RESEARCH ARTICLE **(Open Access)**

Determination of added dye in orange fruit juices of *Citrus sinensis* cultivar with a simple analytical method

LUZIANA HOXHA, ONEIDA KYCYK, RENATA KONGOLI, ERANDA MANE *

Food Research Centre, Department of Biotechnology and Food, Agricultural University of Tirana, Tirana 1030, Albania

*Corresponding author e-mail: eranda.mane@ubt.edu.al

Abstract

Orange juice is a daily food randomly consumed and could be easily presented like an imitation of original products. Identification of its imitation has a big importance for juice authenticity. In some cases, it's used the colouring agent Tartrazine (E_{102}) a monoazo dye, permitted as food additive in EU, but hazardous for human health, due to allergic reactions and hyperactivity increasing especially of children in high levels. E_{102} consists essentially of trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-(4-sulfonato-phenylazo)-H-pyrazole-3-carboxylate and subsidiary colouring matters together with sodium chloride and/or sodium sulphate as the principal uncoloured components. The intention of this study was evaluation of a new simple method to determine the presence and the quantity of synthetic colour added in commercial imported fruit juice. By a spectrophotometer UV-VIS was measured absorbance in maximal wavelength, 426 nm, of five commercial orange juices samples, using as reference control sample an orange fresh juice sample. The concentration of tartrazine in some samples were found from 50-170mg/L, while the maximal permitted level of E_{102} concentration is 100 mg/L (Referred to EU standards, 2011). This study is an advanced step for a quick determination of tatrazine level; otherwise need to profound this argument in the future.

Key words: orange juice, tartrazine, imitation, spectrophotometer.

1. Introduction

Orange juice is very popular all over the world including Albania. Since orange juice is usually consumed, the necessity for this product is increased. Sometimes manufacturers use various ways for commercial orange juices imitation including the use of different additives such are flavourings, colours, vitamins and minerals. Saving the colour of commercial orange juice are used some permitted food colorant, such is tartrazine.

Since the visual aspect plays a determinant role for food selection by the consumers, tartrazine in most countries stand out as one of the widest used additive in food industry in order to enhance sensory response in concordance with the consumer's desire [12]**.** Tartrazine is used mainly for colouring several foods such as juices, jams, soft drinks, cereals, snack foods, chips, biscuits, ice creams, sweets, canned fish etc. Tartrazine content must be declared on label when used, and its content must not exceed the limit established by European Regulation (EEC, 2011), the maximum accepted level by this Regulation for E $_{102}$ in fruit juice is 100 ppm [16]. The Acceptance Daily Intake (ADI) for tartrazine is 7.5 mg/kg/day [15, 25].

Tartrazine (E_{102}, or Colour Index 19140, also known as FD&C Yellow no. 5) belong to the azoic dyes class and is in fact tri sodium salt of 5-hydroxy (1-p-sulphophenyl 4-(p-sulphophenylazo) pyrazol-3-carboxylicacid) (Figure 1). It is characterized by the presence of a chromophoric azo-group, which is determined as carcinogenic activity [5]. Also, it is very soluble in water and with a low solubility in organic solvents. Tartrazine offer a lemon yellow colour and is added in order to realize all diverse yellow-shades. Maximum absorbance was measured at 426 nm wavelength. It is very stable in all the pH range, at light, air and slightly high temperatures when exposed to.

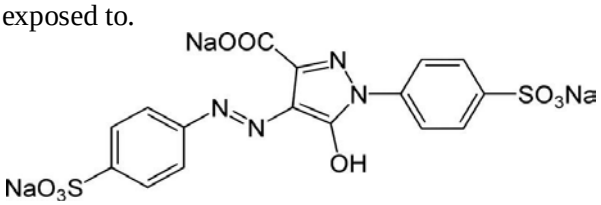

Figure 1: Structural formula of tartrazine

Tartrazine is not just a colour but is a complex product containing many different chemical compounds. In 1884 tartrazine was one of the first synthetic pigments that was patented [22]. Today, it is a common additive found in foods, beverages, medicines, vitamin supplements, cosmetics, toiletries and other non food products. Therefore, this synthetic colour has been the subject of numerous toxicological investigations [1, 11, 17, 21]. Generally, detailed toxicity studies on various food colours and additives

products are missing [29]. The metabolite of tartrazine can generate reactive oxygen species (ROS), which in turn, accelerate the oxidative stress [4]. Out of all the food colourings in current use, tartrazine has been the one of most implicated in causing adverse reactions [28], such as recurrent urticaria, angioedema, and asthma and is frequently implicated in behavioural problems [9]. The most common symptoms linked with tartrazine sensitivity are urticaria and asthma but symptoms are very individual and specific [13]. Recent studies show that tartrazine has significant adverse effects on neurobehavioral parameters [19, 24]. Apart from the metabolism of the dye, a 50mg dose of tartrazine led to increased or accelerated urinary excretion of zinc in hyperactive children. The effect of zinc in hyperactivity is not known [26]. Symptoms of yellow azo dye sensitivity can occur by either ingestion or continuous exposure. A variety of immunologic responses have been attributed to tartrazine ingestion, including anxiety, migraines, clinical depression, blurred vision, itching, general weakness, heat waves, feeling of suffocation, purple skin patches, and sleep disturbance [2]. Certain people exposed to Yellow Azo dyes experience symptoms of sensitivity even at extremely low doses, some for periods up to 72 hours after exposure [11]. In children, asthma attacks and hives have been claimed, as well as links to thyroid tumours, chromosomal damage and hyperactivity [14]. The prevalence of tartrazine sensitivity is not known, but it has been suggested to be 1 in 10 000 [3]. Nevertheless, the actual mechanism of the tartrazine hypersensitivity reaction remains unknown [19].

Therefore, the presence of this colour additive is necessary to be monitored in ongoing bases, in order to be not a danger for the consumers with intolerance or sensitivity. Accurate and precise determination of tartrazine in commercial products represents a special issue for food quality and safety [20]. The presence of tartrazine within certain limits or its absence in juices decisively determines the quality of this product. Its presence in high amount could harm seriously the human body. The use of tartrazine is at least controversial because it is only of esthetical role. Furthermore, it has been related to health problems mainly in children that are considered a very vulnerable group [7]. In some cases the use of tartrazine is also indicative of foodstuff imitation such as in their addition to fruit juices [18].

Several analytical methods have been achieved in order to determine tartrazine. In this study is applied a quick method using UV-Vis spectrophotometer. The intention of this study was evaluation of a new modified method to determine the presence and the quantity of synthetic colour added in commercial imported fruit juice.

2. Materials and methods

There were chosen five (5) commercial orange juices samples (Code No. CJ_1-CJ_5), randomly from different markets in Durres & Tirana, Albania. Three of them were imported fruit juices and two of them were extracted from the fresh orange juice (Code No. FJ) of Saranda, Albania. The control sample was squeezing and followed by filtration, in order to obtain clear juice. Tartrazine was provided by Neranxi Co., Albania.

Absorbance was recorded in UV-Vis spectrum using Spectrophotometer (BIOCHROM-LIBRA S22) equipped with 1.0 cm quartz cells; the absorbance of each diluted tartrazine was measured at 426 nm. Titrable acidity was measured based in ISO 750:1998 method. For pH determination of orange juices under study was used pH meter (Model: HANNA pH 211). A thermostated shaker (Vibramax 100 Hei- dolph), with a constant speed of 300 rpm at 20°C ± 1°C was used for better adsorption. For grade Brix determination was used the refractometer

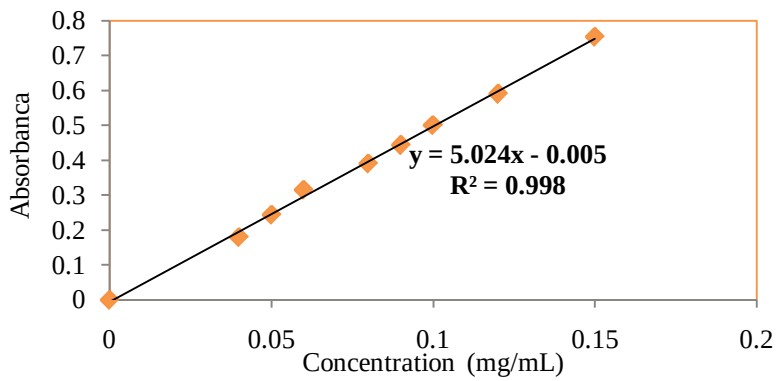

Figure 2: Standard calibration curve of tartrazine

The commercial and fresh orange juices samples before being analyzed were filtered on vacuum with microfiltration membrane (pore size of 0.45 μm). A stock solution of standard tartrazine (1mg/mL) was made by weighing 0.0100 g ± 0.0005 g of colour and diluting up to 10 mL, working standard solutions were prepared by diluting the stock in eight (8) different concentrations 0.04, 0.05, 0.06, 0.08, 0.09, 0.10, 0.12 and 0.15 mg/mL, in order to obtain the calibration standards. All experiments were done at room temperature. A calibration curve (Figure 2) was plotted using the correlation between concentration of working standard solutions (mg/mL) and absorbance (recorded at 426 nm)

After, preliminary vacuum filtered were done for all commercial and fresh orange juices samples, was recorded absorbance at 426 nm by spectrophotometer. All measurements were in three parallel samples. All calculation and chart plotting was made using medium values and standard deviation.

3. Results and discussion

Due to the presence of different natural organics acids, orange juices have generally acidic properties with a pH value around 3.5 [6] In this study the result obtained shown the pH of fresh orange juice was 3.74, and for the commercial orange juices were found to be in range 2.76-3.78.

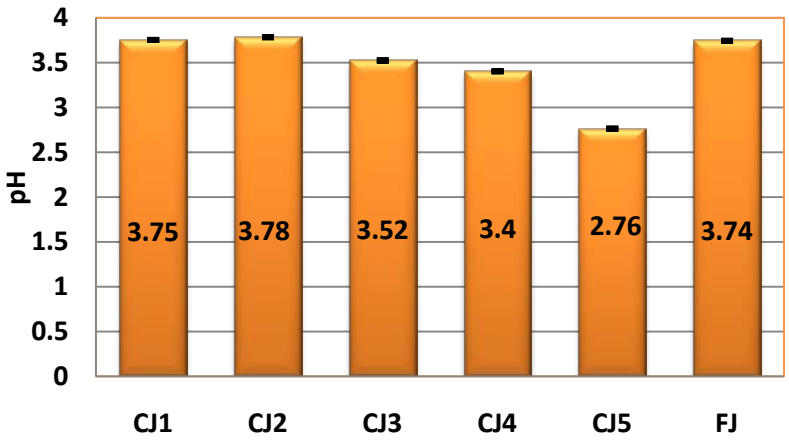

Figure 3: pH value measured by pH meter

In the Figure 3 are shown the values of pH for both commercial and fresh orange juices. Two of orange juices showed values that are near the pH of fresh orange juice respectively samples with code CJ_1 and CJ_2. On the other hand, three of them show value of pH below of fresh orange juice. The result pH values measured are similar from those by [23]. A lower pH of commercially made juice might be due to the presence of acidic additives which can cause acidity in the stomach and erosion of the tooth enamel [27].

Total citric acid determined as shown in Figure 4, which was expressed in g/L citric acid, ranged from 3.78 to 7.87g/L in commercial orange juices samples, while in fresh orange juice it was 7.07g/L. Based on the results of this work, two of the five samples must contein citric acid added in small amount respectivly in samples CJ_1 and CJ_2, while in three others samples the presence of total citric acid is lower. Total titrable acidity resulted in the same trend of pH values in samples analysed, it means that a lower citric acid values is connected with a lower pH values too. Instead this may indicate the presence of accidify additives [27].

Total soluble solid is expressed in Brix value. It is recognized that in different countries, the Brix level must differ from the Maximum Brix level between 11.8 and 11.2 [8]. Based on the results obtained for the fresh orange juice Brix value was 12.9, also indicating a high presence of sugars (pH >3.5), while for the samples CJ_1 and CJ_2 Brix values were 11.7 and 11.8, which are close to the maximum Brix level, also for three others commercial juices samples CJ_3-$CJ_5$the content of sugars resulted lower Brix value, respectively 9.6-10.45.

In figure 6 are shown the results of concentration of tartrazine in commercial orange juices (expressed in mg/mL), which were measured in three parallel samples. The concentration of tartrazine ranged 50-170 mg/L. According to the results all samples have

colour additive, and in reality no one of them was declared on label. In this case commercial orange juices must have an imitation in colour. According to national and international legislation declaring tartrazine on label is obligated.

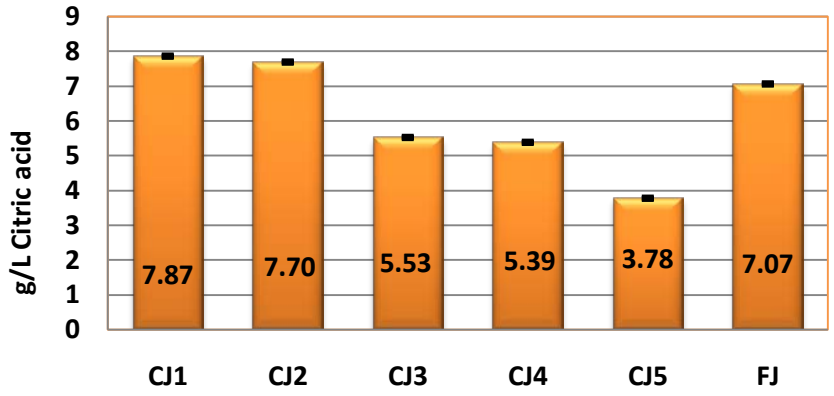

Figure 4: Total titrable acidity expressed in g/L citric acid

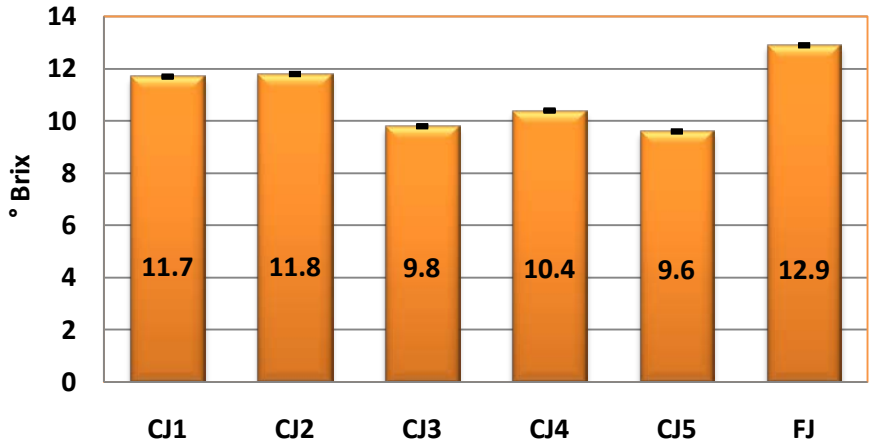

Figure 5: Total soluble solid measured by refractometer

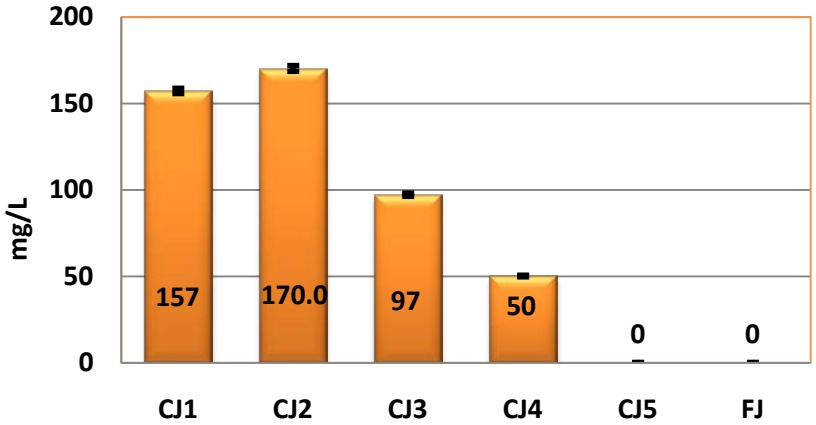

Figure 6: The concentration of tartrazine (mg/L) in orange juices

Referring to Figure 6 the presence of E_{102} in two of five commercial orange juices were higher level than Maximum Permitted Level (100 mg/mL) [16], respectively samples CJ_1 and CJ_2. Two other samples (CJ_3 and CJ_4) the level of tartrazine lied into the limits established [16]. While sample CJ_5 didn't contain tartrazine, which in fact was not declared on label, but there were declared other colour additives, that didn't show absorption in 426 nm wavelength.

4. Conclusion

Considering the results obtained in this work we can conclude that:

The content of tartazine in two of five commercial orange juices was higher than level established. The method used giving accurate results in a short time, using spectrophotometer. This is a new modified method, accurate, fast, and cheap and can be used in any laboratory of analytical chemistry, and has to be tested in the future for analyzing other fruit juices and other foodstuff.

5. Acknowledgements

The authors gratefully acknowledge to Food Research Centre, Faculty of Biotechnology and Food, Agricultural University of Tirana, Albania, for their financial support and their technical expertise.

6. References

1. Ali M A, Bashier S A: **Food Additives & Contaminants** 2006, **1**:1.
2. Amin KA, Abdel Hameid H, AbdElsttar AH: **Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats.** *Food ChemToxicol*2010, **48**(10): 2994.
3. Anon N: **Tartrazine: a yellow hazard**. *Drug Ther Bull* 1980; **15**: 53-55.
4. Bansal AK: **Modulation of N-nitrosodiethylamine induced oxidative stress by vitamin E in rat erythrocytes.** *Human Experimental Toxicology* 2005, **2**:297-302.
5. Borzelleca JF, Hallagan JB,. **Chronic toxicity/carcinogenicity studies of FD& C Yellow No.5 (Tartrazine) in rats**. *Food Chemistry Toxicology* 1988, **26**: 179-187.
6. British Soft Drinks Association. (*http://web.archive.org/web/20060826064558)*, *12 September, 2006*.
7. Clydesdale F M: **Color as a factor in food choice.** *Critical Reviews in Food Science and Nutrition* 1993, **33**(1): 83–101.
8. CODEX: **Codex general standard for fruit juices and nectars**. *Codex Stan* 2005, **247**: 1-19
9. Collins-Williams C: **Clinical spectrum of adverse reactions to tartrazine.** Journal of Asthma 1985, **22**(3):139-43.
10. Commission Regulation (EU) No 1129/2011, European Parliament *Official Journal of the European Union*, 2011 L 295.
11. Daniel J W: **Toxicology and Applied Pharmacology**, 1962, **4** (5): 572-594.
12. Diacu E, Ene C P: **Simultaneous determination of tartrazine and sunset yellow in soft drinks by liquid chromatography**. *Revista de Chimie* 2009, **60**(8): 745-749.
13. Dipalma JR: **Tartrazine sensitivity**. *American Family Physician* 1990, **42**(5):1347-50.
14. Donna M: **Food additives and hyperactive behavior in 3-year-old and 8/9-year-old children in the community: a randomized, double blinded, placebo-controlled trial**. The lancet 2007, **370** (9598): 1560-1567.
15. EFSA: **Scientific Opinion on the re-evaluation of Tartrazine (E_{102}) as a food additive**. *European Food Safety Authority Journal* 2009, **7**(11): 1331.
16. European Parliament and Council Directive 94/36/EC of 30 June 1994 on Maximum Permitted Levels of use of Tartrazine in beverages and foodstuffs according to the European Parliament and Council Directive 94/36/EC: C**olours for use in foodstuffs**. *Official Journal* 1994 **L 237**: 0013 – 0029.
17. Golka K, Kopps S, Myslak Z W: **Carcinogenicity of azo colorants: influence of solubility and bioavailability**. *Toxicology Letters* 2004, **151** (1): 203–210.
18. Kiseleva M G, Pimenova V V, Eller K I: **Optimization of conditions for the HPLC determination of synthetic dyes in food**. *Journal of Analytical Chemistry of the URSS* 2003, **58**: 685–690.
19. MacCara M E, Pharm D: **Tartrazine: a potentially hazardous dye in Canadian drugs.** *Canadian Medical Association journal* 1982, **126**(8): 910-914.
20. Mot A C, Soponar F, Casoni D, Cobzac C S, Sarbu C: **Simultaneous spectrophotometric determination of some food dyes from mixture using principal component regression.** *Revista de Chimie Bucurest* 2009, **60**(7): 647-652.
21. Puttemans M, Dryon L, Massart D: **Isolation, identification and determination of food dyes following ion-pair extraction. Quantitative aspects of the extraction method, qualitative and quantitative applications.** *Journal - Association of Official Analytical Chemist* 1982, **65:** 737-744.

22. Race Sh: **Tartrazine**. *First edition published* 2003, 4.

23. Sultana A, Haque M, Shoeb M, Islam M. S, Iqbal M, Mamun R, Nahar N: **Presence of yellow 6, an artificial colour additive in orange juice.** *Journal of Bangladesh Chemical Society* 2012, **25**(1): 80-86.

24. Tanaka T: **Reproductive and neurobehavioral toxicity study of tartrazine administered to mice in the diet**. *Food and Chemical Toxicology* 2006, **44** (2) 179-187.

25. Walton K, Walker R, Van DS, Jjm S, Castell JV: **The application of in vitro in the derivation of the acceptable daily intake of food additives.** *Food and Chemical Toxicology* 1999, **37**:1175-1197.

26. Ward N I: **Assessment of chemical factors in relation to child hyperactivity**. Journal of Nutritional and Environmental Medicine 1996, **7**: 333-342.

27. Wongkhantee S, Patanapiradej V, Maneenut C, Tantbirojn D: *Journal of Dentistry* 2005, 1-7.

28. Wood R M: **Analytical Methods for Food Additives**. *Boca Raton, CRC Press* 2004, **1**:10-11.

29. Food Reactions (2010). http://home.iprimus.com.au/foo7/additives.html