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Examining the Existence of Synthetic Dyes in the Nuts Offered in Marivan County, West of Iran

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KEYWORDS

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ABSTRACT: These days, making use of synthetic dyes in producing and processing food, such as nuts, is enhancing due to customer attention. Considering that the application of this type of dyes is not permitted in accordance with the current regulations of the country, controlling nut products in terms of dye is required and overriding. In this study, sampling was performed randomly in 10 nut distribution centers at one-week intervals. Samples were tested respecting the type of dye. A Thin-Layer Chromatography (TLC) method was applied to identify the types of dye. After conducting tests on different samples of nuts, it was recognized that different synthetic dyes such as tartrazine, quinoline yellow, and Ponceau 4R were used in the sample nuts. Of the 50 samples tested and analyzed, 23 samples (46%) had non-permitted synthetic dye, and 27 samples (54%) had permitted synthetic dye. The frequency distribution of synthetic dye among different nuts was significantly different. Furthermore, consumption of almond is associated with lower risk of permitted and non-permitted synthetic compared to pistachio ($p< 0.05$). Application of these types of dye, due to their glamorous appearance, will be significantly improved in the future. Therefore, with regard to the high consumption of synthetic dyes in food and their adverse effects on health, it seems that measures like increasing the level of awareness of producers and consumers about the effects of consumption or non-consumption of these compounds as well as continuous monitoring of units by health inspectors are necessary.

INTRODUCTION

Nowadays, synthetic dyes, as additives, are considered among the compounds used in food, voluntarily or involuntarily [1]. The dye of a food product makes the most important expectations about the taste of food in people. The dye of the product must be sustainable during its useful cycle, as the fading dye can make people not being interested [2]. Synthetic dyes are added to food products to make them appealing and compensate for the natural dye change of food products. The dye is conductive in understanding the quality of food and stimulating the desire

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to eat it [3]. Common dye additives are red 40, yellow 5, and yellow 6, constituting 90% of all used dye additives. Other cases consisted of blue 1, blue 2, red citrus 2, green 3, orange B, and red 3 [4]. The substantial reasons for using more of such synthetic dyes are associated with their remarkable stability and slight price relative to natural dyes [5, 6]. Although not all synthetic dyes are harmful, their excessive use in food or other products culminates in several problems. The maximum permissible amount of dyes in any food should be 0.1 g per km of that food [7]. Synthetic dyes in food products impose toxic and carcinogenic impacts on a human being [8-10], and others can bring about allergic reactions in people during consumption [11]. Excessive use of concentration of these synthetic dyes probably results in frequent headaches in adults and increased hyperactivity in children [5, 12]. The nut is one of the types of foods with synthetic dyes in its processing. Nuts include almonds, pistachios, hazelnuts, sunflower seeds, cashews, and so on [13]. These products may be offered either raw or roasted. Natural and often synthetic dyes are used in roasted products to enhance the quality of the appearance of nuts. The majority of synthetic dyes added to nuts may lead to severe skin allergies in people with a history of allergies [10, 13]. Hence, it is supposed to use those dyes permitted in this type of product. This study attempted to investigate the presence of synthetic dyes in nuts offered in Marivan city, west of Iran in 2019.

MATERIALS AND METHODS

This was a descriptive-cross-sectional study conducted in 2019. The desired samples were collected through a simple random sampling method from the private centers offering nut in Marivan city. Then, colorimetric experiments were conducted in the food laboratory of food and drug deputy of Qazvin University of Medical Sciences using TLC method, which was one of the methods of the 2634 Iranian National Standard [14]. Given the conducted researches in this area and the prevalence of 17.2% and based on the used sample size formula and the main variable as the primary target in terms of prevalence in the offered nuts and increasing the accuracy of the study, the sample size was determined to be 50 [15].

Sampling

Initially, 10 nut supply centers were randomly selected in Marivan city, and 5 types of nuts (pistachios, almonds, cashews, Japanese seeds, and pumpkin seeds) were then selected from each center (50 samples totally). This was done by the researcher and without the intervention of the seller of the dyed and roasted nuts domestically produced and sold in bulk. Samples were collected by random sampling method using the correct principles of food sampling at one-week intervals and sent to the laboratory for surveying color additives and the detection of frequency of synthetic dyes (permitted or non-permitted).

Sample preparation

Degreasing and purification

First, 15 g of each sample was transferred to the laboratory, weighed, and dissolved in 2 ml of 100% ammonia. After 24 hours, the upper layer of the solution in the dissolved sample was slowly removed and transferred to humans. The obtained sample was then placed in a steam bath until remaining about 20% of the sample. In the purification phase, 5 ml of the sample was dissolved in 100 ml of double-distilled water. To acidify this mixture, 1 ml of 37% hydrochloric acid was added to the sample. In the following, some white wool fibers were used to absorb the dye, so that the wool fibers were located within the sample container and the sample container was boiled to the boiling temperature in the steam bath. After 1 hour and ensuring complete absorption of the dye, the fibers were transferred to another container, and about 1 ml of 65% ammonia and 50 ml of double-distilled water were added to the container and boiled in a steam bath. Following 1 hour and ensuring the isolation of dye from the fibers to the alkaline environment, the sample was placed in an oven to be dried. All laboratory steps were carried out with respect to the instructions of the General Directorate of Laboratory Affairs with 740 Iranian National Standard [16].

Chromatography Stage

TLC method was used with the aim of identifying the type of dye. In the chromatography stage, a silica gel plate with dimensions of 20×20 was used. The solvent was a mixture of acetic acid, distilled water, and butanol. Identification of dye was determined based on the comparison of the dyes and movement of the sample dyes and the movement of standard dyes' stain on the plate assigned with observing the movement of the dyes in the solvent [16].

Statistical analysis

The R program 4.0.0 was used for data analysis. Chi square test was employed to compare the frequency distribution among different groups. Monte Carlo and Bayesian logistic regression was used for comparing the value of dye in the studied groups.

RESULTS

After completing the experiments on several samples of nuts, it was cleared that different synthetic dyes, including tartrazine, quinoline yellow, and Ponceau 4R were observed in the studied nuts. Out of the 50 samples tested and analyzed, 23 samples (46%) had non-permitted synthetic dye and 27 samples (54%) had permitted synthetic dye. Considering the rules and regulations of the Ministry of Health and the National Standard No. 740, using the permitted synthetic food dyes in nuts was not allowed. Hence, given the existence of synthetic dyes in pistachios, pumpkin seeds, cashews, and Japanese seeds, the above nuts did not observe the national standard and were not consumable. In most samples, only almonds were reported (except sample number 2) without synthetic dyes.

Using permitted and non-permitted synthetic food dyes offered in Marivan is exhibited in Figure 1. In this Figure, permissible food dye was equal to 70% of the samples in seven collected samples of pistachios in Marivan city. Nevertheless, in samples numbers 4, 6, and 9 (30%), there was a non-permitted synthetic dye of tartrazine.

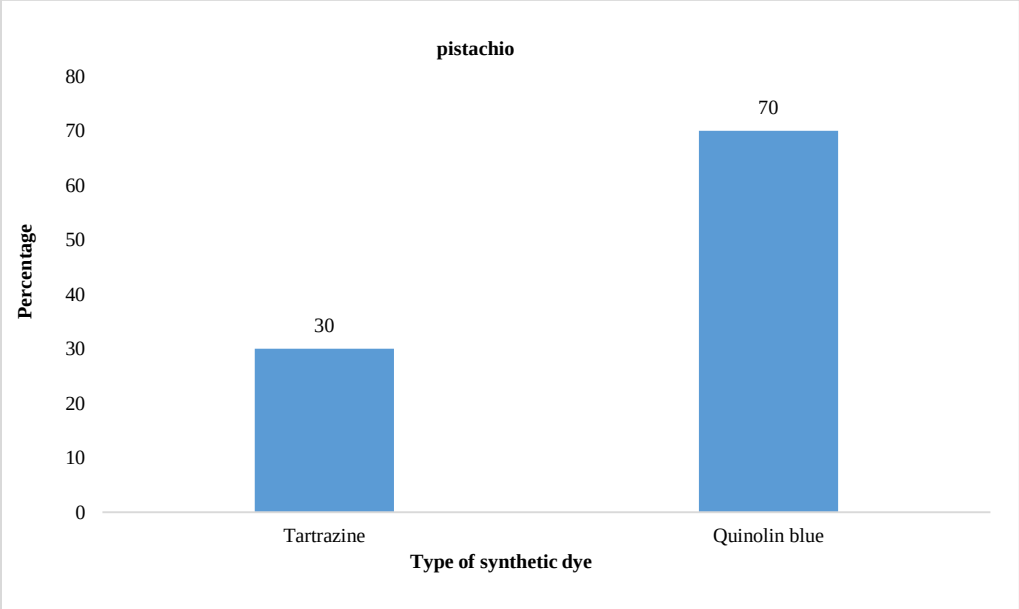

Figure 1. Frequency distribution of the use of permitted and non-permitted synthetic food dye in pistachios offered in Marivan city.

Figure 2 exhibited the frequency of the use of permitted and non-permitted synthetic food dye in almond trees offered in Marivan city. As observed, there was no unauthorized food dye in the ten samples of almonds collected in Marivan city. Permitted synthetic dye of quinoline yellow was seen only in sample number 2, and the other samples (90%) had no synthetic dye. Therefore, 10% of the almond trees samples had a permitted food dye, and there was unauthorized synthetic food dye in none of the samples.

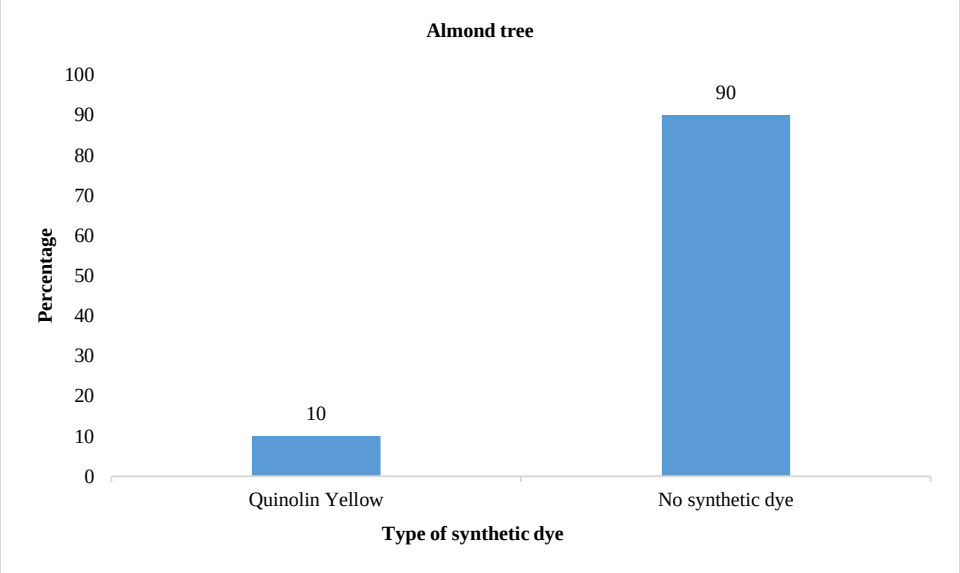

Figure 2. Frequency distribution of the use of permitted and non-permitted synthetic food dye in almond trees offered in Marivan city.

Figure 3 exhibits the frequency of the use of permitted and non-permitted synthetic food dye in cashews offered in Marivan city. It was clear that there was a non-permitted synthetic food dye called tartrazine in nine samples of cashews collected in Marivan city, and only one sample (sample number 7) was without synthetic dye. Therefore, 90% of the samples had the non-permitted synthetic dye of tartrazine, and no sample was observed with permitted synthetic dye.

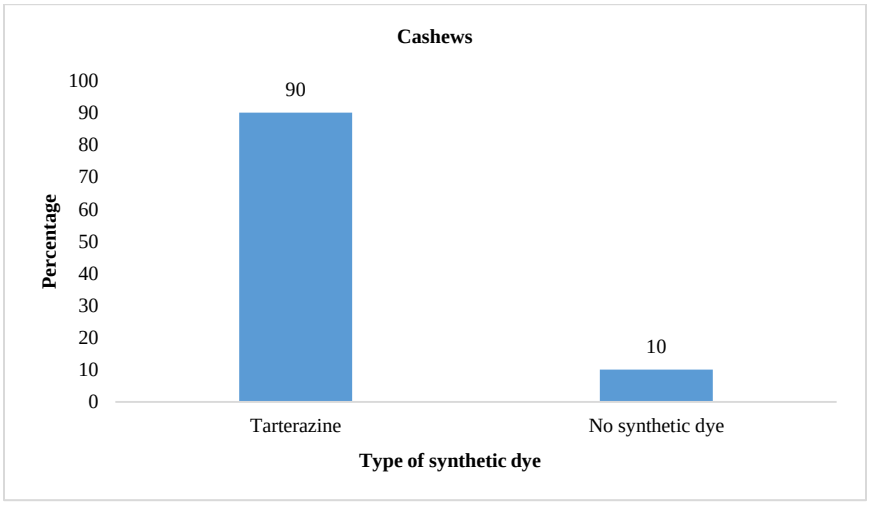

Figure 3. Frequency distribution of the use of permitted and non-permitted synthetic food dye in cashews offered in Marivan city

Figure 4 shows the frequency of the use of permitted and non-permitted synthetic food dye in Japanese seeds offered in Marivan city. The permitted synthetic food dye of Ponceau 4R was used in all samples of Japanese seeds collected in Marivan city. However, none of the samples contained any non-permitted synthetic dyes. Consequently, it was revealed that 100% of the samples had permitted food dye of Ponceau 4R.

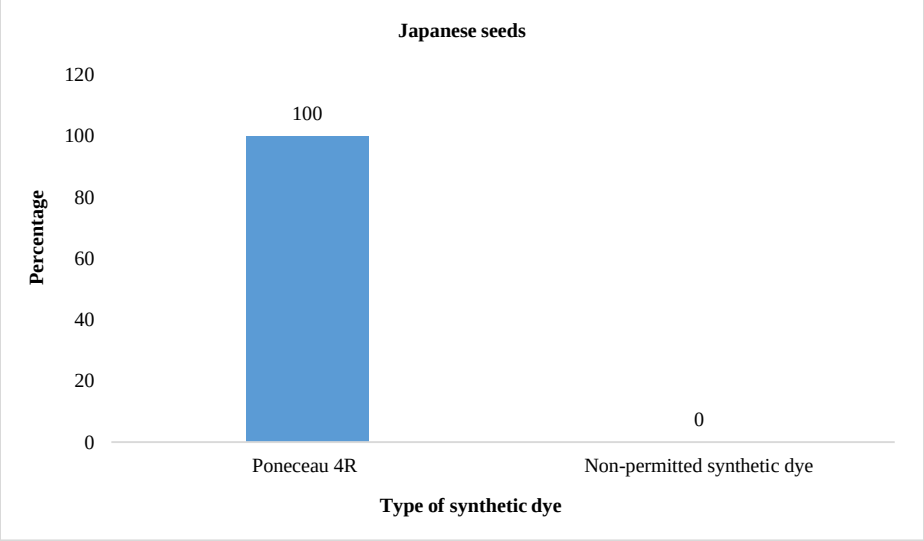

Figure 4. Frequency distribution of the use of permitted and non-permitted synthetic food dye in Japanese seeds offered in Marivan city

Figure 5 illustrates the frequency of the use of permitted and non-permitted synthetic food dye in pumpkin seeds offered in the city of Marivan. Clearly, the non-permitted synthetic dye was observed in all samples. Therefore, 100% of the samples had a non-permitted food dye of tartrazine.

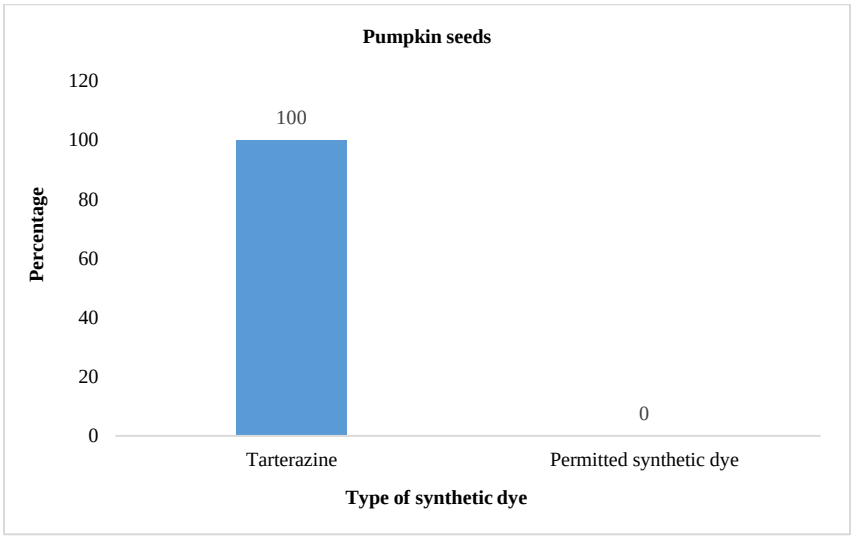

Figure 5. Frequency distribution of the use of permitted and non-permitted synthetic food dye in pumpkin seeds offered in Marivan city

The distribution frequency of dyes in different type of nuts is illustrated in Table 1 based on the number of samples. As it was obvious, the highest number of pistachios (70%) had permitted synthetic food dye. Moreover, almond samples

(90%), Japanese seeds (90%), cashews (100%), and pumpkin seeds (100%) were with no synthetic dye, with non-permitted synthetic dye, permitted synthetic food dye, and permitted inedible synthetic dye, respectively. Besides, based on the exhibited results in Table 1, the distribution of the type of dye used among different nuts was statistically significant (P <0.001).

Table1. Frequency distribution of synthetic dye among investigated nut samples

Nut Type	Dye type (%)			p-value
	No synthetic dye	Permitted synthetic food dye	Non-permitted synthetic food dye	
pistachios	0 (0%)	7 (70%)	3 (30%)	<0.001
almond	9 (90%)	1 (10%)	0 (0%)	
Japanese seeds	1 (10%)	0 (0%)	9 (90%)	
cashews	0 (0%)	1 (10%)	0 (0%)	
pumpkin seeds	0 (0%)	0 (0%)	1 (10%)	

Put differently, the consumption rate of permitted synthetic dye in almond nuts was significantly lower than pistachios (OR=0.009, P=0.002) (Table 2). Moreover, the consumption rate of non-permitted dyes in almond nuts was significantly lower than pistachios (OR = 0.002, P = 0.005) (Table 3). There was no significant difference between the other nuts and pistachios nut (as a reference level for regression analysis) in terms of consumption rate of the permitted and non-permitted dye.

Table 2. The frequency distribution of permitted synthetic dye usage in different nut samples compared to pistachio

	OR(95% CI)	P value
pistachios	1	-
almond	0.009 (0, 0.175)	0.0020
cashews	0.028(0.001,1.427)	0.0799
Japanese seeds	2.922(0.105,83.151)	0.5344

Table 3. The frequency distribution of non-permitted synthetic dye usage in different nut samples compared to pistachio

	OR(95% CI)	P value
pistachios	1	-
almond	0.002(0,0.137)	0.0051
cashews	0.581(0.031,10.661)	0.7316
Japanese seeds	2.685(0.085,85.12)	0.5603

DISCUSSION

Foods, among nuts, due to their high nutritional value, were remarkably considered in human nutrition. These products might be supplied in two forms of raw or roasted. Besides salt, colorant materials were often applied in roasted products to enhance the quality of their appearance. Since food dye was effective in attracting consumer attention, the use of synthetic dyes in nuts was raising. On the other hand, violators may color low quality and moldy nuts products to deceive the consumers. Problems caused by this type of low quality product could be referred to as fungal toxins such as aflatoxin, eventuating to liver disorders. Moreover, the existence of unhealthy dyes gave rise to severe skin allergies. The Ministry of Health, as well as the Institute of Standards and Industrial Research, prohibited

the use of any synthetic dye in the processing of any type of nut.

The results stemmed from this study displayed that pistachios, almonds, cashews, Japanese seed, and pumpkin seeds enjoyed 100%, 10%, 90%, 100%, and 100% synthetic dyes, respectively. Furthermore, 30% pistachios, 90% almonds, and 100% pumpkin seeds contained tartrazine. Based on the Iranian National Standard, tartrazine was counted as one of the non-permitted synthetic dyes.

In the study by Heshmati et al. [15], samples of cashews, pistachios, pumpkin seeds, Japanese seeds, and sunflower seeds had synthetic dyes considered to be 39.3%, 35.7%, 21.4%, 22.2%, and 7.2%, respectively. These values were less than the values of the present study. Ashuk et al. [17] declared that the most non-permitted synthetic dye in the aqueous saffron extract was attributed to tartrazine and they reported the synthetic dyes in more than half of the samples. Among the synthetic dyes, the dye of saffron had the greatest similarity to saffron itself. Due to its cheapness, stability, and likeliness to saffron, this dye was particularly popular among producers and suppliers. Tartrazine, more than other azo dyes, resulted in allergic reactions such as asthma and eczema.

Regarding the permitted dyes available in the studied nut samples, it was illustrated that the Ponceau 4R dye was prevalent in 100% of the Japanese seed samples. The quinoline blue dye was seen in 90% of the almond tree samples and 70% of the pistachio samples. In the study by Rezaei et al. [18], the amount of tartrazine and Ponceau 4R dye in the cookie, ice cream, and saffron samples were equal to 57.1% and 8.57%, respectively. Moreover, Sultan Dalal et al. [19] recognized that 89% of the samples of traditional juices in Tehran had synthetic dyes. Meanwhile, 46% of the samples in the present study used synthetic dyes, which were less than the other studies. It was worth noting that, based on the instructions of the Ministry of Health and the Iranian National Standards Institute, the use of any type of synthetic dye in the processing of nuts was not allowed. Thus, these units were not required to possess the necessary production license and health code for using synthetic dyes [20].

CONCLUSIONS

Furthermore, although the cost of saffron dye was much higher and not economically frugal for the seller, the production units were permitted to use these kinds of natural-based dyes. Dyes could bring along various side effects such as urticaria, carcinogenicity, asthma, high blood pressure allergies, weakening of the immune systems, and vitamin B6 deficiency. Moreover, induction of hyperactivity in children, urticaria, and rashes was due to the use of the synthetic dyes, especially tartrazine [21]. Although the number of studied samples in this paper was not considerable, the high usage of synthetic dyes in some nut products showed that these products were supposed to be more monitored in their production and processing lines. Continuous monitoring of supply-level products, providing food safety training for manufacturers, and treating seriously with the violating units, the use of synthetic dyes was necessary to be lessened. The researcher intended to mention that the group of children was exposed to the threat of using these products. As a result, through establishing codified policies, health care trustees should have the potential to control their rate of use.

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Conflict of Interest

The authors declare no conflict of interest.

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