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Recovery of Retinol in Soft-Drink Beverages Made with Fortified Unrefined and Refined Sugar: Implications for National Fortification Programs[1]

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Retinol (vitamin A) from fortified unrefined sugar was almost completely lost in the production of soft drinks, largely because of its adsorption onto activated carbon and diatomaceous earth that are used to reduce color and to eliminate odors and organic impurities in the purification process. Where refined sugar rather than unrefined sugar was used, the chemical purification step was avoided and 67% of the initial retinol remained in the final product. Nevertheless, even with refined sugar, retinol losses of up to 45% of the postproduction level occurred within 1 week of production although there were no further losses between 1 and 6 weeks postproduction. In other words, only 30% of the total retinol from fortified refined sugar remained in bottled soft drinks after 1 week after production. Retinol accounts for about 90% of the total after costs of a national sugar fortification program, which legislates that all sugar for domestic use be fortified. The use of unfortified sugar in the soft drink industry would result in considerable savings. Between 10 and 30% of national sugar consumption usually goes to this industry. For Guatemala and Honduras, the latter saving would amount to US$ 666,000 per year, which is 17% of the total program costs.

INTRODUCTION

Vitamin A is an essential micronutrient that is found naturally in animal foods as preformed retinol and in plant foods as vitamin A precursors, e.g., β-carotene. Both preformed retinol and provitamin A are fat soluble and sensitive to air, oxidizing agents, ultraviolet light, and low pH values (Aurand *et al.*, 1987; Harris, 1988). As with all kinetic reactions, preformed retinol and provitamin A losses are catalyzed by increasing temperature and the presence of mineral ions.

Retinol can be synthesized more easily and less expensively than β-carotene and is preferred in food fortification. Commercially available retinol ranges from oil-based formulations that can be added to oils and fats to dry-gelatin beadlets, which contain retinol as well as emulsifying and antioxidant agents that enable them to be added to dry or water miscible food (Borenstein, 1981). Among the water-miscible formulations, Hoffmann-La-Roche's and BASF's cold water-soluble retinol beadlets are used to fortify the sugar produced in Central America with vitamin A.

Although the stability of synthetic retinol in different fortified foods has been measured, the data are not always easy to interpret because of the diverse ambient conditions, time periods, and forms of retinol that were used in the different studies. Despite this limitation,

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TABLE 1

Sugar Use in Guatemala (1995) and Honduras (1995)

	GUATEMALA		HONDURAS	
USE	Amount (qq)[1]	%	Amount (qq)	%
Soft drinks	1,052,043	13.8	946,067	22.8
Bakeries	140,808	1.8	195,387	4.7
Confectionery	336,700	4.4	123,603	3.0
Other	652,714	8.6	266,223	6.4
SUB TOTAL INDUSTRY	2,182,265	28.6	1,531,280	36.9
Retail	5,437,864	71.4	2,621,136	63.1
TOTAL NATIONAL	7,620,129	100.0	4,152,416	100.0

[1] 1 qq = 100 lbs ≃ 45.4 kg.

oily foods such as margarine and butter have been shown to retain 85–98% of their initial retinol levels after 6 months of storage at room temperature (23–25°C or 73–77°F). Under similar conditions, dry foods including flours, dry milk, powdered beverages, and cereals have been found to retain at least 76% of their original retinol levels during the same period. Solid foods, in which the retinol was dissolved during preparation, e.g., cookies, retained at least 73% of the initial value after 5 days and liquid foods retained between 60 and 75% of the original level after 6 months (De Ritter, 1976; Bauernfeind, 1981; Bauernfeind and Lachance, 1991; O'Brien and Roberton, 1993).

In studies focusing on fortified sugar, Morales de Canahuí (1995) found the retention of retinol in vitamin A-fortified sugar (15 μg/g) after 6 months of storage varied from 62 to 72% depending on ambient conditions. Guamuch (1995) observed that cookies directly fortified with retinol retained 90% of the original level after baking and, after 15 and 45 (maximum shelf life) days of storage, retinol retention was 95 and 77%, respectively, of the postbaking level. The retinol levels in cookies after 15 days of storage were similar to those for white bread after 5 days of storage (Cort *et al.*, 1976; Parrish *et al.*, 1980; Borenstein, 1981).

In 1995, Guatemala and Honduras consumed over 7.6 and 4.1 million quintals of sugar, i.e., 346,000 and 188,000 MT, respectively (Table 1). The food industry used just under 30% of all sugar consumed in Guatemala and just over 35% in Honduras. Soft drinks accounted for a 14% of all sugar consumed in Guatemala and 23% in Honduras. The combined consumption of sugar for bakeries and confectionery was between 6 and 8%, while other food industries used between 6 and 9% of all sugar consumed.

Legislation in Guatemala, Honduras, and El Salvador (where data on the destination of sugar could not be obtained) requires that all sugar for human consumption, regardless of its type or final use, be fortified with retinol. In practice Honduras allows the production of unfortified sugar for food industry use. There is, however, an ongoing discussion in the food industry as to whether fortified sugar should be used

in the baking and the soft-drink industries, because these two industries account for most of the sugar destined for the food industry, e.g., 51% in Guatemala (Association of Sugars Producers of Guatemala (ASAZGUA), personal communication) and 74% in Honduras (Association of Sugar Producers of Honduras, personal communication) and any losses could have significant financial implications. Guamuch (1995) demonstrated that retinol losses were within an acceptable range for baked foods. The only available data for soft drinks are those reported by Bauernfeind (1981), in which 60% of the initial retinol level in a grape-based soft drink was retained after 6 months of storage. The type of production process in the latter study, however, was not specified and the retinol did not originate from fortified sugar.

To answer the question of whether retinol in fortified sugar remains after manufacturing soft drinks, two experiments were developed. The first tested whether retinol added to unrefined sugar, i.e., unrefined sugar that is chemically whitened, is destroyed in the manufacturing of a low acid drink (Rica Roja study). The second tested whether retinol added to refined sugar is destroyed in the same process in a similar type of beverage (Mirinda study).

MATERIALS AND METHODS

The color of refined sugar varies between 47 and 57 color units, whereas that for unrefined sugar is 90 color units or higher. In Central America, soft-drink manufacturers must meet the specifications set out by the parent company. The maximum permissible sugar color is 60 units. However, for highly colored products, such as Rica, Pepsi Cola, and Coca Cola, unrefined sugar can be used if the color and impurities are removed using activated carbon and diatomaceous earth. For classic soft-drinks, such as 7-Up and Mirinda, refined sugar is mandatory. Soft-drink manufacturers in Central America, particularly in Guatemala, prefer to use unrefined sugar in the manufacturing process because it is less expensive to treat and clarify unrefined sugar than it is to purchase refined sugar. In other countries, e.g., Honduras, Coca Cola has its own sugar mill that produces sugar of the required quality.

Soft-drink manufacturers in Guatemala and sugar producers in both Guatemala and Honduras were contacted and invited to participate in the study. Embotelladora del Pacífico, a subsidiary of PEPSICO in Guatemala, agreed to collaborate in the stability studies. The Rica Roja study was carried out in the Cuyotenango plant in the Pacific lowlands and the Mirinda study at the plant in Guatemala City. The Sugar Producer Associations of Guatemala and Honduras provided data on sugar use in their respective countries. A carefully prepared batch of fortified refined sugar was provided by ASAZGUA, Guatemala, for the Mirinda study and locally available sugar was used in the Rica Roja study.

Rica Roja was selected for the first study because it is made with unrefined sugar, which is subject to chemical treatment during production. Furthermore, its pH (4.51) is among the highest for a soft-drink; thus, retinol losses from exposure to an acid environment are reduced. Mirinda (pH 3.81) was used for the second experiment that involved refined sugar, because production levels were sufficiently small to perform the study at a minimum cost. Table 2 shows some characteristics of these two soft-drinks.

Retinol Assays

Fortified sugar. The retinol content of fortified sugar was determined spectrophotometrically after the vitamin A was extracted from the sugar solutions using hexane (Dary and Arroyave, 1996).

TABLE 2

Characteristics of Rica Roja and Mirinda

Parameter	Rica Roja	Mirinda
pH	4.51	3.81
Brix degree	11.0	13.1
Ingredients:	Sugar (11.3%)	Sugar (13.7 %)
	Carbon dioxide	Carbon dioxide
	Grenadine essence	Natural extracts
	Amaranthus (FD&C No. 5)	Yellow colorants (F&C No. 5 and 6)
	Citric acid	Citric acid
	Sodium benzoate	Ascorbic acid
		Sodium benzoate

Soft drinks. The retinol content of soft-drinks was determined using a spectrophotometric method, involving solid-phase extraction that was developed at the Institute of Nutrition for Central America and Panama (INCAP). This method was validated against the HPLC method for determining retinol in fortified sugar. Soft-drink samples were degassed and a 4-mL aliquot diluted with 1 mL of acetonitrile before the retinol was extracted. Retinol was separated from the soft-drink matrix by solid-phase extraction using a Varian 5500 LC equipped with a UV detector set at 325 nm and 0.002 UA/mV. The solution was injected into a C_{18} column (SP-18-5), 5 μm particle size and 150 × 4 mm ID, at a flow rate of 1.5 mL/min. The mobile phase was 100% methanol. Retinyl palmitate was quantified using an external standard. The quantification was based on the separation of retinol using a C_{18} Sep-Pak cartridge (Sep-Pak Vac 1cc, Waters), in which the retinol was adsorbed on to the cartridge's resin while the other compounds (colorants, sugar, and impurities) passed through. After the cartridge was washed with 2 mL of a mixture of isopropyl alcohol and water (40:60, v/v), retinol was released from the cartridge using 2 mL of absolute isopropyl alcohol. Absorbance was read, both before and after irradiation with ultraviolet light, at 325 nm using a Perkin Elmer UV/Vis Lambda 3B spectrophotometer. Retinol content was estimated using an extinction coefficient value of 0.094 μg^{-1} cm^{-1} mL.

The characteristics of the spectrophotometric method determined at INCAP were detection limit, 0.10 μg/mL; quantitation limit, 0.17 μg/mL; coefficient of variation, 7%; and recovery, 71%. Recovery was constant and included in the calculations. Where the results of the spectrophotometric method were lower than the quantitation limit, the extraction process described above was used, but retinol was released from the cartridge with 1 mL of isopropyl alcohol. The solution was injected in a Varian Vista 5500 Liquid Chromatographer equipped with an ultraviolet detector set at 325 nm, using a Nova-Pak C18 column of 3.9 ID ×75 mm, 4-μm particle size (Waters, Millipore) with 100% methanol as the mobile phase. The HPLC method had the following parameters: detection

limit, 0.03 μg/mL; quantitation limit, 0.08 μg/mL; coefficient of variation, 7%, and recovery, 71%.

Sampling

In the Rica Roja study, 30-g samples were taken from three different levels of the fifty-nine 50-kg sacks of fortified unrefined sugar used to prepare the batch of beverage. The three samples of each sack were pooled and thoroughly mixed before the retinol content was determined. In the Mirinda study, 30-g samples were taken from 10 randomly selected 50-kg sacks of fortified refined sugar. More intense sampling was unnecessary because this type of sugar was fortified in a closed mixer and, under this system, the retinol homogeneity in sugar is very good. In both studies, the retinol level in the sugar samples was analyzed just before syrup preparation.

The manufacturing process for soft-drinks comprises three main steps: (A) preparation of a simple syrup, in which specified amounts of sugar and water are placed in a jacketed tank at 80°C with low steam pressure for 30 min. Where *unrefined* sugar is used, activated carbon and diatomaceous earth are added to eliminate impurities, odor, and flavor and to lighten the color of the sugar. The simple syrup is then passed through a filter that eliminates both the activated carbon and the diatomaceous earth, and then it is filtered through a polish filter to a cold tank. Where *refined* sugar is used, the simple syrup is filtered through a press cellulose filter to eliminate any solid impurities from the sugar, and then it goes to a cold tank. Once the simple syrup is in the cold tank, the Brix degree is measured to confirm that the sugar concentration is correct. (B) The syrup is passed through a heat exchanger, where it is pasteurized before going into stainless-steel tanks. At this point, beverage-specific essences are added to the pasteurized simple syrups to form the final syrup. The final syrup remains in the tanks for 24 h to mature. (C) Carbon dioxide is added to the flavored syrup, and then water is added to meet the specifications for the final concentration of the soft-drink.

In the Rica Roja study, 350-mL clear bottles were removed at 2-min intervals from the production line immediately after filling until a total of 48 bottles was sampled. The retinol content of one-half of the sample bottles was determined within 24 h of production. The remaining bottles were refrigerated at 4°C and their retinol level was determined after 1 and 2 weeks of storage. In the Mirinda study, 2-L clear bottles were removed every 15 min immediately after being filled until 32 samples were obtained. The initial retinol content was determined in four samples and the remaining bottles were divided so that an equal number ($n = 7$) were assayed after 1, 2, 3, and 6 weeks of refrigerated storage at 4°C.

RESULTS

Retinol stability using fortified unrefined sugar (Rica Roja production). The mean and standard deviation retinol level in the unrefined sugar used to prepare a batch of Rica Roja was 9.0 ± 7.1 mg/kg. The large standard deviation was not unexpected and is mainly the result of the large variation in the sugar fortification procedures followed in Guatemala. Table 3 shows that all (99.6%) the retinol in fortified unrefined sugar was lost during the manufacturing process. To determine the extent to which this was due to the pH level, degassed solutions of Rica Roja bought in the local market were directly fortified using retinol palmitate beadlets at a concentration of 1 μg/mL retinol, i.e., 350 μg retinol per bottle, which was equivalent to adding about 10 μg/g fortified sugar during production. The retinol content of the enriched solution was tracked for 29 h, after which 62% of the

TABLE 3

Retinol Added and Retention in Rica Roja and Mirinda

	Rica Roja			Mirinda		
	Amount	Retinol content ($\bar{X}\pm$SD)	Total retinol (g)	Amount	Retinol content ($\bar{X}\pm$SD)	Total retinol (g)
Fortified sugar	2,951.4 kg	9.0±7.1 mg/kg	26.56	1,861.4 kg	9.3±1.3 mg/kg	17.31
Syrup	1,100 gal	--	--	720 gal	--	--
Prod'tion	67,128 (350ml bt) = 24,195 L	4.6±3.4 μg/L[1]	0.11	6,816 (2L bt) = 13,632 L	852±74 μg/L	11.6
Retained retinol	--	--	0.4%	--	--	67%

[1] HPLC method, described under Materials and Methods.

added retinol remained (Table 4). These data suggest that most of the retinol losses occurred during syrup preparation when retinol adheres to the activated carbon and diatomaceous earth; thus, the vitamin A remains bound to these two substances during filtration.

Retinol stability using fortified refined sugar (Mirinda production). The mean and standard deviation retinol in the refined sugar used to prepare the Mirinda were 9.3 ± 1.3 mg/kg. Table 3 shows that two-thirds of the original retinol in the fortified refined sugar was retained after manufacturing. Table 5, however, shows that 50% of retinol remaining after production was lost during the first week of storage, but there appeared to be little loss after that.

DISCUSSION AND CONCLUSIONS

The data presented in this paper show that retinol in fortified unrefined sugar is lost during soft-drink production. Most of the loss occurs when the syrup is prepared because the solubilized retinol adheres to the activated carbon and diatomaceous earth that are added as purifiers.

Soft drinks, such as Mirinda, that are made with fortified refined sugar, retained two-thirds of the initial retinol level after manufacturing. This finding supports the assertion that most of retinol loss in fortified unrefined sugar is due to its binding to active charcoal and diatomaceous earth. Where fortified refined sugar is used, retinol losses are the result of destruction in a low pH environment. This destruction continues after bottling, and after 1 week of bottling the retinol level remains stable, perhaps because of the anoxygenic environment in the CO_2-rich drink. These results support earlier claims (Bauernfeind, 1981) that retinol is stable in soft-drinks stored for up to 6 months, which were based on experiments that were unlikely to have involved chemical purification of the syrup.

TABLE 4

Stability of Retinol Directly Added to Rica Roja

Time	Retinol	Retained retinol
(hours)	($\bar{X}$±SD μg/bottle)	($\bar{X}$±SD %)
	n=3	
0	348±32	100±9
1	316±14	91±4
2	315±29	91±8
4	290±06	83±2
5	324±19	93±5
24	245±11	70±3
25	269±01	77±0.3
26	257±03	74±1
28	252±03	72±1
29	217±11	62±3

Note. Retinol palmitate was added to clear bottles as a solution of CWS-250 (cold water soluble), and the compound was used to fortify sugar. The beverage was capped and refrigerated at 4°C until the subsequent analysis.

In the experiments presented here, exposure of the retinol to a higher pH (4.51) in Rica Roja resulted in less loss than in the lower pH (3.81) Mirinda drink—38% versus 33%, respectively. These results were opposite to those expected and it is speculated that this was due to a lower exposure of Mirinda to experimental oxygen during pasteurization, because of the large volume and tightly closed apparatus. The experiment with Rica Roja was carried out in opened bottles.

Assuming that beverages such as Mirinda retain 0.40 μg/mL retinol throughout most of their storage, one bottle (350 mL) would provide 140 μg of retinol. This amount is 23% of the FAO/WHO (1988) recommended daily allowance (RDA) for an adult male and 28 and 35% of the RDA for women and children, respectively; thus, soft-drinks made with fortified refined sugar could be an important source of vitamin A for those consuming them.

While soft-drinks made with fortified refined sugar could be a safe way to deliver retinol there are important financial implications for the sugar industry that is mandated to fortify sugar. First, only 30% of soft-drinks are made with refined sugar and all the retinol in fortified unrefined sugar is lost. Second, only 30% of the retinol in the fortified refined sugar remains after the second week of storage. Retinol palmitate beadlets (7.5% retinol) cost US$ 37/kg and sugar is to be fortified at a level of 15 mg/kg. Given that about 2

TABLE 5

Retinol Stability in Mirinda during Storage

Week	Retinol	Retained retinol	n
	($\bar{X}\pm$SD μg/L)	($\bar{X}\pm$SD%)	
0	1705±148	100	4
1	827±121	49±7	7
2	660±20	39±2	7
3	759±85	45±5	7
6	829±70	49±4	7

Note. Sealed clear bottles were refrigerated at 4°C until analyzed.

million quintals (90,000 MT) of sugar are used per year by the soft-drink sector in Guatemala and Honduras (Table 1), this represents a potential loss of 1350 kg retinol (or 18,000 kg of fortificant), which is equivalent to about US$ 666,000/year.

Retinol in fortified unrefined sugar survives the baking process and the quantity of sugar used by this sector is relatively small, less than 5% in Guatemala and Honduras; thus, it would not be prudent to set up a system whereby the baking sector gets unfortified sugar, especially as there are many bakeries throughout the country and controlling leakage of unfortified sugar into the retail market would be difficult. The amount of sugar going to the confectionary sector is too small to influence the decision of whether that sector should use fortified or unfortified sugar.

Overall, in countries that mandate all sugar destined for human consumption to be fortified with vitamin A, it would be appropriate for the soft-drink sector to be exempt from having to use fortified sugar, given that much of it is lost during the production and storage of the beverages irrespective of whether refined or unrefined fortified sugar is used.

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