Identification and Determination of Synthetic Dyes in Grape Juice in Closed Package

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ABSTRACT

Ten samples of grape juice packaging in closed package each of which cost 4-17 Baht were analyzed. Four samples were found to have consisted of nonpermitted organic synthetic dyes. Two samples were permissible dyes but their amounts were over the permissible levels. Other 4 samples did not consist of any organic synthetic dye. The dyes were identified by paper chromatography, using 4 different developing solvents and confirmed by comparing their visible absorption peaks with permissible standard dyes. Column chromatographic technique was used to purify food samples and silicagel 60 (0.2-0.5 mm) was used as the adsorbent. The amounts of dyes were determined by visible spectrophotometric method. Indigo carmine 96.09 ppm and ponceau 4R 280.18 ppm were found in one sample. The other sample consisted of indigo carmine 88 ppm and carmoisine 320.9 ppm. This method showed average percent recovery of 111.7, relative standard deviation of 9.2 and the limit of detection was 2 ppm.

Key words: Synthetic dyes, Grape juice

INTRODUCTION

Food dyes are made from organic synthesis. They are useful because colored foods look tasteful. Food dyes are toxic from the dyes themselves and from contaminated trace metals receiving from their producing process. Toxic dyes are amaranth, tartrazine and sunset yellow. Lymphosarcoma and abnormal growth can occur in albino rats taking amaranth, so it is a forbidden dye. Amaranth and tartrazine can also stain stomach, small and large intestine of albino rats, so food absorption is interfered (Sujumnong, 1994). From these reasons, the types and amounts of dyes that can be used in soft drink are declared in Thai Food Act 2004. (Thai Food Act 2004: Food Additives). Permitted red colors are carmoisine 70 ppm, ponceau 4R 50 ppm, erythrosine 70 ppm, permitted yellow colors are tartrazine 70 ppm, sunset yellow FCF 70 ppm and permitted blue colors are fast green FCF 100 ppm, brilliant blue FCF 50 ppm and indigo carmine 70 ppm.

From our preliminary investigation of pesticide residue in vegetable juice, fruit juice and green tea solution (Nantachit and Wongpayapkul, 2007), the mixtures

of synthetic red and blue colors were found in grape juice which was expensive sample instead of natural color. From this information, analysis of the types and amounts of organic synthetic dyes was the objective of our investigation.

MATERIALS AND METHODS

Materials

Paper and column chromatographic techniques were used to identify and purify food dyes. Whatman filter paper No.1 and silicagel 60 (0.2-0.5 mm) were used as the adsorbent. Beckman model DK-2A UV spectrophotometer was used to determine absorption peaks and absorbances of food dyes.

Ten grape juice samples each of which cost 4-17 Baht were used. They were bought from supermarket and consisted of concentrated grape juice, grape juice mixed with combined fruits, grape juice mixed with coconut jelly and grape juice mixed with caragenan.

Chromatographic conditions

Four developing solvents (Stahl, 1969) were used in paper chromatographic technique. They were:

Developing solvent I (DVS I) was the mixture of n-Propanol + ethyl acetate + water (6+1+3)

Developing solvent II (DVS II) was the solution of concentrated ammonia 5 ml which was diluted to 100 ml with distilled water. Sodium citrate 2 gm was dissolved in this solution.

Developing solvent III (DVS III) was the mixture of n-Butanol + absolute ethanol + water (5+2.5+2.5)

Developing solvent IV (DVS IV) was the mixture of iso-Butanol + absolute ethanol + water (3+6+3)

Identification of food dyes

Grape juice 50 ml was evaporated on boiling water bath until it reached constant weight. If the sample consisted of coconut jelly or caragenan, it should be filtered before evaporation. 1 gm of dry sample was weighed and dissolved in small amount of ammonia solution, pH 10. Sample solution was passed through silicagel 60 (0.2-0.5 mm) column and eluted with ammonia solution, pH 10. The eluate was concentrated by evaporating on boiling water bath. Concentrated sample dye was identified by comparing its Rf-value with permissible standand red and blue colors, using 4 developing solvents. Permissible-color samples that were found were confirmed by comparing their visible absorption peaks with permissible standard dyes.

Validation

1. Specification

After sample dyes were identified from paper chromatography, the absorption curves of known permissible sample dyes were compared with standard dyes

in aqueous solution to confirm that the permissible sample dyes and standard dyes were of the same type.

2. Linearity and range

The standard dyes in the concentrations ranging from 2-15 ppm were accurately prepared by diluting 0.1% stock standard solution with distilled water. Visible absorbances of standard dyes were plotted against their concentrations to construct the calibration curve.

3. Accuracy and precision

Synthetic mixtures of standard carmoisine at 100% level and known sample No.3 were prepared. Five spiked samples were purified as the identification method and their amounts were determined by comparing their absorbances with the calibration curve. The percent recovery and the relative standard deviation (RSD) were calculated.

Determination of the amounts of permissible sample dyes

Only the amounts of permissible sample dyes were determined. The concentration of the eluate from chromatographic column, as mentioned in the identification method, was prepared in the range that the absorbances at their λ_{max} could be measured. The amounts of sample dyes were calculated from calibration curve and their dilution factors.

RESULTS

Identification results

Rf-values of 10 samples in 4 developing solvents are shown in Tables 1 and 2. Types of dyes in 10 grape juice samples are shown in Table 3.

Table 1. Rf-values of red dye samples in 4 developing solvents.

Dyes	Rf-values			
	DVS I	DVS II	DVS III	DVS IV
Standard dyes				
1. Sunset Yellow	0.47, 0.45	0.53,0.57	0.39, 0.32	0.48
2. Ponceau 4 R	0.39, 0.33	0.57, 0.57	0.14, 0.17	0.36
3. Carmoisine	0.52, 0.51	0.14, 0.18	0.35, 0.34	0.47
4. Erythrosine	0.90, 0.91	0.05, 0.04	0.79, 0.87	0.90
Sample dyes				*
5. Sample No.1	0.38, 0.40	0.63, 0.63	Spot 1 = 0.18, 0.27 Spot 2 = 0.32, 0.40	
6. Sample No.2	0.32	0.52	0.14	0.35
7. Sample No.3	0.53	0.10	0.39	0.51
8. Sample No.4	0.57	0.14	0.32	0.49
9. Sample No.5	0.20	0.20	0.12	0.29
10. Sample No.6	0.37	0.57	0.13	0.34
11. Sample No.7	0.10	0.11	0.06	0.08
12. Sample No.8	0.13	0.28	0.08	0.13
13. Sample No.9	0.21	0.71	0.07	0.20
14. Sample No.10	0.20	0.31	0.09	0.12

Table 2. Rf-values of blue dye samples in 4 developing solvents.

Dyes	Rf-values			
	DVS I	DVS II	DVS III	DVS IV
Standard dyes				
1. Indigo carmine	0.31	0.25	0.19	0.31
2. Brilliant blue	0.50	0.86	0.36	0.58
3. Fast green FCF	0.41	0.94	0.27	0.55
Sample dyes				
4. Sample No. 1	-	-	-	-
5. Sample No. 2	0.35	0.21	0.14	0.28
6. Sample No. 3	0.31	0.31	0.17	0.29
7. Sample No. 4	0.22	0.34	0.24	0.38
8. Sample No. 5	0.16	0.14	0.12	0.25
9. Sample No. 6	0.15	0.26	0.11	0.22
10. Sample No. 7	0.10	0.11	0.06	0.08
11. Sample No. 8	-	-	-	-
12. Sample No. 9				
13. Sample No. 10				

Table 3. Types of dyes in 10 grape juice samples.

Sample number	Types of dyes
Sample No.2	Ponceau 4R + Indigo carmine
Sample No.3	Carmoisine + Indigo carmine
Sample No.1	Non-permitted dyes
Sample No.4	Non-permitted dyes
Sample No.5	Non-permitted dyes
Sample No.6	Ponceau 4R + Non-permitted dye
Sample No.7	No organic synthetic dye added
Sample No.8	No organic synthetic dye added
Sample No.9	No organic synthetic dye added
Sample No.10	No organic synthetic dye added

For confirmation test, absorption peaks of permissible sample dyes and standard dyes in aqueous solution were measured as shown in Table 4.

Table 4. Absorption peaks of permissible sample dyes and standard dyes in aqueous solution.

Descriptions	Absorption peaks (nm)	
	Red color	Blue color
Ponceau 4R	509	-
Carmoisine	510	-
Indigo carmine	-	612
Sample No.2	509	612
Sample No.3	510	612

Determination results

Linearity and range

A linear response of standard ponceau 4R, carmoisine and indigo carmine was observed over the concentration range of 2-15 ppm. Calibration curve of each standard dye was constructed and shown in Figures 1, 2 and 3.

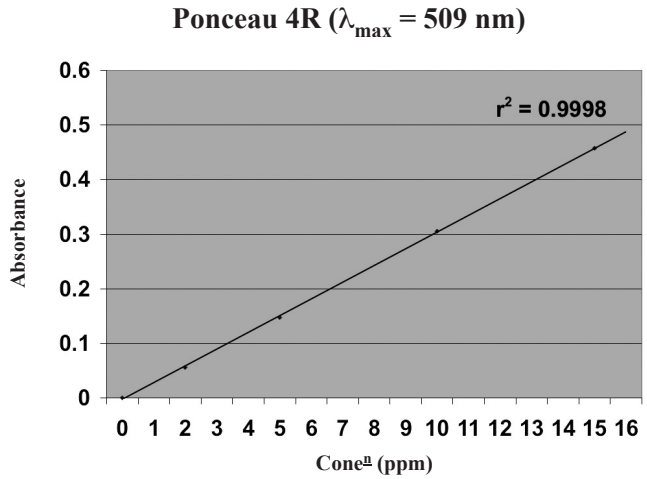

Figure 1. Calibration curve of Ponceau 4R.

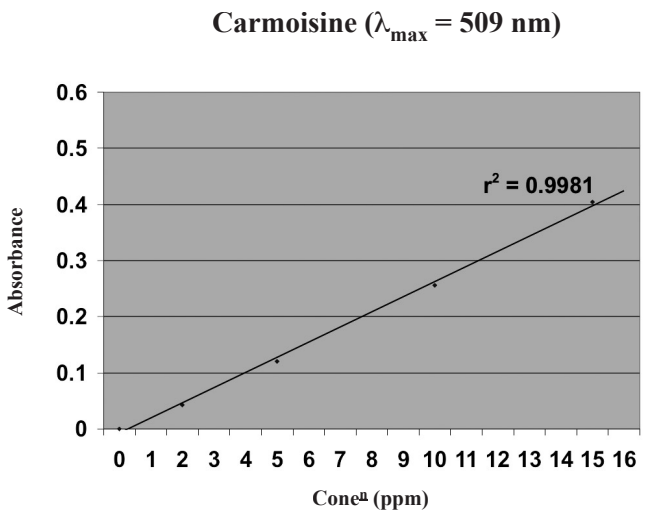

Figure 2. Calibration curve of carmoisine.

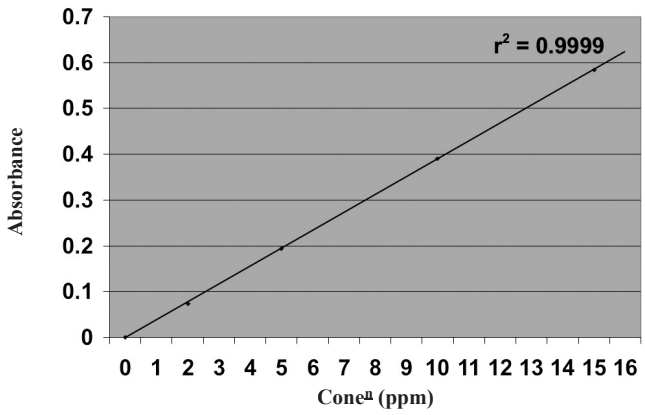

Figure 3. Calibration curve of Indigo carmine.

Accuracy and precision

The mean percent recovery of standard carmoisine from synthetic mixtures of food sample number 3 was 111.7 + 9.2 (n = 5), as determined by visible spectrophotometric method and shown in Table 5.

Table 5. % Recovery, RSD (relative standard deviation) and limit of detection using visible spectrophotometric method.

Dyes added (mg)	Dyes found (mg)	% Recovery
24.9	28.2	113.2
25.1	28.7	114.3
25.2	28.4	112.7
21.1	23.6	111.8
20.1	21.4	106.5
Mean		111.7
RSD		9.2
Limit of detection (ppm)		2.0

Amounts of permissible dyes found in grape juice samples

From 10 samples of grape juice, 4 samples were non-permitted dyes, the other 4 samples contained no organic synthetic dyes and only 2 samples were permissible dyes which were samples No.2 and 3. Sample No.2 contained ponceau 4R 280.18 ppm and indigo carmine 96.09 ppm. Sample No.3 was carmoisine 320.9 ppm and indigo carmine 88 ppm.

DISCUSSION AND CONCLUSION

Silicagel 60 (0.2-0.5 mm) was used as the adsorbent of the column chromatographic method in purification step instead of polyamide which was the adsorbent in Pearson's method (Pearson, 1976). Silicagel 60 (0.2-0.5 m.m.) is 10 times cheaper than polyamide. The mean percent recovery of the proposed method was 111.7, RSD was 9.2 and limit of detection was 2 ppm. Adsorption process is the mechanism of purification step. The sample dyes can be eluted by ammonia solution because acidic dye will form salt with ammonia solution which is a water-soluble compound as the equation:

$$NH_3 + Dye - SO_3H \rightarrow Dye - SO_3NH_2$$

From the Rf-values of 10 samples in 4 developing solvents (Stahl, 1969), sample numbers 1,4,5 and 6 were found to be consisted of nonpermitted dyes. Sample numbers 2 and 3 were permissible dyes and were confirmed by comparing their absorption peaks with standard dyes. The amounts of dyes used in these two samples were over the permissible limit in Thai Food Act 2004. Sample number 2 consisted of ponceau 4R 280.18 ppm and indigo carmine 96.09 ppm. Sample number 3 consisted of carmoisine 320.9 ppm and indigo carmine 88 ppm. The permissible limit of ponceau 4R is not over 50 ppm, carmoisine not over 70 ppm and indigo carmine not over 70 ppm in soft drinks. Sample numbers 7,8,9 and 10 were not found any synthetic dyes following the producer labels.

From this investigation, 40% of grape juice samples were added with nonpermitted dyes, 20% of samples were permissible dyes but their amounts were over Thai Food Act 2004 (Thai Food Act 2004: Food Additives) limit. No synthetic organic dye was found in 40% of samples as the food labels indicated. The Ministry of Public Health should be more interested in controlling the use of organic synthetic dyes in food and the consumers should be more concerned about the food labels whether the dyes were added or not added in the food.

ACKNOWLEDGEMENTS

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REFERENCES

Nantachit, K., and L. Wongpayapkul. 2007. Determination of pesticide residue in vegetable juice, fruit juice and green tea solution in closed package. Chiang Mai University Journal 6(1):43-47.

Pearson, D. 1976. The chemical analysis of foods. 7th Edition, Churchill Livingstone, London. p.53-59.

Stahl, E. 1969. Thin-layer chromatography. 2nd Edition. Springer-Verlag, New York. p.622-624.

Sujumnong, P. 1994. Food law and standard of food. Bangkok O.S. Printing House, Bangkok. p.244-246.