significance level.

3 Results and discussion

The formulation was arranged according to the results of chemical and sensory analyses which were carried out and after addition of minerals and vitamin C to raw milk. The results of the chemical analyses are given in Tables 1 and 2. After supplementation trials, sensory analyses were carried out on the control and Spm groups. For both groups "like" criteria were chosen. In the study of the chemical properties of raw milk; moisture, protein, vitamin C, iron and zinc levels were similar to literature data (Table 1), while the values for ash, fat, carbohydrate and energy levels were lower than the literature values. The results for raw milk, control and Spm groups show that the levels of protein, fat, carbohydrate and energy were highest in the control samples. As a result of pasteurisation, 0.95% moisture loss occurred in the control group. Several studies have indicated that heat treatment to foods decrease vitamin C concentration [20, 21]. On heat treatment a 5.26% reduction occurred in the concentration of vitamin C. As supported by literature data [22, 23], no change was observed in levels of minerals during heat treatment. Also no change occurred in the pH value of the raw milk group and control group (Table 2). In the Spm group protein, fat, carbohydrate and energy levels were decreased related to the statistical variation in the analysis and/or the added vitamin C's (a known reducing agent) small influence on the accuracy of the analysis techniques.

In the study of Ranhotra and co-workers [24], 38 ppm of iron sulphate was added to milk and stored at +4 °C in a refrigerator for 1 week. No change was reported in the sensory properties of the product. In another study, skim milk was supplemented with 5000 IU/vitamin A, 30 ppm of iron and aromatized. After 6 h at room temperature, the colour and aroma of the supplemented skim milk was acceptable in sensory evaluation. It was also reported that the iron level was stable during storage [25]. It is known that iron supplementation to prevent anemia affects the bioavailability of zinc negatively. To prevent a decrease in the bioavailability of zinc, the iron-to-zinc concentration ratio should not exceed 2 [26]. In our work this is assured. Lavigne and co-workers [16] carried out a study of milk supplemented with vitamin C and iron. When the vitamin C level exceeded 100 mg/100 mL, pH decreases but no signifi-

Table 3. Changes of pH and vitamin C levels of control and Spm groups during storage (5 days, +4 °C)

(n = 4)	Control group	Spm group
1st day		
pH[a)]	6.54 ± 0.01[a)]	6.37 ± 0.0.1[a)]
Vitamin C (mg/100 g)[b)]	1.43 ± 0.01[1]	27.20 ± 0.02[1]
2nd day		
pH	6.54 ±0.01[a]	6.37 ± 0.01[a]
Vitamin C (mg/100 g)	1.36 ± 0.01[2]	26.28 ± 0.01[2]
3rd day		
pH	6.54 ± 0.01[a]	6.37 ± 0.01[a]
Vitamin C (mg/100 g)	1.05 ± 0.02[3]	25.41 ± 0.02[3]
4th day		
pH	6.50 ± 0.01[b]	6.35 ± 0.01[b]
Vitamin C (mg/100 g)	0.52 ± 0.02[4]	24.97 ± 0.01[4]
5th day		
pH	6.48 ± 0.01[c]	6.35 ± 0.01[c]
Vitamin C (mg/100 g)	0.42 ± 0.01[5]	24.64 ± 0.02[5]

a) 5 days vitamin C levels were evaluated and significant differences ($P < 0.05$) of vitamin C levels between days were found and given as superscript letters (a–c).
b) 5 days pH levels were evaluated and significant differences ($P < 0.05$) of pH levels between days were found and given as superscript numbers (1–5).

cant change is observed in the microbiological quality and the free fatty acid levels. A further study of milk supplemented with ascorbic acid and iron showed that longer heat treatment and 7 days storage in the refrigerator caused 35–40% decrease in the levels of vitamin C. It was also reported that the storage period and conditions did not affect the fatty acid and protein composition of the supplemented milk. However, it was pointed out that the best way for the supplementation of foods is encapsulation of vitamins. At the beginning of our research, raw milk was supplemented with 50 mg/100 g ascorbic acid standard. However, this increased the acidity and decreased the sensory quality. Therefore, new trials were carried out at 45, 40, and 30 mg/100 g of vitamin C. The trials showed that 30 mg/100 g of vitamin C does not cause an increase in acidity and does not negatively affect the sensory quality. That high concentration of ascorbic acid decreases the pH value has been reported by others [27].

According to the ANOVA and the tukey test during 5 days storage at +4 °C in the refrigerator significant changes occurred between the 1st and the 5th days in the levels of vitamin C and pH of control and Spm samples as shown in Table 3 ($p < 0.005$). Our findings indicated that during 5 days storage 71% and 9.41% of vitamin C losses occurred in control and Spm samples, respectively. These losses could be related to temperature changes during 5 days storage in a frequently used home refrigerator and may be related to the oxygen dissolved in the milk which is influenced by process steps like mixing and filling bottles. Another study reports that fat content and pasteurisation conditions do not affect vitamin losses. In addition, during 7 days storage, 25–45% of vitamin C is lost. In various investigations it is reported that 74 °C pasteurisation temperature at 16 s causes only small losses of vitamin C [16, 28]. Therefore, these parameters were chosen in our study. According to Bosset *et al.* (1991) 2-stage homogenisation does not affect vitamin C concentration significantly, and in our study a homogenisation procedure was not applied [29]. Sensory quality is the most important preference factor for consumers. In our work the control group was compared with the Spm group. A small colour difference was determined related to the iron addition to the Spm group. However, both groups were similar and evaluated with "like" criteria. A panellist remarked that only in a comparative situation could this difference attract attention. Ingredients such as artificial colour and aroma were not added to the product. Taste parameter panellists pointed out that the control group had a slightly sweeter taste than the Spm group. This may arise from the added vitamin C to Spm samples. It was also emphasised by the panellists that this difference would be an unimportant factor for consumers. Our findings are also supported by Hicks *et al.* and Palani *et al.* [30, 9].

In conclusion, supplemented pasteurized milk could be preferred in the dairy industry owing to the ease of application and low cost. For consumers it could be a new alternative because of the high nutritive value which could ameliorate nutrient deficiencies in the Turkish population, as well as the good sensory quality which could be easily acceptable to Turkish taste.

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