

A study on the Extraction and Stability of Yellow and Orange Pigments from *Daucus Carota* subsp. *Sativus* and *Curcuma Longa* for Food Coloring

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Abstract— This paper was an experimental project where it was done to extract of yellow and orange pigment from carrot and turmeric by using supercritical fluid extraction and hydro-distillations and also to investigate the effect of natural and synthetic colour on different condition of storage, temperature, light, toxicity and food products. The extraction by using hydro-distillation is chosen because the amount of colorants is higher using hydro-distillation. A few tests had been discovered to know the stability of natural colorants and compared with the synthetic ones. From the tests, the absorbance values were obtained. The value of absorbance use to compared between the natural colorants and synthetic colorants. The stability of the orange pigment is affected due to the carotenoids contained and yellow pigment is affected due to the curcuminoid contained in both colorants respectively. Both carotenoids and curcuminoids are affected by the UV light and temperature.. In term of toxicity, the concentration of elements and heavy metals in the natural orange and yellow pigments were lower than the synthetic ones. Natural colorants were safer than synthetic colorants for human consumptions in their daily lives. The study suggested that both orange and yellow pigments are well kept in the dark and suitable temperature condition to keep the pigment from color loss.

Keywords— Colorants, Stability, Carrot, Turmeric, Absorbance, Supercritical fluid extraction and Hydro-distillations.

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I. INTRODUCTION

Colorants are utilized as a part of nourishment to influence it to feel all the more appealing and appetizing, which are vital variables while picking nourishment off the racks. The colorants are utilized to upgrade existing hues that can be lost either amid the produce or over the time span of usability, or even to credit new ones to it. Food colors can be characterized into three gatherings; characteristic food colorants, which allude to ones that are incorporated normally; nature-indistinguishable colorants, which despite the fact that orchestrated in ventures, emulate the regular ones lastly the counterfeit/manufactured colorants [14]

In a focused worldwide market, the slightest costly technique for food conservation is constantly supported, and by and large, food added substances are picked over the others. Besides, food added substances are basic to empower the food business to influence food to meet the inexorably difficult market and lawful requests [14] Food added substances could be partitioned into 6 gatherings of

particles which are additives, nourishing added substances, shading operators, enhancing specialists, texturizing specialists and various operators [10]. In this research, we are focusing on the colouring agents properties.

The natural color of foods is due primarily to carotenoids, anthocyanins, betanin and chlorophylls, either as inherent food constituents or as food or feed additives (Delia B et al., 2012). These compounds have attracted significant consideration late years, not due to their shading properties, but rather because of their potential wellbeing advancing impacts [8]. Their event and levels in food, alongside the components that impact the arrangement, have been generally explored. Preparing impacts have been effectively considered. Despite the exceptional look for plant and microbial sources and endeavors to expand yield, couple of regular nourishment shading added substances have achieved the market. Lack of stability is a major deterrent that we focus on in our research.

Employing natural colors is the current marketing trend because of consumer's concern about the safety of artificial food dyes, reinforced by possible health benefits of the natural pigments (De Pascual et al., 2014). Substitution of the previous by the last mentioned, be that as it may, is testing since natural colorants are normally less steady, more exorbitant, are not as effortlessly used as simulated hues. It is require more material to accomplish equal shading quality and has restricted scope of tints.

A. Carotenoids

Carotenoids are another gathering of normally inferred nourishment colorants with a prestigious effect and request, for the most part because of their conspicuous shading characteristics and bioactive properties, among other medical advantages [2]. These shades have been additionally progressively utilized for corrective and pharmaceutical purposes, with accessible supplements containing both individual and blends of these fixings [12]. In any case, their nourishment colorant traits are additionally exceedingly valued.

Carrot is a biennial plant of the plant aggregate Umbelliferae (or Apiaceae) that additionally incorporates parsley, anise, caraway dill, and a huge number of different species. Be that as it may, dissimilar to most plants, the capacity foundation of numerous carrot cultivars shows a trademark shading because of the amassing of abnormal amounts of carotenoids. Youthful carrot roots are pale yet after the main month of development they begin gathering carotenoids to achieve largest amounts in around 3 months, just before optional development is finished [13].

It is likely that wild carrot plants had uncolored foundations of a severe taste and a woody center however were at first developed in view of their sweet-smelling leaves and seeds. The foundation structure originating from statistic and early development history still endures in at present developed carrot germplasm. A present, carrots (i.e. develop *D. carota* roots) are accessible in a scope of hues, albeit orange assortments are generally famous.

B. Curcumin

Curcumin is the essential shade of shading. It is normally utilized as a part of different food businesses for shading. Curcumin has a serious yellow shading and is insoluble in water and in ether, is debased in antacid arrangement and is hazardous to mellow, a factor that for the most part restrains its bundles in foods [1]. For the shading of numerous foods stock wherein an egg-yellow shade is required, its hue is simply excessively unpracticed and each other more orange shading wishes to be utilized as a part of total to enhance this impact.

Aside from shading, it is moreover utilized as a part of healthy skin and hair mind restorative stock as it's far antibacterial in nature. It is in like manner used in Ayurvedic solution as pain relieving, calming, antitumor, antiallergic, cell reinforcement, disinfectant, in treating iron deficiency, diabetes, heartburn, gallstones, foods harming, animosity flow.

Turmeric (*Curcuma longa* L.) is one of the most essential spices used all over the world. It is called "the golden spice of life". Turmeric can be the "cheap saffron" for common people because it has same yellow coloring cost effectively. It can be used as substitute to replace saffron. Tropical and subtropical climate in countries and region are suitable for this plant widely cultivated. Turmeric is known for its aromatic rhizomes and is commonly used as a condiment, preservative, flavoring and coloring agent or in folk medicine [4]. In some research, there is presence of phenolic compound which classified as curcuminoids in the turmeric.

II. METHODOLOGY

A. Materials

The preparation of extracted color is different by using supercritical fluid extraction and hydro-distillation. The method to observe the stability of the colorants is by testing on their storage time, light and temperature. The colorants were store in different condition and temperature for stability test. The food products were test by using the extracted colorant in preparation of muffins and observed by its color, taste and texture. And lastly to study the toxicity, the extracted colorants were tested if there were any metal that harmful to human body.

B. Experimental

The colors need to be extracted before stability test by using supercritical fluid extraction and hydro-distillation.

For supercritical fluid extraction, the samples were prepared by drying the carrot and turmeric and grind it until reach size of 600 μ m. After the samples preparations, Approximately 50 g of dried carrot and turmeric respectively were introduced into the basket of the extraction vessel. The extraction vessel including the basket of carrot or turmeric is purged with CO₂ for 2 min to remove any moisture or impurities from the pump filter [9]. After purging, the exit valve is closed and the water batch is heated to the required temperature using the thermostat, and then pressure is set to the desired value using the control panel of the SF10 pump. Time is recorded upon reaching the desired temperature and pressure, where the extraction can take place [7].

For hydro-distillation, the small pieces of carrot or turmeric placed in a blender with 500 ml of water and blended well. After that, another 500 ml of water was added to mix and blended it again. The mixture that had been blend later was transferred to hydro-distillation (Clevenger- type) flask, resulting in total mixture 1500 ml of total water and 300 g of sample mix with ratio of water to sample (5:1). The distillation was run until 40ml of color collected. Samples were collected then kept in a freezer at -5°C before run for further analysis.

After the extraction of color using supercritical fluid extraction (SFE) and hydro-distillation, the stability of the color were test based on its storage time, light and temperature. But unfortunately,

the extraction by supercritical fluid extraction extracted small amount and not enough for further tests.

For evaluation of the colorants, the colorants will be tested:

- Light test
- Storage
- Temperature
- Food product
- Toxicity (trace element and heavy metals)

The light tests were examined by preparing 4 samples bottles and color that had extracted by SFE and hydro-distillation were filled about 20 ml each. 2 sample bottles were placed with presence of light and 2 without presence of light at room temperature. The samples were test with spectrophotometer at absorbance 600 nm.

For storage test, 4 samples bottles were prepared and color that had extracted by SFE and hydro-distillation were filled about 20 ml each. 2 sample bottles were wrapped using aluminum foil and another 2 did not. One from each samples (wrapped and unwrapped), were kept in refrigerator at 4°C. Another 2 from each samples (wrapped and unwrapped) were kept in open area at room temperature. The samples were test with spectrophotometer at absorbance 600 nm

For temperature test, 6 samples bottles were prepared and color that had extracted by SFE and hydro-distillation were filled about 20 ml each. Each samples were heated at different temperature (25, 30, 40, 50, 60, 70) degree Celcius. The samples were test on the color intensity by using spectrophotometer at absorbance 520 nm. The light, storage and temperature tests were examined for at day 7,14,21,28 for one month period and pictures were taken each day of test.

The food products were tested by using the extracted color in in the making of the muffins. The parameters measured using the sample muffins are colors, texture and taste of the muffin. After the baking of the muffins, few parameters were measured. Colors of the muffins were observed and pictures were taken for comparison of color intensity. For texture and taste of the muffins, about 5 people were volunteered to taste and observed the texture. There will be screening of taste and texture range from 1 to 5 for example; 1 for very bad to 5 very good.



Figure 1: Colorant Added In Baking Mixture

The toxicity was tested to ensure either the color will harm the human body or not. It was determined by measured the metals in the color extracted. The sample preparation for trace and heavy metal were ground to a fine powder and dried at 55-70°C for 6-8 h in a controlled environment, to remove moisture [3]. Accurately weighed sample of 3.0 g immediately after drying and placed in a flask and treated with 3 ml of concentrated HNO₃ for 4-5 hour. A mixture HNO₃ and HClO₄ in a ratio of 2:1 (3 ml/g of sample) was added.

The mixture then been heated at 120-130°C for 5-6 h, until fumes stop and until the resulting solution is clear. Then, 10 ml of milli-Q

water was added and boiled again for 10-15 min and the volume was reduced to half, cooled to room temperature and filtered using Whatman filter paper no. 42. The filtrate was mixed and increases the volume to 50 ml with Milli-Q water. A blank was also prepared for every sample in the same way. The experiment was repeated for several times.

The atomic absorption measurements were performed using Thermofisher AA 303 atomic absorption spectrophotometer with hollow cathode lamp light source. For analysis for all metals, oxy-acetylene flame was used. Mercury, Hg were determined using hydride generator, where the metal will convert into their volatile hydride forms using sodium borohydride and concentrated HCl. The vapour of hydride generated in the system was sent to the optical cell using peristaltic pump. Determination of Hg was carried out by the way of cold analysis. The standard instrumental configuration and experimental condition maintained for the analysis of Nickel Ni, Lead Pb and Mercury Hg (Nema, Maity, Sarkar, & Mukherjee, 2014).

III. RESULTS AND DISCUSSION

A. Light Test

Light Test for Orange Colorant

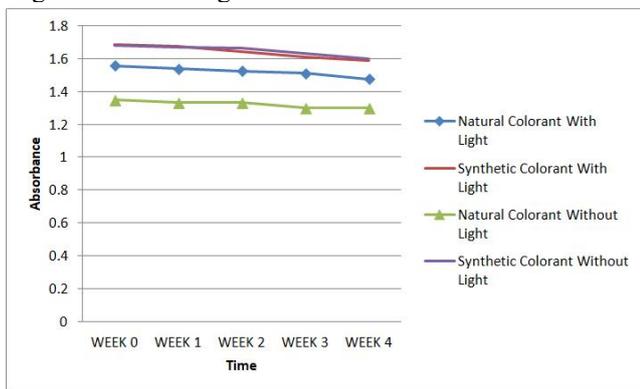
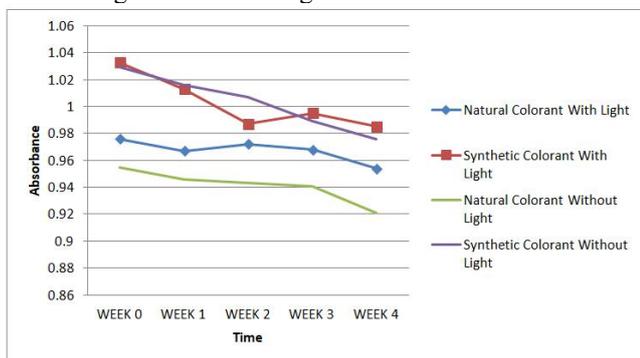


Table 4: Light Test for Orange Colorant

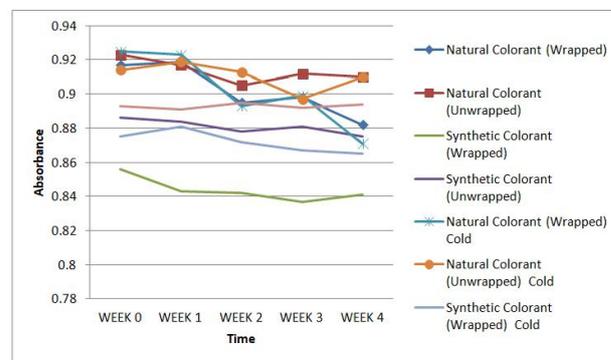
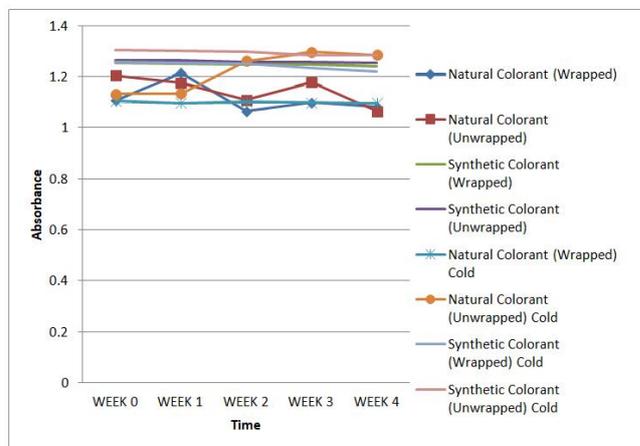


The different between these two conditions of the natural orange colorants is the percent of the absorbance decreasing. At presence of light, the orange natural colorant decreased by 5% meanwhile without the presence of light is 3.7%. The synthetic orange colorants the percentage decreased 5.7% with presence of light and 5% without presence of light. In the other hand, the percentage of the yellow natural colorant at presence of light decreased by 3.5% and without presence of light is 2.25%. The synthetic yellow colorants percentage decreased by 5% with presence of light and 4.6% without presence of light.

The results were supporting previous research, which the light transmitted through the colorant increased as the

absorbance value decreased also known as photo-degradation. It means that the color started to fade away after been exposed too much of lights. The exposure of lights effects the amount of caretenoids and curcuminoids in both natural colorants (Angkana et al., 2008). The photo-degradation of both caretenoid and curcuminoids can be reduced when kept in dark. If the natural colorants compared to the synthetic one, the percentage decreased is a bit higher. This shows that the stability of the natural colorants in terms of light is lower than the synthetic one

B. Storage

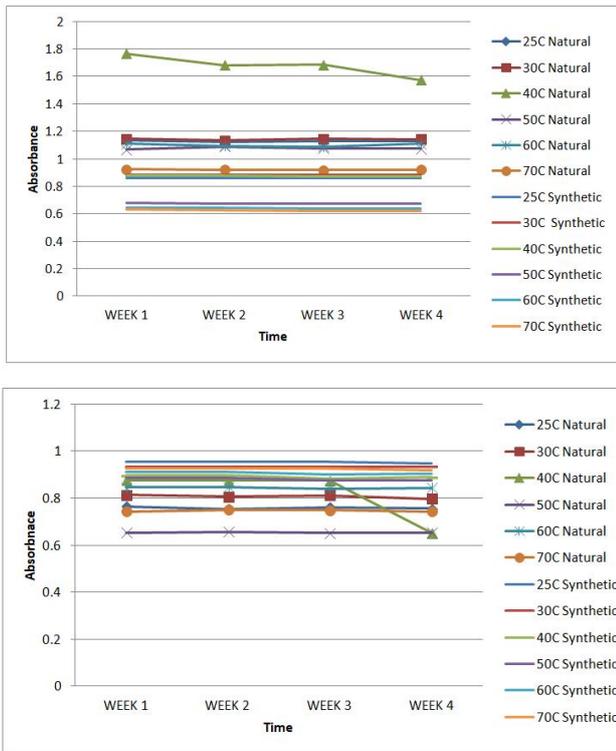


The different in this test based storage in room and cold temperature, with and without presence of light. Both orange and yellow colorants are affected by different conditions.

As the colorants kept in the cold temperature, the stability of the colorants can be sustained for longer time especially with no presence of light. The caretenoid level tend to remain unchanged in low temperature conditions. but different with yellow natural colorants, the curcuminoids tend to decreased in low temperature condition [11]. The wrapped and unwrapped condition is same as the light test previously. As the exposure of light increased, the absorbance decreased and effect the pigment of the colorants to become less pigment over time.

Compared to the synthetic colorants, the colorants tend to sustain compared to the natural one. this is because the synthetic colorants of orange, sunset yellow FCF and yellow synthetic colorants contain tartrazine are not affected so much by temperature. The molecule of both synthetic colorants remained the same. The change over time is because the synthetic coloring was already dissolved in water and dilution of it made it unstable in different condition over time.

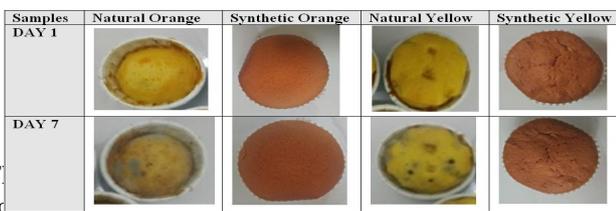
C. Temperature



The different of temperature conditions do effects the stability of both natural and synthetic colorants. The caretenoids in natural orange colorants and curcuminoids in natural yellow colorants are easily change in different temperature conditions. According to Angkana (2008), the caretenoids tend to decrease in low temperature conditions while the curcuminoid increase in low temperature condition. So the colorants were measured in different temperature 25°C, 30°C, 40°C, 50°C, 60°C and 70°C.

The higher the temperature condition, the higher the absorbance of the colorants decreased. Based on the result the temperature of the natural orange colorant only clearly decreased at temperature 25°C. But at other temperatures the value of absorbance is slightly unchanged. Same goes to the synthetic one and natural yellow colorant. The synthetic yellow colorants shows different results which the decreased of absorbance can be seen after week 4. The result for the orange colorants supposed to increase as the temperature increased but it had decreased. Compared to the yellow colorants which supported the study by Angkana (2008) as the lower the temperature the lower the value of cucuminoids.

D. Food Product



The food products started to spoil day 6 but not fully spoiled at day 7. The texture changing slowly compared to food product with natural colorant. The texture remained soft and firm till day 7 same goes with the smell. The smell of the food

products remained good until day 6. There were presence of microorganism at day 6 but not so many compared to the food product with natural colorants. That is why the food product with synthetic colorant take some time to spoil.

The content of the food products that contained natural colorants tend to spoil faster than synthetic colorants. The caretenoid in the colorant slowly degrade. The degradation of caretenoids effect the structure of the food cellular and severity[15]. Other than that, it is easily spoiled as the natural colorants are sensitive to the environment. The bright color like orange is prefer to acidic environment to have a longer shelf-life. That is why the food products with the natural coloranrs easily degrade and spoiled compared to synthetic one

E. Toxicity

| Colorant | Elements | | | | |
|-------------|-----------------------|-------|--------|---------------------|-----|
| | Trace Element (mg/gr) | | | Heavy Metal (mg/gr) | |
| | Cu | Ni | Fe | Pb | Hg |
| Orange (NC) | 0.571 | 0.338 | 5.543 | 0.069 | N/A |
| Orange (SC) | 2.775 | 5.178 | 10.564 | 1.376 | N/A |
| Yellow (NC) | 0.005 | 0.413 | 6.754 | 0.092 | N/A |
| Yellow (SC) | 3.132 | 6.989 | 9.113 | 1.453 | N/A |

*NC = Natural Colorant *SC = Synthetic Colorant

The Copper (Cu) content varies from 0.005 to 3.132 mg/gr. The colorants with low concentrations of Cu is natural orange and yellow colorant which are 0.005 mg/gr and 0.571 mg/gr respectively. The synthetic orange and yellow colorants have high Cu concentration of 2.775 mg/gr and 3.132 mg/gr. For human consumption, 10 ppm is the acceptable limit. So both synthetic and natural colorans are safe for used but natural one is way more better. High consumption can lead to hypertension, uremias and sporadic fever [5].

The Nickel (Ni) content varies from 0.338 to 6.989 mg/gr. The colorants with low concentrations of Ni is natural orange and yellow colorant which are 0.338 mg/gr and 0.413 mg/gr respectively. The synthetic orange and yellow colorants have high Ni concentration of 5.178 mg/gr and 6.898 mg/gr. The safe amount in human is 3 to 7 mg/day. Excess of Ni in human can lead to asthma, nausea or epidemiological symptoms like cancer of nasal cavity [6].

The safe amount of Iron (Fe) for human consumptions is 8 to 11 mg/day. The Nickel (Ni) content varies from 5.543 to 10.564 mg/gr. The colorants with low concentrations of Fe is natural orange and yellow colorant which are 5.543 mg/gr and 6.754 mg/gr respectively. The synthetic orange and yellow colorants have high Ni concentration of 10.564 mg/gr and 9.113 mg/gr. Low intake of Fe may cause anemia, tiredness and pallid physique, while high intake may results into hepatic megaly, cardiac infraction and nephric malfunction [6].

During the present study, Lead (Pb) content varies from 0.069 to 1.453 mg/gr, which falls within safety limit at 1.5 ppm for human consumption. The colorants with low concentrations of Fe are natural orange and yellow colorants which are 0.069 mg/gr and 0.092 mg/gr respectively. The synthetic orange and yellow colorants have high Pb concentration of 1.376 mg/gr and 1.453 mg/gr. Todd (1996) emphasized that most of the accumulated Lead is sequestered in the bones and teeth. This causes brittle bones and weakness in the wrists and fingers. Lead that is stored in bones can reenter the blood stream during periods of increased bone mineral recycling [13].

Even though both colorants are safe for used because there are still within the acceptable limit of human consumptions, the both

natural colorants are way better than the synthetic one. This is because the concentration of the heavy metals of natural colorants is lower than the synthetic one. This shows that the natural colorants are safer than synthetic one.

IV. CONCLUSION

To extract the natural colorants, hydro-distillations is preferable than supercritical fluid extraction because the amount of colorants is higher using hydro-distillation. Natural colorant can be good colorants than synthetic one for food product but further studies needed to improve the stability of the pigment natural colorants. The stability of both orange and yellow natural colorants are more easily affected by different conditions. Light and temperature are conditions which are affecting the stability of the colorants. Presence of light and changes of temperature can effects the loss of pigment of the colorants. Other than that, both orange and yellow colorants are safer than synthetic colorants. Both natural and synthetic colorants are safe because its element and heavy metals concentrations are still within the acceptable limit for human consumptions. Even though both are safe, the synthetic colorants have higher concentration of metals compared to natural colorants.

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