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Research Article

TARTRAZINE (E102) CONTENT IN WEANING INFANTS FLOURS "ANAGOBAKA POWDER" IMPORTED FROM WEST AFRICA COUNTRY FOR LOCAL MARKET IN ABIDJAN DISTRICT – CÔTE D'IVOIRE

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ABSTRACT

Our diet is increasingly composed of processed foods, which almost always include one or more additives. To evaluate the tartrazine rate in this weaning infants flours Anagobaka powder by the HPLCmethod. Eleven (11) boxes of "Anagobaka" powderwere selected on the local market as follows, 4 from Adjamé, 4 from Abobo and 3 from Treichville and onebottle of local weaning flour. Another bottle of weaning flour purchased from a shopping center was used as reference flour. The dyes are extracted by the wool tart method and the tartrazine contents were determined by the HPLC method. These results showed that the reference weaning flour and local flour are except for tartrazine. All samples of Anagobaka weaning flours analyzed have tartrazine contents ranging from 408,785 ± 48,638 to 2065,561 ± 237,837 mg.kg-1. These levels do not respect the recommended standard of 300 mg.kg-1. They are higher than the European estimate of the maximum theoretical consumption of tartrazine by a child who is based on a no-adverse-effect level of 390 mg.kg-1 body weight per day. These high levels of tartrazine in these "Anagobaka" weaning flours can induce allergenic, carcinogenic and mutagenic potential in infants and young children who consume them.

INTRODUCTION

Our diet is increasingly composed of processed foods, which almost always include one or more additives. Additives are substances added to food products to enhance their appearance, flavor, taste, color, texture, nutritional value and preservation. Currently, more than 300 additives are used in the European Union, classified into about 20 categories according to their technological effects on food (FAO/OMS, 1974). Additives have been shown to be safe at the proposed levels of feed use. Most additives are considered harmless today, others are rather doubtful or even dangerous according to studies reports. More and more books and health experts denounce the toxicity of a large number of food additives, which while authorized and very useful to industry, are often dangerous for health. Thus, synthetic food colors are part of the additives. These synthetic dyes are the most used for their physicochemical properties (Himri *et al*., 2011). For these reasons, the European Food Safety Authority ensures that the consumer does not exceed the acceptable daily intake (ADI) of additives including dyes authorized in the European Union (AESA, 2007). However, some synthetic dyes may have a toxic potential by consuming them unattended, resulting in a risk for the consumer to exceed their ADI (Sayed *et al.,* 2012). Of thesesynthetic colors, tartrazine is one of the most common. It is used in the food industry to conquer markets. It is a water-soluble azo dye that is used as an additive to color foods, drugs and cosmetics (Dinç *et al.,* 2005). The Scientific Committee for Food SCF confirmed the Acceptable Daily Intake (ADI) of 7.5 mg.kg-^{1} of body weight initially attributed to tartrazine by the Joint Expert Committee on Food Additives (FAO/WHO,2006), based on a no-adverse-effect level of 750 mg.kg^{-1} body weight per day.

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The European estimate of the maximum theoretical tartrazine consumption by children is lower than the ADI established for tartrazine (52% ADI), either 3.9 $mg.kg^{-1}$, based on a no adverse effect dose 390 $mg.kg^{-1}$of body weight per day(AFSSA, 2004).The maximum level in solid foods is 300 $mg.kg-^{1}$(De Reynal and Multon, 2009). Studies have shown that tartrazine is responsible for respiratory allergy problems by triggering certain adverse reactions such as urticaria, eczema and asthma (Inamata *et al.,* 2006). This substance is responsible for the exacerbation of atopic dermatitis and angioedema, as well as the gastrointestinal disorders described in individuals primarily sensitive to aspirin (Devlin et David,1992). It appears that tartrazine is involved in hyperactivity and behavioral disorders in children (Mccann *et al*., 2007) and decreased fertility in male swiss mice (Mehedi *et al*.,2009). It results in mutagenic and genotoxic actions involving cellular Deoxyribonucleic AcidDNA (Sasaki *et al*., 2002). The growing concern of industrialized countries about allergenic, carcinogenic, mutagenic and teratogenic contaminants in food products is shared by health authorities in developing countries.

In Côte d'Ivoire, as elsewhere in Africa, weaning flours are used as nutritional supplements for the feeding of children from six months of age, in the context of food diversification. The quality of the infant flours used during this period is therefore of great importance. Local or imported industrial infant flours are of better nutritional quality but remain inaccessible to the majority of households with low purchasing power. These mothers get their supplies from distributors, who are Nigerian traders, from cheap industrial flour. Mothers as well as these traders have no knowledge of the nutritional and sanitary quality of these products. The latter provide infant flours made in Nigeria from the Custard Powder brand in all districts of the District of Abidjan, hence the name "Anagobaka". Anago is the term commonly used in Côte d'Ivoire to designate people from Nigeria, while the cereal porridge is locally called "Baka". "Anagobaka" flours are fine-textured cream powders based on corn starch in which salt, flavors, colors, vitamins and minerals are added with or without the addition of egg yolk (Ihekoronye et Ngoddy, 1985; Okoye *et al*., 2007). Also the dyes used in these flours are synthetic dyes (Tartrazine, sun yellow) whose concentrations are not mentioned on the label. Moreover, data on the concentration of synthetic dyes in Anagobaka flours are not well known. The objective of this study is to evaluate the tartrazine content in these Anagobaka flours by the high-performance liquid chromatography (HPLC) method.

MATERIAL AND METHODS

Material

Biological Material

The biological material used consists of flours marketed on the markets of the communes of the District of Abidjan and coded as follows: 1st letter = name of the commune / 1st digit = N° of the sample and 2nd digit = the year of sampling.

Imported "Anagobaka" weaning flours, local artisanal flour and weaning flour used as a reference flour were purchased in a commercial center.

Technical Material

It consists of a Waters Alliance 2998 PDA detector HPLC chain equipped with:
A variable flow pump of 0.1 to 2 ml /min, an analytical column ODSC18 (150x4.6 mm id, 5 µm) Zorbax SB-AQ type specially adapted for very aqueous mobile phases, a RP guard column (C18, 5 µm), a UV-Vis detector with variable wavelengths, a 10 µl injection loop, an autosampler, a column oven and a chain control, acquisition and management system. data.A laminar flow hood (JOUAN, MSC12, No. 39609600), a 0.45 µm nylon filter (Acrodisk® type), a Hamilton® filter-type syringe, wool specks (05 for each sample).
Reagents

The reagents used consist of: acetic acid, ammonia solution, concentrated ammonia solution, ammonium acetate and methanol were purchased from Merck (Darmstadt, Germany), the standard solution of 0.1% tartrazine was purchased from Sigma-Aldrich (Wisconsin, WI, USA) and distilled water.

Criteria for the Selection of Municipalities

The communes were selected according to the way of life of the inhabitants, the density of the population and the accessibility of the markets

Criteria for Selecting Samples

The choice was focused on an industrially produced rice weaning flour. Local home-made weaning flour and manufactured, mislabelled, imported weaning flours known as "Anagobaka" and marketed in the markets of Abidjan District.

Table 1 Description and codification of the flours analyzed

N°	Trade name of the analyzedproducts	Codification of products	Type of products
01	Boleropineapple	ADJ/001/18	Flours of Anagobaka (Custard Powder)
02	Fincap	ADJ/003/18	
03	Jone Family	ADJ/004/18	
04	Tollex	ADJ/006/18	
05	Bolerostrawberry	ABO/001/18	
06	Egg Banana	ABO/003/18	
07	Queen Royale	ABO/005/18	
08	Prime	ABO/006/18	
09	Family Milk	TREICH/001/18	
10	Lady B	TREICH/002/18	
11	Glad Family	TREICH/003/18	
12	Corn flour	COCO/001/18	Artisanal flour
13	Cerelac of rice	COCO/002/18	Industrialflour

1ere lettre = (ADJ) nom de la commune
1er chiffre = (001) N° de l'échantillon et 2e chiffre (18) = l'année de prélèvement.

Methods

METHODOLOGY

Eleven (11) samples of "Anagobaka" weaning flask boxes were purchased from merchants in markets in the District of Abidjan as follows: 4 boxes purchased on Adjamé markets (ADJ / 001/18, ADJ / 003 / 18, ADJ / 004/18, ADJ / 006/18, 4 boxes purchased in Abobo markets (ABO / 001/18, ABO / 003/18, ABO / 005/18, ABO / 006/18), 4 boxes purchased at Treichville markets (TREICH / 001/18, TREICH / 002/18, TREICH / 003/18), 1 bag of 300g of COCO /001/18 local flour and a box of reference flour COCO / 002/ 18 were purchased at

a Cocody shopping mall and shipped to the Pasteur Institute of Côte d'Ivoire (Table 1).These flours were collected in sterile stool boxes using a sterile spoon around the bunsen burner flame in a sterile area and stored at room temperature to determine the concentration of tartrazine.

Extraction of Dyes in flour by the wool Mouchetmethod (Macek, 1972)

Preparation of the Wool

Strands of unbleached wool are boiled for 2 min in an ammonia solution. The wool is then thoroughly rinsed with water and then dried in an oven for 30 min.

Fixing Dyes on wool

One (1) g of each flour sample was poured into a vial and then supplemented with 100 ml of distilled water. The mixture obtained was homogenized by stirring at 5000 rpm for 3 min, then the supernatant was recovered in a beaker. An acidic medium favors the fixing of the dyes. To do this, a few drops of pure acetic acid are added to 25 ml of the supernatant obtained under the hood. Five strands of wool were introduced into the beaker and then heated for 5 min. The strands of wool are finally removed from the beaker and rinsed with water.

Recovery of Dyes

The extraction is done in basic medium. In fact, the dyes which are fixed on the wool must be disgorged by an ammonia solution. A very gentle boiling is done until the solution turns pink.After removing the wool, the volume of the solution is reduced to 10 ml by evaporation of water under the hood.This solution containing tartrazine is subject to a chromatographic analysis.

Dosage of Tartrazine by HPLC

Preparation of Standard Solutions

The standard solution was prepared by dissolving 1 g of tartrazine in a graduated flask with 100 mL of distilled water to obtain a concentration of 0.01 $g.ml^{-1}$. A calibration range of 0.05 to 5 $mg.ml^{-1}$ was freshly prepared by diluting the standard solution with distilled water.

Chromatographic Conditions

The separation was carried out by the liquid chromatograph equipped with a gradient pump capable of mixing several solvents, a vacuum membrane degasser, a 10 µl loop injector, and the UV detector. An ODS analytical column C-18 (150 x 4.6 mm i.d. mm, 5 µm). The mobile phase is composed of ammonium acetate (40 mM, pH 5) which is the solvent A and methanol (98%), the solvent B. The extracts obtained from the flour samples are injected into the column using a cellulose acetate membrane filter syringe with a pore diameter of 0.45 µm. The flow rate of this analysis is kept constant at 1 ml / min. In order to achieve a good resolution, an elution gradient program will be examined and is as follows: at time t = 0 at t = 2 min 90% of the solvent A and 10% of the solvent B; at time t = 5 at t = 8 min, 75% of the solvent A and 25% of the solvent B; at time t = 10 at t = 12 min, 25% of the solvent A and 75% of the solvent B; at time t = 13 at t = 15 min, 90% of the solvent A and 10% of the solvent B were obtained. The program of the optimized final elution gradient mode was 10% of the solvent B as initial measurement. The column was kept at room temperature and initially packaged for 5 min before each run. The UV detector was optimized under the following conditions: the fixed wavelength was fixed at 430 nm for tartrazine, the injection volume was 10 µL, the duration of the analysis was 15 min and the time of retention at which the peak chromatogram appears was 2.026 min (Kiseleva *et al.,* 2003). The programming system used Empower software. The standard solution of 0.1% tartrazine was prepared in distilled water and kept in a dark place before use. After each injection, an automatic rinsing system is made using purified water.

Validation

Linearity of the chromatographic response as a function of the standard concentration of 0.05 to 5 mg. ml^{-1}.

Serial dilutions of the tartrazine standard solution are subjected to chromatographic analysis.

The samples were subjected to chromatographic analysis to determine the percentage of recovery.

The determination of the detection limit (signal-to-noise ratio of 3) and quantification (signal-to-noise ratio of 10) was evaluated from serial dilutions of the tartrazine standard solution.

Statistical Analysis

For microbiological studies, the different results obtained are the average of three repetitions and are expressed in the form of averages ± standard deviations. The data were subjected to a one-factor analysis of variance (ANOVA) using GraphPad prism.7 software.The Dunnet test with a statistical significance threshold of $p < 0.05$ was used for the comparison of averages when the analysis of variance reveals significant differences.

RESULTS

Dosage of Tartrazine by Calibration

The chromatographic peak of tartrazine was identified at a retention time of about 2.026min (Fig 1). A range of linearity was defined over the concentration range of 0.05 to 5$mg.mL^{-1}$ of tartrazine with a coefficient of determination $R^2 = 0.999$. The line of the regression equation was established: Y = 4213 X - 83.21 where Y is the chromatographic response and X is the concentration in mg/g (Fig 2). The concentration of each sample is deduced by the transfer on this curve of the peak area of the unknown solution (Table 2). The recovery percentage of tartrazine was 83%. The limit of detection was 0.001 mg. mL^{-1} and the limit of quantification was 0.009 mg. mL^{-1}.

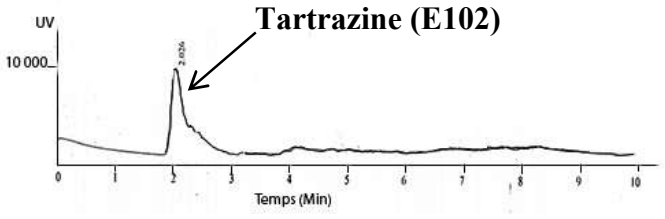

Fig 1 Thechromatographic peak of the tartrazine standardsolution at 0.5 mg.ml-1 (source: in HPLC, Waters Alliance 2998 PDA procedure)

Table 2 Concentration of tartrazine standards as a function of surfaces

Standard concentration (mg. g-1)	Surfaces
0,05	22 ± 1,16
0,5	2186,33 ± 2,33
2	8550 ± 28,87
3	12142 ± 6,09
5	21135 ± 5,78

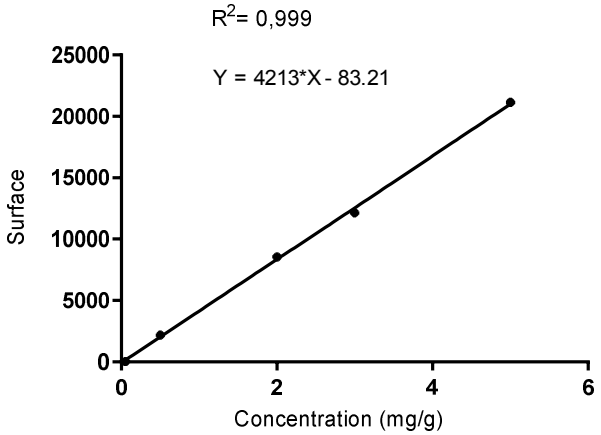

Fig 2 Straight calibration of standard concentrations according to surfaces

Tartrazine Content

The results of the tartrazine concentrations of the differents marks of infantile flours of weaning analyzed by the method of HPLC are indicated in table 3. The tartrazine content was not determined in the reference flour and local flour, indicating that there is no tartrazine in these two flours. In all "Anagobaka" flours, however, tartrazine levels ranged from 408.78 ± 48.64 to 2065.56 ± 237.84 mg.kg^{-1}. These levels do not meet the recommended standard of 390 mg.kg^{-1}. The highest tartrazine content was 2065.56 ± 237.84 mg.kg^{-1} found in *Queen Royale* brand flour, exceeding six times the recommended standard and lowest grade 408.78 ± 48.64 found in the *Bolero Strawberry* brand flour.

Table 3 Tartrazine content in the weaning flours analyzed

Flour infantiles	Peaks	Concentration of tartrazine (mg.kg^{-1})	P Value
Bolero pineapple	2382±152,67	585,14±47,18***	0,0001
Fincap	3634±198,00	882,32±70,38****	0,0001
Jone Family	2064±74,67	509,66±25,58**	0,005
Tollex	3335±206,00	811,35±71,85****	0,0001
Bolerostrawberry	1639±141,33	408,78±48,64^{ns}	0,3108
Egg Banana	2424±200,67	595,11±63,52****	0,0001
Queen Royale	8619±669,33	2065,56±237,84****	0,0001
Prime	2745±146,67	671,31±47,07****	0,0001
Family Milk	6261±333,33	1505,86±117,63****	0,0001
Lady B	4667±194,00	1127,51±61,30****	0,0001
Glad Family	4062±339,33	983,91±105,26****	0,0001
Corn flour	-	<LD	-
Rice Cerelac	-	<LD	-
Norm	-	300	-

****: The more the asterias increase, the more significant difference is important at the threshold of p <0.05
LD: Limit of Detection
NS: No Significant

DISCUSSION

Several methods are used for the extraction of synthetic dyes in food products. These are the liquid-liquid, solid-liquid and wool speckle methods. In this study, we proceeded to the use of the unbleached wool according to the protocol explained previously. Indeed, among all the other constituents of dissolved flours or syrup, only dyes have a chemical affinity for the molecules that make up wool. The wool tuple method for the extraction of the dyes made it possible to recover 83% of tartrazine, which was then assayed by the high-pressure liquid chromatography method. Several experimental and analytical methods are used for the identification of synthetic colors. Because of its specificity, sensitivity, speed and reliability the HPLC method can be used for the determination of synthetic colors in routine. The retention time of tartrazine determined in this work is 2.026 min. This retention time is similar to that reported by Amin *et al.* (2014) who used a C18 separation column, an isocratic mode of elution and a mobile phase different from ours, during the chromatographic analysis of the tartrazine content in artisanal yogurts from Côte d'Ivoire. These satisfactory results from the validation tests have shown that the chromatography method is well suited for the determination of tartrazine in "Anagobaka" weaning flours purchased in the markets of four communes of the Abidjan district. The application of the method to the control of tartrazine content to confirm its presence in these "Anagobaka" weaning flours above the no-adverse-effect level. Thus, a potential risk of allergic reactions and attention deficit hyperactivity disorder can occur in children who are most at risk (Stevens *et al*., 2010; Kamel et El-lethey, 2011).

Reference and local flours are free of tartrazine. There is a significant difference (P<0.05) between standard and "Anagobaka" flours analyzed exempt *Bolero Strawberry* brand flour. The tartrazine content of *Queen Royale* brand "Anagobaka" flour is four times that found in the "Anagobaka" flour brand of *Jone Family* and *Bolero Strawberry* and higher than that of other "Anagobaka" flour brand Abidjan District. The *Queen Royale, Family Milk, Lady B,Glad Family* and *Tollex* flours are still the ones with the highest levels of tartrazine, posing a threat to the health of the consumer. All of these "Anagobaka" weaning flours tested exceeded the recommended maximum level in foods (300 mg.kg-^{1}). These high levels of tartrazine obtained in the present study can be explained by the desire to make these infant flours more attractive (flavor, color, fragrance and taste) especially for infants and young children; the lack of knowledge about synthetic food dyes and in particular their use in food production and the lack of knowledge of synthetic colors by the general population and producers of "Anagobaka" flours in particular.

Several clinical trials have confirmed the fact of sensitivity to tartrazine in atopic or non-allergic people. Many adverse reactions to tartrazine are noted among which rashes are the most common. These may include eczema, urticaria, purpura, atopic dermatitis, especially in children, cutaneous pruritus and angioedema which are physiologically demonstrated by a high content of sulfidoleukotrienes that play an important role in the inflammation of the skin (Juhlin *et al.,* 1972;Krafcik *et al.,* 2003; Lee, 2004). Asthma is also linked to pulmonary

symptoms on the clinical picture of respiratory complications of tartrazine. Tartrazine stimulates the appetite of the food in which it is incorporated (Collins *et al*., 1992). Mice treated with 1% and 2.5% from tartrazine show aggression and vigorous agitation in addition to irritation of the skin. The weight gain results showed a significant increase in weight over time in the 1% treated group of rats followed by a reduction in body weight during the last week of testing (Borzelleca et Hallagan,1988). This suggests that the decrease in weight is due to the presence of tartrazine in the diet, which decreases caloric intake (Himri *et al*.,2011). Egnon *et al*. (2016) showed that "Anagobaka" fed rats had low values for urea and especially creatinine, which could justify renal dysfunction. Also, these rats had a low cholesterol production associated with triglyceride deficiency hence the induction of hyperactivity leading to deformation of the liver. According to the results of the study Aboel-Zahab *et al*., (1997), the administration of a mixture of synthetic dyes to rats for one month revealed a pigmentation of the portal vein and Kupffer cells in the liver and interstitial tissues as well as renal tubular cells. In studies by Sasaki *et al.*, (2002) azo food dyes such as tartrazine, Allaru red and coccin induce DNA damage in the liver and kidney at doses of 500 $mg.kg^{-1}$. Yahagi *et al*., (1975) found that azo dyes and their derivatives are mutagenic and carcinogenic and suggested that these effects may involve DNA modification. This non-compliance with the recommended standard, as well as the health hazards of these synthetic dyes, point to the need for a sanitary quality control, including tartrazine, of all Anagobaka flours and other flours imported and commercialized in Côte d'Ivoire.

CONCLUSION

This study has shown that the HPLC method used after dye extraction by the wool tart method is a reliable method for the determination of tartrazine content in weaning flours. Tartrazine levels determined in "Anagobaka" flours samples are above the recommended standard. These data show that there is an excess of tartrazine in these "Anagobaka" weaning flours. The consumption of these flours is potentially allergenic, carcinogenic and mutagenic in infants and young children who consume them. Measures must be taken to strictly control the use of synthetic dyes in the weaning flours marketed in Côte d'Ivoire and to specify the acceptable daily intake.

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