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Food awareness of college students consumers towards unhealthy snacks: A case study of food colorant at "Bundaran ITS" landmark of Institut Teknologi Sepuluh Nopember (ITS) Surabaya during Ramadhan festival

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Abstract. The high number of registered college students of Institut Teknologi Sepuluh Nopember (ITS) in 2018/2019 academic year as well as their neighbour polytechnics, i.e. Politeknik Elektro Negeri Surabaya (PENS) and Politeknik Perkapalan Negeri Surabaya (PPNS), makes them as potential consumers in food industry. Most of them purchase food or beverages from the inside-campus food service, canteens. However, during the Ramadhan festival, most of the canteens are closed. Therefore, many seasonal food stalls are open for business at "Bundaran ITS" starting at 15.00 until 20.00 WIB (West Indonesian Time Area), providing food or beverages for breaking the fast. Therefore, a survey was conducted to check the healthiness of the food, meaning the food colorant additive especially red colorants. Moreover, this study also examined relationships among healthy food awareness, behavioural intention toward healthy foods, and actual behaviour of the ITS consumers. Thirty-seven (37) samples were collected during two periods of survey, at 9-12 May 2019 and 28-29 May 2019. The collected samples focused on the samples that might have red colorants. The collected samples were classified into three categories: beverages (32.43%), ketchups (54.05%), and snacks (13.51%). Gratefully, none of them contained Rhodamine B.

1. Introduction

Indonesia Government through the Minister of Health Regulation Issued a regulation (Permenkes) No.1168/MenKes/PER/X/1999 about illegal food additives; including Rhodamine B, methanyl yellow, dulsin (synthetic sweater), and potassium bromate (hardener). However, due to the high availability and accessibility of commercially food additives in high grade with relatively affordable prices, the application of the food additives will increase. Both the consumers and food industries should be aware of the faked food additives, or the addition/substitution of illegal compounds in the legal food additives. Therefore, public awareness to the importance of unhealthy foods should be developed.

Rhodamine B (red colorant), methanyl yellow (yellow colorant), formalin or formaldehyde and natrium tetraborate or boraks are the illegal food additives that are commonly found in Indonesia. Although they are illegal, due to their color stability and long-lasting power as cheaper preservative, they are often found in the food. Recently, it was reported that Rhodamine B was positively identified in some snacks in the traditional market i.e. Genteng, Soponyono, Wonokitri at South Surabaya area [1]. Rhodamine B, formalin, borax, and methanyl yellow were also found in seaweed, dried anchovies, dried squid, wader fish, sausages, and milk candy, in other areas of Surabaya [2]. Fortunately, in the report of the children's snacks sold in elementary schools in Sukolilo area, Surabaya (survey period of March-April 2018) [3], no Rhodamine B was identified in tomato ketchup, chili sauce, sausage, burger meat, and corned beef. Rhodamine B (CI No. 45170 Food Red 15) is a restricted red colorant in Indonesia according to Permenkes RI No. 239/MenKes/Per/V/1985 as well as Ponceau 3R (Acid Red, CI No. 16155), Ponceau SX (CI. 14700 Food Red 1), and Ponceau 6R (CI No. 16290 Food Red 8). However, three samples of tomato ketchup contained amaranth. Though this red colorant is not banned in Indonesia, its toxicity to the human health is recorded in the US and is officially denoted as an illegal dye [4].

The huge number of students at 2018/2019 academic year in Institut Teknologi Sepuluh Nopember (ITS) in Surabaya, ca. 18,698, as well as the two polytechnics i.e. Politeknik Elektro Negeri Surabaya (PENS) and Politeknik Perkapalan Negeri Surabaya (PPNS), ca. 3,855 and 2,479 students respectively for PENS and PPNS. It was necessary for ITS to care for the health of their students and staffs. Hence, paying attention to their daily snacks and food could be considered as caring for their health. Therefore, surveys were conducted by our team analysing the healthiness of the food, especially red colorants to the food sold at "Bundaran ITS" during Ramadhan festival 2019 (in May 2019). Picturing the behaviour intention of the ITS students and staff toward healthy food, a survey using Google forms was also conducted in parallel.

"Bundaran ITS" is a landmark spot of ITS. Annually, in every Ramadhan festival, this spot is full of many seasonal food stalls, approximately 100 food stalls, starting at 15.00 until 20.00 WIB (West Indonesian Time Area), offering food or beverages for breaking the fast [5]. Therefore, there was an obligation for ITS to do the survey, maintaining the health of their students and staff trough monitoring the healthiness of the food sold around the area.

2. Materials and methodology

2.1. Materials

Samples such as beverages, ketchups, and snacks were bought from the "Bundaran ITS" during the Ramadan festival (in May 2019). The samples that were collected were the ones that might contain red colorant which identified by its appearances. Standard compounds of Rhodamine B, Amaranth, and Red Allure were commercially purchased from Sigma-Aldrich (India) in pro analyst grade whereas both Erythrosine and Ponceau 4R in pharmaceutical grade were from Morton Chemical (Netherlands). Silica gel 60 F 254 20×20 cm in size (Merck, Darmstadt, Germany) was used for qualitative analysis of dyes. In case of solvents such as ethanol (EtOH), ammonia (NH3) (25%, v/v), and isopropanol (iPrOH) were purchased from Merck (Darmstadt, Germany) in pro analyst grade. A spectrophotometer T60 (PG Instrument, Leicestershire, United Kingdom) was used to quantify the red dyes.

2.2. Method of sampling

The samples collected at "Bundaran ITS" were suspected as containing red colorant which were identified by the appearances. They were classified into three groups, namely beverages (B), ketchups (K), and snacks (S). The sampling periods were conducted twice: 9-12 May and May 28 2019. At each period of sampling, same samples were bought and collected.

2.3. Extraction of red colorants from the sample matrix

Three grams (3 g) of each sample was crushed, homogenized with 5 mL 70% EtOH (v / v) or 70% isopropanol, swirled in 10 minutes until all the red color came out. Furthermore, centrifugation (3000 rpm, 10 minutes) and the clear solution obtained was then separated by filtration [6]. The red colorant was then qualitative identified by applying the filtration to Thin Layer Chromatography (TLC) (**subsection 2.4**) and was further quantified by a Spectrophotometer (**subsection 2.5**).

2.4. Qualitative analysis of red colorants by Thin Layer Chromatography

Thin Layer Chromatography (TLC) was conducted for red colorants separation and identification. TLC plates (10 cm ×10 cm) and Silica gel 60 F 254 were prepared and marked 1.5 cm and 0.5 cm respectively from the base of the plate to the top of the plate. A hundred microliter (100 µL) extract of each sample solution (**subsection 2.3**) was applied to the TLC plate in duplicates as well as the standards solution. In case of mobile phase of TLC, two combinations of mobile phase composition were observed, finding the optimum separation of five different kinds of red dyes, i.e. Rhodamine B, Amaranth, Ponceau 4R, Erythrosine, and Red Allure. They were EtOH: Ammonia (9:1, v/v/) and isopropanol: Ammonia (4:1, v/v).

2.5. Quantitative analysis of red colorants by Spectrophotometer UV-Vis

A calibration curve was previously conducted for quantifying the red colorant. Calibration curves were made by serial dilutions of a standard stock solution of red dye. Standard stock solutions were made with an accurate weight of 3 g and diluted to 5 mL EtOH 70% (v/v). Calibration was carried out using 11 concentrations (7.0, 6.0, 5.0, 4.0, 3.5, 3.0, 2.5, 2.0, 1.5, 1.0, and 0.50 ppm) at maximum wavelength of each red colorants. The quantification of red colorants was only conducted to the identified red colour by TLC (**subsection 2.4**).

2.6. Questionnaires form for identifying the healthy food awareness of consumer

Healthy food awareness measured by surveying the behaviour intention and the actual behaviour of the consumer, i.e. students and staff of ITS. The survey was conducted using Google forms consisting of three parts; representing the demographic respondent, the behaviour intention, and the actual behaviour of the respondent/consumer. Eleven questions were elaborated to mimic the behaviour intention while for the actual behaviour of respondents to the healthy food were captured using six questions. The behaviour intention questions were marked as scores. The scores value were as follows: 1 = extremely disagree, 2 = disagree, 3 = neutral, 4 = agree, 5 = extremely agree.

3. Results and discussion

Having an accurate analysis of the red colorant, a combination between separation-qualitative and a quantitative analysis of the red colorant need to be applied. First, thin layer chromatography was (TLC) applied; separating as well as differentiating each of red colorants, i.e. Rhodamine B, Amaranth, Ponceau 4R, Erythrosine, and Red Allure. After the red colorants were qualitatively identified, Spectrophotometer UV-Vis quantified them. The Spectrophotometer could not solely quantify specifically the red colour because some of the red colorants had similar range of absorbed colour; giving a false positive quantification. Complemented colour of red that absorbed colour of blueish green had the maximum wavelength of 490-500 nm. While red colour of wine that absorbed colour of green had maximum wavelength of 500-560 nm (**Figure 1**).

Developing the sample in the mobile phase, isopropanol: Ammonia (4:1, v/v) performed better separation of the red colorants compared to EtOH: Ammonia (9:1, v/v/) (**Figure 2**). Isopropanol: NH3 (4:1, v/v) performed the best separation to all five of red colorants. On the other hand, both amaranth and red allure had overlapped spot when the TLC plate was developed with EtOH: Ammonia (9:1, v/v/) (**Figure 2**). Hence, Isopropanol: NH3 (4:1, v/v) was chosen as the most appropriate mobile phase to clearly separate and identify the Rhodamine B, Amaranth, Ponceau 4R, Erythrosine, and Red Allure.

Figure 1. Variance of red colorants of Rhodamine B (A), Amaranth (B), Ponceau 4R (C), Erythrosine (D), and Red Allure (E).

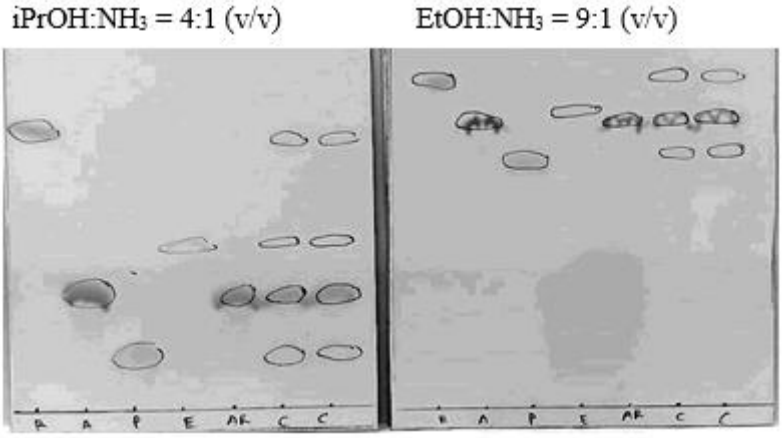

Figure 2. Mobile phase variation of thin layer chromatography (TLC).
Note: Spot of Rhodamine B (R), Amaranth (A), Ponceau 4R (P), Erythrosine (E), and Red Allure (AR), each spot was 100 ppm. While C spot is mixed standard of all the red colours standard.

3.1. Online survey results of the healthy food awareness of consumer

One hundred and twenty-six respondents filled the questioner. Mostly, ca. 68.30%, were either students or staff/employee of ITS. In average, they were 20-30 years old. Most of them, ca. 86.50%, were aware and knew about the usage of food additives. They could also mention correctly the two kinds of food additives in minimum. Sweeteners and preservatives were the most popular food additives mentioned by the respondents, followed by flavouring/flavour enhancer, colouring, and emulsifier.

The score scales (**subsection 2.6**) were applied to measure the behaviour intention of healthy food respondent. Respondent, ca. 84.90%, was extremely concerned about the halal food, followed by the cleanness of the food (76.20%), highly nutritious food (48.40%), affordable food price (42.90%), tasty food (42.90%), and appetite-able food (41.30%).

Moreover, 92.9% (117 respondents) already knew that food additives could alter the properties of the food and beverages (including the flavour, the storable lifetime, shape etc.). They realized (93.7%, 118 respondents) that there was a maximum dosage for food additives addition. They also realized and were aware (97.6%, 123 respondents) of the effects to the human health when an excessive usage of food additives were applied. Hence, the respondents were already aware about the healthy food. It means they have a behaviour intention to the healthy food. Unfortunately, their awareness did not directly reflect to their actual behaviour to the healthy food.

Around 50% of respondents, 59 respondents from the 126 were used to buying food and beverages at “Bundaran ITS” during Ramadan festival. Meanwhile, 30 respondents frequently bought food and beverages at “Bundaran ITS” ca. 3-4x during Ramadan. Deep-fried food with sauce (81.40%) such *siomay*/steamed seasoning flour with fish or chicken floss, *cireng*/fried-seasoning flour, *otak-otak*

fish/fried-seasoning flour with fish extract; colourful beverages (62.70%) such *dawet* were the favourite type of food that respondent bought. Therefore, ITS need to monitor the quality of the food which are sold around the area of ITS; maintaining the health of their student and staff.

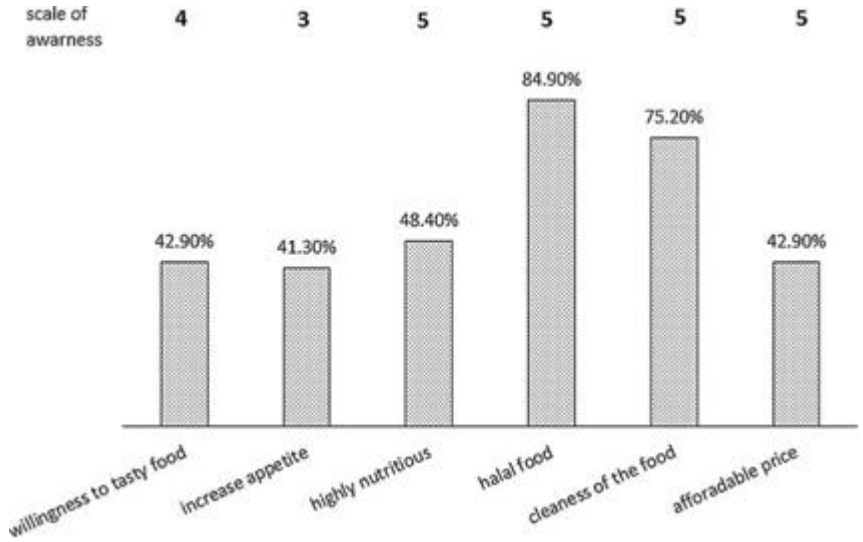

Figure 3. The scale of awareness of respondent to their daily food consumptions. The scores value of awareness are as follows: 1 = extremely disagree, 2 = disagree, 3 = neutral, 4 = agree, 5 = extremely agree.

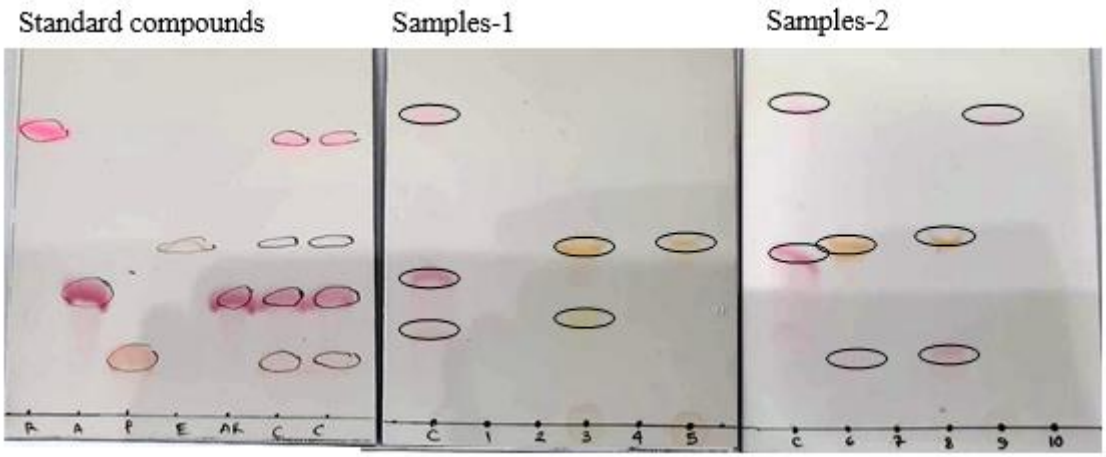

Figure 4. TLC Spot of some "Bundaran ITS" samples. Spot Rhodamine B (R), Amaranth (A), Ponceau 4R (P), Erythrosine (E), and Red Allure (AR), each spot was 100 ppm. While C spot is mixture of all red colorants standard.
Note: Samples code: 1 = snack-*siomay*; 2 = snack-*cireng*/fried-seasoning flour; 3 = ketchup of the first sample of fried rolled egg; 4 = *otak-otak* fish/fried-seasoning flour with fish extract; 5 = ketchup of *otak* fish/fried-seasoning flour with fish extract; 6 = ketchup of cireng/fried-seasoning flour; 7 = ketchup of fried meat ball; 8 = ketchup of the second sample of fried rolled egg; and 9 = ketchup of *lumpia*.

3.2. Survey results at the ITS round about during Ramadhan

"Bundaran ITS" is a favourite spot during Ramadhan festival for either food stalls or students and staff/employees of ITS to break their fast. The place is always crowded with people and mostly causes traffic jams starting from 16.00-18.00 WIB. Approximately more than 50 street food stalls are there.

Thirty-seven (37) samples were taken from "Bundaran ITS" in two periods: 9-12 and 28th of May 2019 at Ramadhan festival 2019. They were grouped into 3 categories, namely beverages (B), snacks (S), and sauces/ketchups (K). Each of them was 32.43%, 13.51% and 54.05%, respectively, for B, S, and K. All of the samples were extracted (**subsection 2.3**), identified qualitatively by TLC (**subsection 2.4**) and the identified red colors spot was further quantified with spectrophotometer (**subsection 2.5**). Gratefully, mostly, both friend snacks (S) and ketchups (K) do not contain Rhodamine B. Only in one sample, ketchup of *lumpia*, Rhodamine B was detected (**Figure 4**). One sample of ketchup also contains methanyl yellow, one sample of noodle and meatball contains boraks (data not shown). Most of the ketchup use Ponceau 4R and Erythrosine as their red colorant (**Figure 4**). Unfortunately, some of them were using beyond the maximum allowable value, 30 mg/kg (The National Agency of Drug and Food Control of Republic Indonesia RI)/BPOM Regulation No.1168/2013 and Permenkes RI No. 37/2013). However, Permenkes RI No. 33/2012 stated than Ponceau 4R (CI No. 16255), Erythrosine (CI No. 45430), and Allure Red (CI No. 16035) are permitted as red food colorants with a limitation on the maximum dosage of the usage.

Table 1. The detected red colorant in the some "Bundaran ITS" samples.

Sample code [a]	Erythrosine (ppm)	Ponceau 4R (ppm)
1. ketchup of the first sample of fried rolled egg (3)	388.68	351.34
2. ketchup of otak-otak fish/fried-seasoning flour with fish extract (5)	720.26	NN
3. ketchup of cireng/fried-seasoning flour (6)	584.86	NN
4. ketchup of the second sample of fried rolled egg (8)	25.34	20.92

[a] sample codes please refer to Fig. 4

4. Conclusion

There is a lack of public awareness to unhealthy food. Even though the consumers realize and are aware of healthy food, shown by the high intention of healthy food, it does not correlate directly to their actual behaviour of healthy food. Therefore, there is still opportunities for irresponsible food stalls to freely sell either sauces/ketchups or food that may contain illegal food additives especially red food colorant.

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