Food Science and Human Wellness 7 (2018) 220–228

Contents lists available at ScienceDirect

Food Science and Human Wellness

journal homepage: www.elsevier.com/locate/fshw

The feasibility study of natural pigments as food colorants and seasonings pigments safety on dried tofu coloring

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ARTICLE INFO

Article history:
Received 10 July 2018
Accepted 20 August 2018
Available online 5 September 2018

Keywords:
Natural colorants
Dried-tofu
The coloring from liquid phase system to solid phase system on dried-tofu

ABSTRACT

In order to improve appetite, attract consumers and even conform to the food culture, food coloring has become one of the necessary links in modern food processing. For example, dried-tofu will be colored by adding artificial food colors (AFCs) such as sunset yellow, cochineal red A or other seasonings like soy sauce. However, the dispute persists about whether AFCs are harmful to health. Some studies indicate AFCs affect children's intelligence and attention, cause hyperactivity, and allergy when children consumed ≥ 50 mg. In addition, researches showed that chemical soy sauce produces a trace of methylglyoxal (MGO) in the manufacturing process, which is related to diseases such as oxidative stress, diabetes, and cognitive deterioration. Therefore, natural pigments are relatively new and promising strategy for replacing high-risk AFCs. Thus, the objective of this study was to use dried-tofu as a natural colorants coloring screening platform, through the concept of three primary colors to discuss the coloring effects of natural colorants in Taiwan in double –phase (liquid phase to solid phase) food coloring system and assess the effects of MGO on PC12 neuron cellular morphology and cell cycle at the dietary exposure in soy sauce. Our results showed that formula G:R = 0.2:0.8 and C:R = 0.08:0.92 were coloring by combined natural colorants had the same eye sensory quality acceptance of consumer and had the intention to purchase. Furthermore, the results from the PC12 cell suggested that dietary exposure of methylglyoxal (<50 μM) in soy sauce did not affect neuron cellular morphology and cell cycle significantly. Overall, Gardenia Yellow, Curcumin, and Radish Red could overcome the application restrictions in multiple-phase food coloring system and simultaneously soy sauce as a coloring agent was safety. It showed the possibility of them as food colorants on dried-tofu.

1. Introduction

Food colorants play an important role in altering or conferring colors to food, in order to increase its attractiveness toward consumers. The consumers' sensory acceptability of color on foodstuff is influenced by many factors, including the local humanities, geography, and social background. However, regardless of a region of its dietary culture, people have a specific expectation of the color of food. These expectations are some from the natural food color, some from the results of processing experience so that some food only within a specific color range can be favored. For instance, apples or the bananas be blue they cannot be accepted. A food color also can be a strategic marketing communications purposes when it is for flavor identification. A novel color in a food context appears incongruous and therefore unacceptable [1]. Taking early 1990's PepsiCo's Crystal Pepsi for example, apart from its clear color, the Crystal Pepsi was made in all other respects, including flavor. However, to cola drinkers, the clear Crystal Pepsi connoted cer-

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Peer review under responsibility of KeAi Communications Co., Ltd.

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https://doi.org/10.1016/j.fshw.2018.09.002

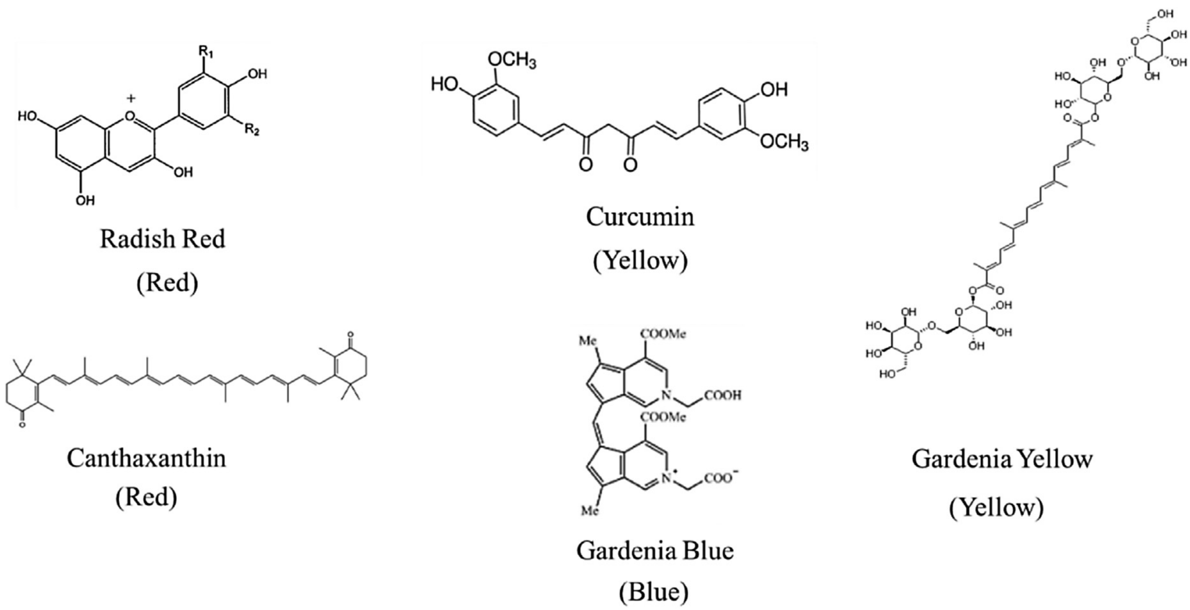

Fig. 1. The structures of natural colorant.

tain "non-cola" flavor expectation. Consequently, the Crystal Pepsi failed to be a successful product [2]. From the above examples, we learn the importance of food color for consumer acceptance. According to the report of Taiwan Food and Drug Administration (TFDA), to fit the food culture of Taiwan, the commercial dried-tofu are used to be coloring by AFCs cochineal red A and sunset yellow [3]. In spite of AFCs have been used for a long time in the food industry, but there are some controversies and disagreements regarding their health effects. The influences of AFCs on children's behavior since 1975 have been studied for more than 35 years. According to previous studies meta-analysis [4–8] found evidence when children intake of AFCs $\geq$ 50 mg, the hyperactivity and allergic symptoms were significantly higher than those who have intake of AFCs <50 mg(sunset yellow:112.5 mg). Nevertheless, the dose has not yet exceeded children acceptable daily intake of AFCs recommended by the U.S. Food and Drug Administration. This leads to numerous regulations throughout the world, which for example in the USA reduced the permitted list of AFCs to seven from 700 being used. [9] Food Safety Authority and the EFSA, but the public advocacy groups and the media promoted a voluntary ban on the implicated colors. In 2008, the EU published regulation 1333 pointing out that some additives should clearly state possible negative effects on concentration activity of children (Council Regulation (EC) 1333/2008) [10]. Soy sauce in dried-tofu coloring is also one of the commonly used methods, but small amounts of methylglyoxal (MGO) were produced in the soy sauce manufacturing process [11]. MGO is a highly reactive dicarbonyl compound. The glycation reaction of MG with protein, lipid, and DNA lead to oxidative stress, diabetes mellitus, cognitive deterioration, etc. [12–14]. Consumer pressure, sociological changes, and technological advances leading to the requirement for natural food colorants have been increasing. Carotenoids, anthocyanin, annatto, and paprika are applications of natural colorants that can substitute their synthetic counterparts, especially in single-phase food coloring system such as in the baking products (solid phase) and drinks (liquid phase) [15]. These natural colorants vary widely in their physical and chemical properties. Because many natural colorants sensitive to oxidation, pH change, light, and their inherent solubility vary widely, these limitation result in the application restrictions in multiple-phase food coloring system. In view of this, the objective of this study will use dried-tofu as a natural colorants coloring screening platform, through the concept of three primary colors to discuss the coloring effects of natural colorants in Taiwan in double –phase (liquid phase to solid phase) food coloring system and assess the effects of MGO on PC12 neuron cellular cytotoxicity and cell cycle. In this study, we have evaluated the feasibility using natural colorants in Taiwan as food colorants on dried-tofu and risk assessment MGO at the dietary exposure in soy sauce. Our aim is to make dried-tofu more suitable the needs of current social food safety and the concept of healthy diet under the food culture of Taiwan.

2. Materials and methods

2.1. Materials

White dried-tofu was purchased from the street market. Curcumin was purchased from SIGMA. Gardenia yellow, radish red, and gardenia blue were purchased from DAYSPRING BIOTECH CO.LTD. Canthaxanthin was provided from Allied Biotech Corporation. The structures of natural colorants are shown in Fig. 1.

2.2. Analysis of the color of natural colorants on dried-tofu

White dried-tofu were coloring by natural colorants at 25, 65,75 and 85 °, during 5,10,15,20,25,30,40,50,60,70,80,90,100,110, and120 min. After coloring, the dried-tofu was divided into four equal parts. Its L, a, b value by Color Difference Meter (model TC-1, Tokyo Den-shoku Co, Japan) was measured. The △E value to filter out the heating temperature and time of the processing conditions was calculated

$$\Delta E = \sqrt{(L1 - L2)^2 + (\alpha 1 - \alpha 2)^2 + (b1 - b2)^2}$$

2.3. The color of natural colorants on dried-tofu and the sensory evaluation of consumers

Commercial dried-tofu and experimental dried-tofu were randomly coded. In order to understand 64 consumers' degree of preference for commercial dried-tofu and experimental dried-tofu, the 9-point hedonic test was conducted by William Latin Square method. For the purpose of realizing the correlation between the degree of preference for the color on dried-tofu and the purchase

Table 1
The Lab value of the Radish Red and the Canthatene were analyzed by a four-way ANOVA.

Factor	Num DF	Den DF	p-value L	a	b	△E
Pigment	1	2	0.0532	0.0615	0.0016	0.0069
Temperature	3	6	0.0005	<.0001	0.0001	0.2216
Time	14	28	0.0004	<.0001	0.9560	0.1370
Pigment*Temperature	3	382	<.0001	<.0001	<.0001	0.0001
Pigment*Time	14	382	0.9665	0.4287	<.0001	0.8440
Temperature*Time	42	382	0.9047	0.2152	0.9112	0.9999
Pigment*Temperature*Time	42	382	0.9953	0.0027	0.9962	1.0000

Statistical significant at *p* value < 0.05.

intention, the consumer purchase intention 5 points investigation was performed at the same time. A better understanding of the eye sensory quality acceptance of consumer about commercial dried-tofu and experimental dried-tofu was Reached.

2.4. Cytotoxicity assay

The effect of MGO on cell viability and toxicity was tested using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Trypan Blue analysis, respectively. Details of the MTT assay followed the procedure set up in the previous study [16]. PC12 cells treated with 0, 0.5, 5, 50, 500 μM of MGO for 48 h measured by MTT assay. Trypan blue assay was PC12 cells treated with 0, 0.5, 5, 50 μM MGO at 2,4,6, and 8 days, the cell suspension was collected and dyed with trypan blue by a 1:1 ratio. Cells were then counted in a hemacytometer by a microscope (Olympus America, Inc., Lake Success, NY).

2.5. Flow cytometry

Incubated PC 12 cells were seeded with the 2 × 105 cells/mL density in the 24 well plate overnight and treated with different concentrations of MGO for 2,4,6, and 8 day and cells were harvested with phosphate-buffer saline (PBS), fixed with iced 100% ethanol, and then stored in −20 °. Cells were resuspended with hypotonic buffer (0.5% Triton X-100 in PBS and 0.5 μg/mL RNase) and then stained with PI (Propidium iodide in PBS 1 mg / mL) at 37 °C for 15 min, individually. Fluorescence intensity was quantified by flow cytometry (Becton-Dickinson, San Jose, CA).

2.6. Statistical analysis

For the sake of the factor, which affected the coloring results, the L, a, b and △E value of the red natural colorants on dried-tofu were analyzed using four-way ANOVA by SPSS. To filter out the heating temperature and time of the processing conditions, the L, a, and b value of the Radish Red were analyzed using Principal components analysis (PCA) and Cluster analysis by XLSTAT. The 9-point hedonic test and the consumer purchase intention 5 points investigation were analyzed using PCA by XLSTAT so as to understand the eye sensory quality acceptance of consumer about commercial dried-tofu and experimental dried-tofu.

3. Results and discussion

3.1. The processing conditions of natural colorants

Through the four-way ANOVA to explore the factor, which affected the coloring results, the results were shown in Table1. It was found that the interaction between different red natural colorants and the different temperature had a significant effect on the L, a, b and △E value. The interaction relationship between different red natural colorants and different time was only in the b value.

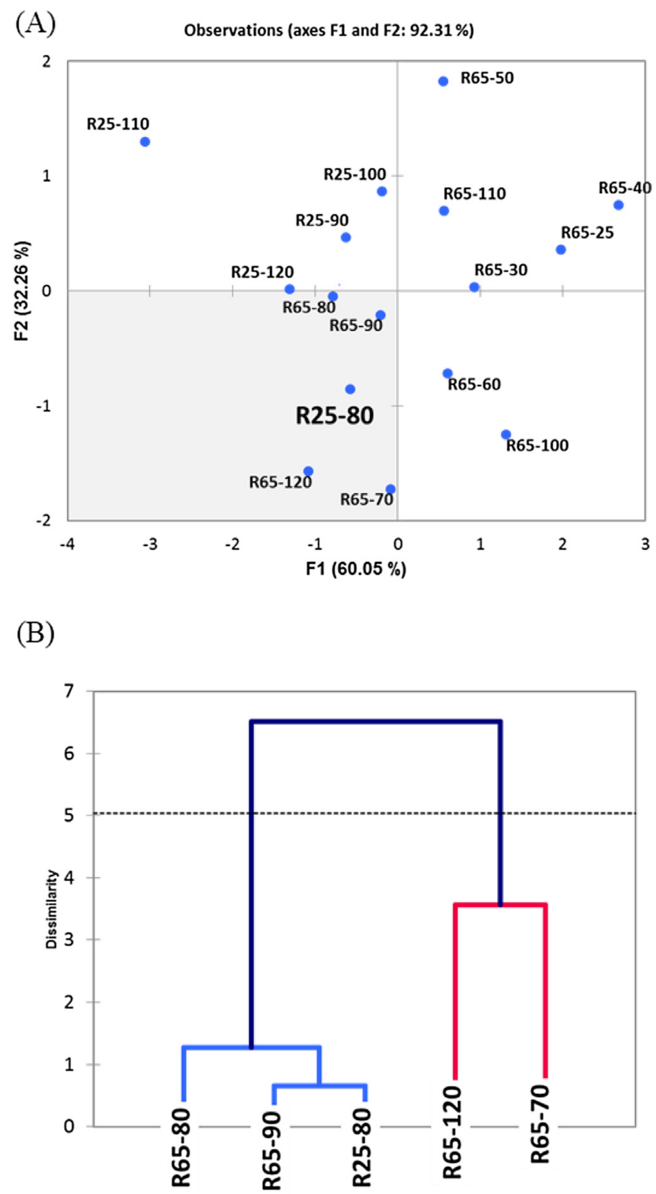

Fig. 2. The group of Radish Red food colorant on dried tofu at 25 and 65 °.
(A) The Lab value of Radish Red food colorant on dried tofu at 25 and 65 ° was analyzed by Principal Component Analysis (PCA) by Pearson (n-1), Correlation biplot and Coefficient Automatic.
(B)The Cluster analysis divided the Lab value of Radish Red food colorant on dried tofu at 25 and 65 ° into groups by Euclidean distance and Ward's method.
The data showed as Rtemperature-time, for example R25-80 mean the processing conditions of Radish Red food colorant was at 25 ° during 80 min.

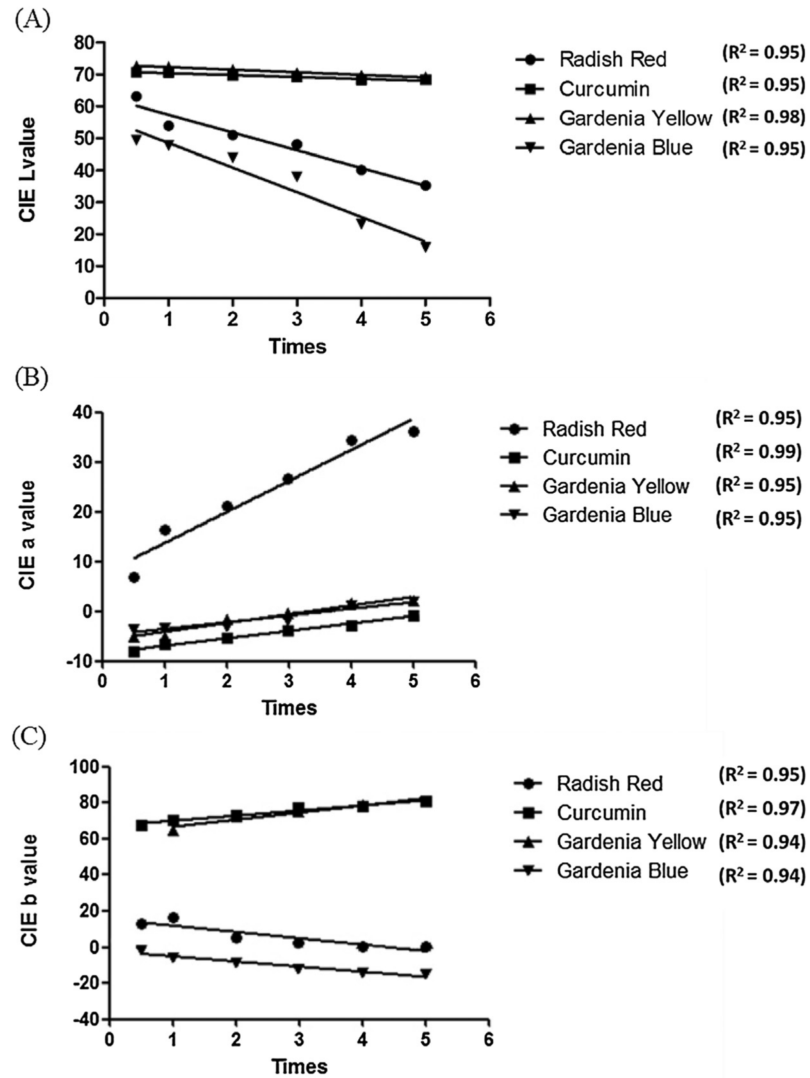

Fig. 3. The different times of uncertified food colorants showed linear correlation.
Different times of each uncertified food colorant on dried tofu at 65 ° 90 min and the (A) L value, (B) a value and (C) b value of different times food colorant showed linear correlation.

It was clear that the coloring results of the red natural colorants, Radish Red and Canthatene were mainly affected by the temperature. The influence of temperature effect of Radish Red under the concentration 1:1000 and 1:250 at 25 ° the performance of redness was much more obvious than at 65, 75, and 85 ° due to the main structure of Radish Red(data not show). Anthocyanin is the main structure of Radish Red so that its thermal stability is poor [17]. While Canthatene under the concentration 1:1000 the performance of redness as the temperature increases became much more obvious (data not shown). Despite canthatene is thermally stable, the color was only slightly attached to the dried-tofu surface. Based on that dried-tofu is a perishable food [18], the temperature should be increased to at least 65 °C in the coloring process to reduce the number of initial microorganism before leaving the factory.

The △E difference between two colors is calculated by the formula:

$$\Delta E = \sqrt{(L1 - L2)^2 + (\alpha 1 - \alpha 2)^2 + (b1 - b2)^2}$$

When $0 < \Delta E < 1$ observer does not notice the difference; $1 < \Delta E < 2$ only experienced observer can notice the difference; $2 < \Delta E < 3.5$ unexperienced observer also notices the difference [19].Through the ΔE value can know the similarities and differences between different coloring time at 25 °. The results showed that the ΔE value between 25° 70 min and 80 min is 4 meaning unexperienced observer notices the difference. However, 25° above 80 min the ΔE were less than 2 ($\Delta E80\text{-}90 = 1.70$, $\Delta E90\text{-}100 = 1.47$, $\Delta E100\text{-}110 = 1.67$, $\Delta E110\text{-}120 = 1.87$) meaning only experienced observer can notice the difference and general observer cannot distinguish the color difference. In order to find the processing conditions of natural colorants, the L, a, and b value of the Radish Red at 25 ° above 80 min and at 65 ° were analyzed using Principal components analysis (PCA) [20]. The results are shown in Fig. 2(A). R25-80 had the highest similarity to the color performance with R65-70, R65-80, R65-90, and R65-120. For finding the most similar groups to the color performance, R25-80, R65-70, R65-80, R65-90, and R65-120 were classification by Cluster analysis. The results are shown in Fig. 2(B). R25-80 and R65-90 were the most similar groups to the color performance. The processing conditions of natural colorants were 65 ° 90 min.

3.2. The color prediction of combined natural colorants from liquid phase system to solid phase system on dried-tofu

In the past study, it was found that the L, a, and b values of the single powders with different weight percentage showed linear

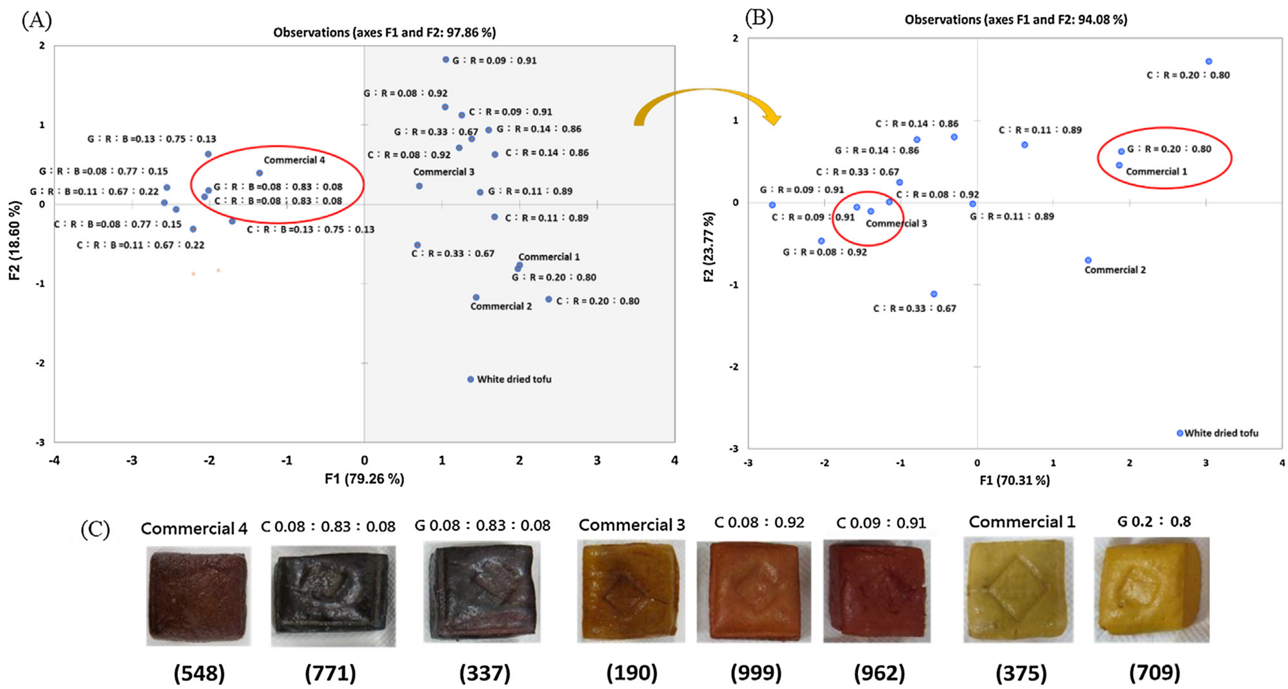

Fig. 4. The principal component analysis (PCA) of food colorant mixed with different ratio at 65 ° during 90 min on dried tofu.
(A)、(B)The Lab value of food colorant mixed with different ratio at 65 ° during 90 min on dried tofu was analyzed by Principal Component Analysis (PCA) by Pearson (n-1), Correlation biplot and Coefficient Automatic. (C) The sensory evaluation samples.

Table 2
The target Lab values compared with the actual Lab value after two food colorant mixing with different ratio.

The mixing ratio	Target value			Actual value			△E
	L	a	b	L	a	b	
C:R = 0.11:0.89	53.27	18.21	12.42	49.96 ± 1.25	15.01 ± 1.37	43.25 ± 3.33	31.17
C:R = 0.20:0.80	57.31	11.77	27.13	57.60 ± 1.75	07.05 ± 0.71	55.07 ± 3.25	28.34
C:R = 0.33:0.67	59.39	09.14	35.22	55.46 ± 1.53	10.00 ± 0.67	50.82 ± 1.07	16.11
G:R = 0.11:0.89	53.47	18.21	12.42	50.24 ± 0.16	18.07 ± 0.87	36.57 ± 0.58	24.36
G:R = 0.20:0.80	57.67	12.07	25.97	56.99 ± 2.35	10.39 ± 1.22	45.37 ± 1.12	19.48
G:R = 0.33:0.67	60.04	10.45	35.05	52.02 ± 1.15	17.35 ± 0.33	57.14 ± 2.10	24.49
C:R = 0.08:0.92	49.69	23.93	07.82	44.08 ± 0.66	22.06 ± 1.57	34.27 ± 1.51	07.82
C:R = 0.09:0.91	38.42	32.51	06.66	43.15 ± 0.82	26.24 ± 1.14	31.93 ± 2.37	06.66
C:R = 0.14:0.86	51.32	18.19	12.66	46.51 ± 0.92	22.46 ± 0.76	40.68 ± 1.61	12.66
G:R = 0.08:0.92	49.83	24.04	07.38	41.93 ± 1.39	26.75 ± 1.32	28.25 ± 0.79	22.48
G:R = 0.09:0.91	38.60	32.87	06.61	36.97 ± 2.80	31.83 ± 1.50	28.73 ± 1.33	22.20
G:R = 0.14:0.86	51.60	22.62	01.03	44.31 ± 0.61	25.04 ± 0.88	39.03 ± 1.97	38.77

C:Curcumin、G:Gardenia Yellow、R:Radish Red、B:Gardenia Blue.
The mixing ratio was the weight percentage of each uncertified food colorant and actual value of Lab shows the mean ± S.D.
The△E value is a measure of change in visual perception of two given colors. When the △E< 2 indicated that general observer does not notice the difference.

correlation and the L, a, and b values of the mixed powders would be linear correlation so that it was possible to predict the color of the mixed powders by a linear formula [21]. As Fig. 3 showed, the different times' natural colorants showed linear correlation, so assumed the L, a, and b values of combined natural colorants from liquid phase system to solid phase system on dried-tofu also showed linear correlation.

And the color on coloring dried-tofu can be estimated by the following linear relationship formula:

(1): $L_{mix} = Y \times L_Y + R \times L_R + B \times L_B$
(2): $a_{mix} = Y \times a_Y + R \times a_R + B \times a_B$
(3): $b_{mix} = Y \times b_Y + R \times b_R + B \times b_B$
$Y + R + B = 1$

L_{mix}、a_{mix}、b_{mix} were the L, a, and b values of combined natural colorants from liquid phase system to solid phase system on dried-tofu.

Y、R、B were the weight percentage of three primary colors (yellow, red and blue)

L_Y、L_R、L_B、a_Y、a_R、a_B、b_Y、b_R、b_B were the L, a, and b values of three primary colors (yellow, red and blue).

The L values of the commercial dried-tofu in Taiwan were among 25–60; values of the commercial dried-tofu in Taiwan were among 5–15; b values of the commercial dried-tofu in Taiwan were between 10-45. Through the above linear relationship, the formula can estimate the weight percentage of three primary colors ratio. The target values and actual values showed in Table 2 and 3. Because of the △E value between the target values and actual values were more than 2 meaning unexperienced observer notices the difference, it indicated that the color prediction of combined natural colorants from liquid phase system to solid phase system on dried-tofu was more complicated in single-phase (solid phase) food coloring system. As the Table 2 showed, in two combined nat-

Table 3
The target Lab value compared with the actual Lab value after three food colorant mixing with different ratio.

The mixing ratio	Target value			Actual value			△E
	L	a	b	L	a	b	
C:R:B = 0.03:0.30:0.50	21.73	11.16	−5.65	13.09 ± 0.32	−0.62 ± 0.51	0.84 ± 0.41	16.00
C:R:B = 0.08:0.83:0.08	39.26	29.46	05.91	21.93 ± 0.47	04.93 ± 1.16	5.64 ± 0.71	30.04
C:R:B = 0.08:0.77:0.15	49.01	26.97	04.71	19.43 ± 0.62	02.07 ± 0.60	2.96 ± 0.15	38.70
C:R:B = 0.11:0.67:0.22	50.37	16.36	08.49	22.62 ± 1.43	00.60 ± 0.56	5.09 ± 1.19	32.09
C:R:B = 0.13:0.75:0.13	50.97	18.79	10.82	26.11 ± 1.00	03.21 ± 0.35	9.90 ± 1.47	29.35
G:R:B = 0.03:0.30:0.50	25.42	11.20	−5.80	14.67 ± 0.20	0.51 ± 0.41	−1.03 ± 0.39	13.59
G:R:B = 0.08:0.83:0.08	39.43	29.79	05.87	19.57 ± 0.58	10.09 ± 0.78	4.61 ± 0.24	28.00
G:R:B = 0.08:0.77:0.15	39.99	27.27	04.67	17.36 ± 0.59	04.27 ± 0.90	0.62 ± 0.30	32.52
G:R:B = 0.11:0.67:0.22	50.59	16.79	08.44	17.51 ± 0.62	02.24 ± 1.03	1.74 ± 0.52	36.75
G:R:B = 0.13:0.75:0.13	51.21	26.67	10.76	21.39 ± 0.77	05.65 ± 0.25	6.99 ± 1.58	36.68

C:Curcumin↘G:Gardenia Yellow↘R:Radish Red↘B:Gardenia Blue.
The mixing ratio was the weight percentage of each uncertified food colorant and the actual value of Lab shows the mean ± S.D.
The△E value is a measure of change in visual perception of two given colors. When the △E< 2 indicated that general observer does not notice the difference.

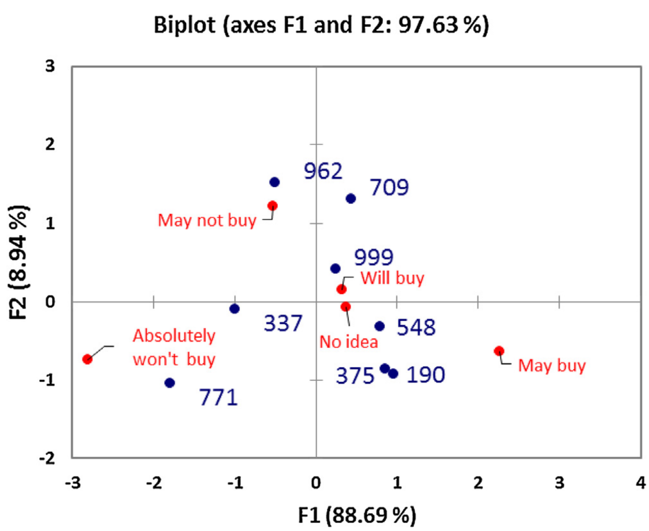

Fig. 5. The sensory quality acceptance of consumers.
The degree of preference about the color of dried tofu samples and purchasing intention for the dried tofu samples were analyzed by Principal Component Analysis (PCA) by Covariance (n-1), Correlation biplot and Coefficient Automatic.

Table 4
The correlation between the degree of preferences and purchasing intention for the dried tofu samples.

	9-point hedonic test	Purchase intention	Pearson correlation coefficient
375	5.86 ± 1.30	3.39 ± 0.93	0.665*
190	6.00 ± 1.35	3.52 ± 0.88	0.683*
548	5.83 ± 1.78	3.42 ± 1.04	0.821*
709	5.42 ± 1.62	3.00 ± 1.03	0.731*
999	5.11 ± 2.02	3.03 ± 1.20	0.761*
962	4.08 ± 1.68	2.42 ± 1.10	0.624*
771	2.55 ± 1.69	1.69 ± 0.98	0.821*
337	3.47 ± 1.98	2.20 ± 1.17	0.766*

Each row shows the mean ± S.D.
Correlation degree: pearson correlation coefficient 1means the correlation degree is perfectly correlated; 0.7-0.99 means the correlation degree is highly correlated; 0.4-0.69 means the correlation degree is moderately correlated; 0.1-0.39 means the correlation degree is modestly correlated; 0.01-0.09 means the correlation degree is weakly correlated.
Statistical significance: *$p <$ 0.01.

ural colorants from liquid phase system to solid phase system, the main factor of the △E value between the target values and actual values were more than 2 was the prediction bias of b values. As the Table 3 showed, in three combined natural colorants from liquid phase system to solid phase system, the main factor of the △E value between the target values and actual values were more than 2 was the prediction bias of L and values. The natural colorants used in this experiment with −OH and −COOH groups, some of them with a charge. Then surmise that the natural colorants used in this experiment are through the hydrogen bond and ion bond to combine with the surface of dried-tofu [22]. The past studies had pointed that the dye through electrostatic force, hydrophobic forces, hydrogen bonds, electron transfer and other non-specific protein bindings, the structure may be a factor influencing the binding ability to the protein [23]. It was assumed that the prediction coloring formula is not accurate due to the number of −OH and −COOH groups and the position of −OH and −COOH groups. When combined natural colorants from liquid phase system to solid phase system on dried-tofu, single natural colorant had a competitive relationship with the binding site, resulting in that combined natural colorants did not transfer to the original proportion from liquid phase system to the solid phase system. In Red-Yellow two natural colorants mixing system found in the yellow natural colorants were more competitive with the binding site on dried-tofu than the red natural colorant. In Red-Yellow-Blue three natural colorants mixing system found in the red natural colorant was the least competitive with the binding site on dried-tofu.

3.3. The coloring effect of combined natural colorants from liquid phase system to solid phase system on dried-tofu and the sensory evaluation of consumers

PCA analysis was used to evaluate the coloring effect of combined natural colorants from liquid phase system to solid phase system on dried-tofu. The result as Fig. 4 showed. The coloring effect of C:R:B = 0.08:0.83:0.08 (771) and G:R:B = 0.08:0.83:0.08 (337) were similar to Commercial 4 (548). The coloring effect of G:R = 0.2:0.8 (709) was analogous to Commercial 1(375). The coloring effect of C:R = 0.09:0.91 (962) and C:R = 0.08:0.92 (999) resembled Commercial 3 (190). For the purpose of realizing the correlation between the degree of preference for the color on dried-tofu and the purchase intention, the consumer purchase intention 5 points investigation was performed at the same time [24]. As the Table 4 showed, there was a high correlation between the degree of preference for the color on dried-tofu and purchase intention. To put it another way, when the degree of preference for the color on dried-tofu was low the purchase intention also was low. A better understanding of the eye sensory quality acceptance of consumers about commercial dried-tofu and experimental dried-tofu was reached by PCA analysis. The results were shown in Fig. 5. The samples 709, 999, 548, 375 and 190 had the same eye sensory qual-

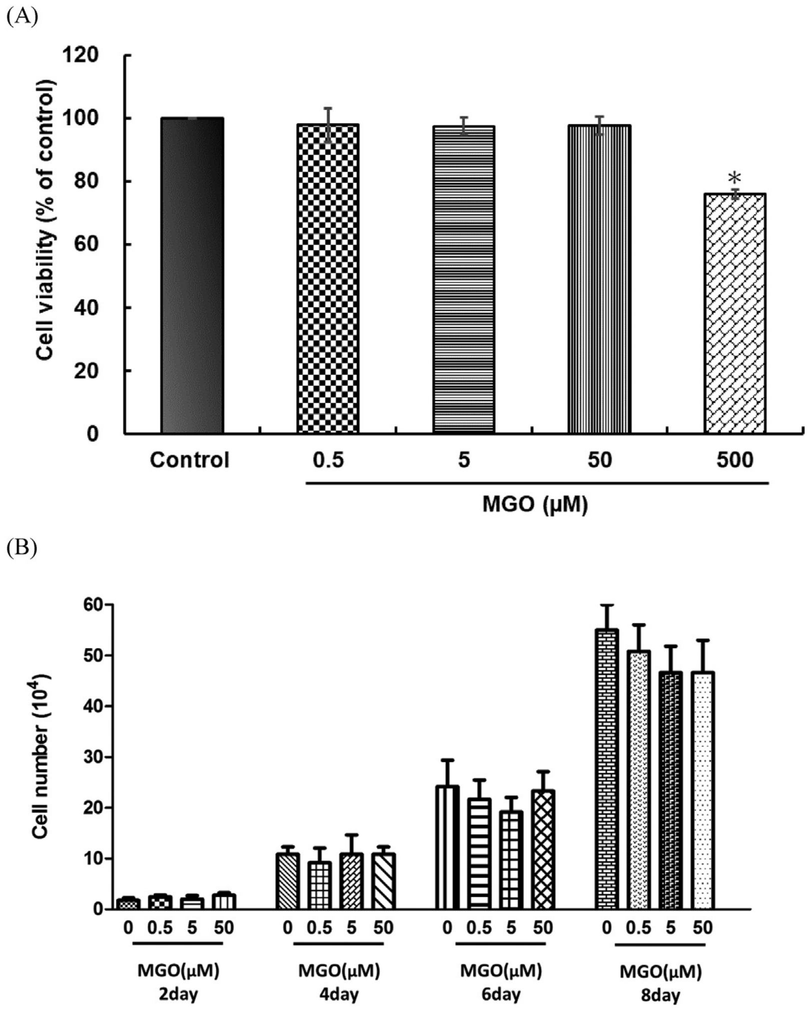

Fig. 6. The effects of methylglyoxal on cell cytotoxicity from 2 to 8 days.
(A)The viability of PC12 cells treated with 0, 0.5, 5, 50, 500 μM of MGO for 48 h measured by MTT assay. (B) The viability of PC12 cells treated with 0, 0.5, 5, 50 μM of MGO for 8 days measured by Trypan Blue assay. The data showed as Mean ± SD, n = 3, *$p <$ 0.05 v.s. control by ANOVA.

ity acceptance of consumer and had the intention to purchase. The sample 709 and 999 were coloring by combined natural colorants. It was shown that the feasibility of natural colorants, which Gardenia Yellow, Curcumin and Radish Red as food colorants on dried-tofu. The combined natural colorants can make dried-tofu more suitable for the needs of current social food safety and the concept of healthy diet under the food culture of Taiwan.

3.4. Effect of low concentration methylglyoxal on PC12 neuron cellular cytotoxicity and cell cycle

Soy sauce often used as the coloring agent of dried tofu contains tiny MGO. Study show MGO cause form high-level oxidative stress, which further leads to neuron cellular apoptosis, therefore there may be disadvantages to health. This study evaluated the risk of damage to PC12 neuron cellular caused by dietary exposure of MGO in soy sauce. The cytotoxicity result as Fig. 6(A) and (B) shown that the concentration was lower than 50 μM for 48 h or 8 days MGO treatment is no significant effect PC12 growth. Furthermore, at the PC12 growth cycle results in Fig. 7(A), (B) and (C) shown the concentration for 0.5–50 μM having no significant difference was observed in the MGO treatment. These results suggested no noticeable toxicity caused by treatment of MGO during a period of 8 days in PC12 cells. Therefore, concerning the risk of low concentration MGO could be preliminary excluded.

4. Conclusion

Through the 9-point hedonic test and the consumer's purchase intention 5 points investigation to understand the eye sensory quality acceptance of consumer about commercial dried-tofu and experimental dried-tofu. In this study, we have found that the samples 709 and 999 coloring by combined natural colorants had the same eye sensory quality acceptance of consumer who had the intention to purchase. It was shown the feasibility of natural colorants, which are Gardenia Yellow, Curcumin and Radish Red as food colorants on dried-tofu. The combined natural colorants of them can be novel coloring materials in the industry of dried-tofu in Taiwan. If the competitive relationship with the binding site and the structures of natural colorants could make a thorough inquiry, the color of combined natural colorants from liquid to solid phase system on dried-tofu would be predicted well and truly. The natural colorants as food colorants on dried-tofu will be more popular. Moreover, if soy sauce is applied as a food colorant, the trace amount of MGO injuring the neuron cellular is without excessive worrying, but it is still not liable to ignore the high concentration of MGO in other foods.

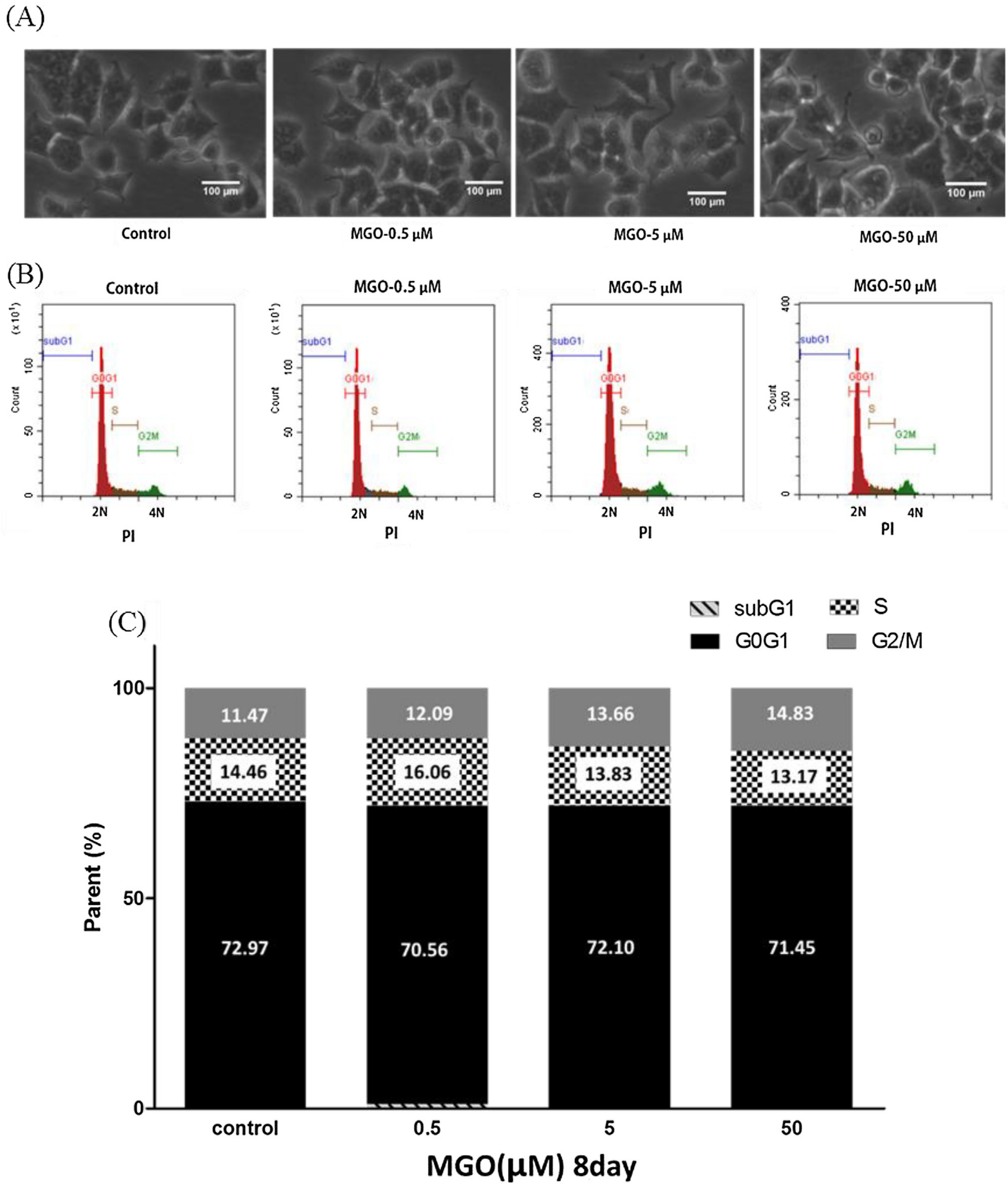

Fig. 7. Cell cycle analysis of PC12 cells treated with 0, 0.5, 5, 50 μM of MGO for 8 days.
PC12 cells treated with 0, 0.5, 5, 50 μM of MGO for 8 days. From left to right was control, PC12 cells treated with 0.5, 5, 50 μM MGO for 8 days. (A) The morphology of PC12 cells. (B)The population histogram of PC12 cells. (C)The cell cycle distribution of PC12 cells. For any of the subG1, G0G1, S, G2/M distributions reported did not differ statistically (by ANOVA) between the control and methylglyoxal treatment.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgement

This study was supportedby the Ministry of Science and Technology [105-2320-B-002-031-MY3, 105-2628-B-002-003-MY3].

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