



Assiut University  
Faculty of Agriculture  
Food Science and Technology Department



# Utilization of Some Food Processing Wastes as a Source of Natural Pigments

*By*

**Hamada Khalaf Hassan Megali**

*B.Sc. Agric. Sci. (Food Science and Technology)  
Faculty of Agriculture, Assiut University (2010)*

**A THESIS**

*Submitted in Partial Fulfillment of the  
Requirements for the Degree of  
Master of Science*

*In Agricultural Sciences  
(Food Science and Technology)*

*Supervised by:*

**Prof. Dr. Soumia Mohamed Ibrahim Darwish (Main supervisor)**

Professor of Food Science and Technology, Faculty of Agriculture, Assiut University.

**Dr. Hassan Ismail  
Abd El-Hakim Ahmed**

Senior Researcher at Food Technology Research Institute, Agricultural Research Center, Giza

**Dr. Mokhless Ahmed  
Mohamed Abd-El-Rahman**

Lecturer of Food Science and Technology, Faculty of Agriculture, Assiut University

**2019**

## CONTENTS

<b>Subjects</b>	<b>Page</b>
<b>1. INTRODUCTION</b>	1
<b>2. REVIEW OF LITERATURE</b>	7
<b>2.1- Color and colorants in foods</b>	7
2.1.1 - Color	7
2.1.2 - Food colorants	7
2.1.3 - Classification of colorants	8
2.1.3.1 - Synthetic colorants	8
2.1.3.1.1 - Negative impacts of synthetic food colorants	9
2.1.3.2 - Natural colorants	10
<b>2.2- Natural pigments from food wastes</b>	11
<b>2.3- Chemical composition of raw materials</b>	11
2.3.1 - Purple carrot pomace	11
2.3.2 - Orange peels	12
2.3.3 - Tomato peels	13
<b>2.4- Mineral content of raw materials</b>	13
2.4.1 - Purple carrot pomace	13
2.4.2 - Orange peels	14
2.4.3 - Tomato peels	14
<b>2.5- Bioactive compound of raw materials</b>	14
2.5.1 - Purple carrot pomace	14
2.5.2 - Orange peels	15
2.5.3 - Tomato peels	15
<b>2.6- Red Pigments</b>	16
<b>2.6.1 - Anthocyanin</b>	16
2.6.1.1 - Structure	16
2.6.1.2- Anthocyanin extraction	20
2.6.1.3 - Identification of anthocyanin for purple carrots by HPLC	21
2.6.1.4- Anthocyanin stability	22
2.6.1.4.1- Anthocyanin stability as affected by pH	22
2.6.1.4.2- Anthocyanin stability as affected by thermal processing	23
2.6.1.4.3 - Anthocyanin stability as affected by light	23
2.6.1.5 - Application of anthocyanin pigment	24
<b>2.7- The yellow pigment.</b>	25
<b>2.7.1 - Carotenoids (<math>\beta</math>-carotene)</b>	25

Subjects	Page
2.7.1.1 - Structure	25
2.7.1.2 - Carotenoids ( $\beta$ -carotene) extraction	27
2.7.1.3 - Identification of carotenoids ( $\beta$ -carotene) from orange peels by HPLC	29
2.7.1.4 - Carotenoids ( $\beta$ -carotene) stability	29
2.7.1.4.1 - Carotenoids ( $\beta$ -carotene) stability as affected by pH	30
2.7.1.4.2 - Carotenoids ( $\beta$ -carotene) stability as affected by heat	32
2.7.1.4.3 - Carotenoids ( $\beta$ -carotene) stability as affected by light	32
2.7.1.4.4 - Effect of crude $\beta$ -carotene extracts addition on sunflower oil oxidative stability	33
2.7.1.5 - Application of carotenoids ( $\beta$ -carotene) pigment	33
<b>2.8.1– Lycopene</b>	34
2.8.1.1 – Structure	34
2.8.1.2 - Lycopene extraction	35
2.8.1.3 - Identification of lycopene extracted from tomato peels by HPLC	36
2.8.1.4 – Lycopene stability	37
2.8.1.4.1 - Lycopene stability as affected by pH	37
2.8.1.4.2 - Lycopene stability as affected by heat	38
2.8.1.4.3 - Lycopene stability as affected by light	39
2.8.1.4.4 - Effect of crude lycopene extracts addition on sunflower oil oxidative stability	39
2.8.1.5 - Application of Lycopene pigment	40
<b>3.MATERIALS AND METHODS</b>	41
<b>3.1- Materials</b>	41
3.1.1 - Raw Materials	41
3.1.2 - Chemicals and reagents	41
<b>3.2- Methods</b>	41
<b>3.2.1- Preparation of samples</b>	41
<b>3.2.2- Analytical methods</b>	42
3.2.2.1 - Chemical composition of raw material	42
3.2.2.2 - Total carbohydrates	42
3.2.2.3 - Minerals determination of raw materials	42
3.2.2.4 - Determination of total phenolic compounds of raw materials	42
3.2.2.5 - Determination of total flavonoids of raw materials	43

Subjects	Page
3.2.2.6 - Determination of antioxidant activity of raw materials	43
<b>3.2.3- Extraction of natural pigments from raw materials</b>	44
3.2.3.1- Extraction of anthocyanin from purple carrot pomace	44
3.2.3.2 - Extraction of $\beta$ -carotene from orange peels	44
3.2.3.3 - Extraction of lycopene from tomato peels	45
<b>3.2.4- Determination of pigments from raw materials</b>	45
3.2.4.1 - Determination of total anthocyanin content from purple carrot pomace	45
3.2.4.2 - Identification and quantification of anthocyanin	46
3.2.4.3 - Determination of carotenoids ( $\beta$ -carotene) content from orange peels	47
3.2.4.4 - Identification and quantification of carotenoids ( $\beta$ -carotene)	47
3.2.4.5 - Determination of lycopene content from tomato peels	48
3.2.4.6 - Identification and quantification of lycopene	48
<b>3.2.5- The adsorption of concentrated pigments on solid supports</b>	48
<b>3.2.6- Stability of anthocyanin, carotenoids (<math>\beta</math>-carotene) and lycopene</b>	49
<b>3.2.6.1 - Stability of anthocyanin</b>	49
3.2.6.1.1 - Effect of pH	49
3.2.6.1.2 - Effect of temperature	49
3.2.6.1.3 - Effect of light	49
<b>3.2.6.2 - Stability of carotenoids (<math>\beta</math>-carotene)</b>	50
3.2.6.2.1 - Effect of pH	50
3.2.6.2.2 - Effect of temperature	50
3.2.6.2.3 - Effect of light	50
<b>3.2.6.3 - Stability of lycopene</b>	50
3.2.6.3.1 - Effect of pH	50
3.2.6.3.2 - Effect of temperature	51
3.2.6.3.3 - Effect of light	51
<b>3.2.7- Rancimat method from orange and tomato peels</b>	51
<b>3.3- Technological methods</b>	52
<b>3.3.1- Preparation of Jam</b>	52
3.3.1.1 - Effect of storage on natural red pigment stability from purple carrot pomace	52

Subjects	Page
<b>3.3.2- Manufacture of ice cream mix</b>	52
3.3.2.1 - Effect of storage at (– 18°C) on natural pigment stability from orange peels	53
<b>3.3.3- Preparation of beef burgers</b>	53
3.3.3.1 - Effect of storage at (– 18°C) on natural pigment stability from tomato peels	54
<b>4.4- Color measurements</b>	54
<b>5.5- Sensory Evaluation</b>	55
<b>6.6- Statistical Analysis</b>	55
<b>4. RESULTS AND DISCUSSIONS</b>	56
<b>4.1- Chemical composition of purple carrot pomace, orange and tomato peels</b>	56
<b>4.2- Mineral content of purple carrot pomace, orange and tomato peels</b>	57
<b>4.3- Bioactive compounds of purple carrot pomace, orange and tomato peels</b>	59
<b>4.4- Identification of pigments</b>	60
4.4.1 - Identification of anthocyanin for purple carrot pomace by HPLC	60
4.4.2 - Identification of $\beta$ -carotene for orange peels by HPLC	62
4.4.3 - Identification of lycopene for tomato peels by HPLC	63
<b>4.5- Stability of extraction natural pigments</b>	64
<b>4.5.1- Stability of anthocyanin pigment</b>	64
4.5.1.1 - Effect of pH on retention of anthocyanin pigment extracted from purple carrot pomace	64
4.5.1.2- Effect of temperature degrees on retention of anthocyanin pigment extracted from purple carrot pomace	66
4.5.1.3 - Effect of light on stability of natural anthocyanin pigment	68
<b>4.5.2- Stability of <math>\beta</math>- carotene pigment.</b>	69
4.5.2.1 - Effect of pH on retention of $\beta$ - carotene pigment extracted from orange peels.	69
4.5.2.2 - Effect of different temperature degrees on $\beta$ -carotene pigment stability under different pH values	70
4.5.2.3 - Effect of time exposed to heat on $\beta$ -carotene stability at different pH values at 100°C	72

## **Contents**

---

<b>Subjects</b>	<b>Page</b>
4.5.2.4 - Effect of light on stability of natural $\beta$ - carotene pigment	73
<b>4.5.3- Stability of lycopene pigment.</b>	74
4.5.3.1 - Effect of pH on retention of lycopene pigment extracted from tomato peels	74
4.5.3.2- Effect of different temperature degrees on lycopene pigment stability under different pH values	76
4.5.3.3 - Effect of time exposed to heat on lycopene stability at different pH values at 100°C	77
4.5.3.4 - Effect of light on stability of natural lycopene pigment	78
<b>4.6- Rancimat method</b>	80
4.6.1- Effect of crude $\beta$ -carotene extract addition on sunflower oil oxidative stability	80
4.6.2 - Effect of crude lycopene extract addition on sunflower oil oxidative stability	81
<b>4.7- Sensory evaluation of processed products with natural pigments</b>	83
4.7.1 - Sensory evaluation of guava jam with natural anthocyanin pigment	83
4.7.2 - Sensory evaluation of ice cream with natural $\beta$ - carotene pigment	84
4.7.3 - Sensory evaluation of beef burger with natural lycopene pigment	85
<b>4.8- Color measurements of processed products with natural pigments</b>	87
4.8.1 - Color measurements of guava jam	87
4.8.2 - Color measurements of ice cream	89
4.8.3 - Color measurements of beef burger	92
<b>4.9- Effect of storage on color intensity of pigment products</b>	94
4.9.1 - Effect of storage on color intensity of guava jam	94
4.9.2 - Effect of storage on stability of $\beta$ - carotene pigments in ice cream at -18 °C	95
4.9.3 - Effect of storage on stability of lycopene pigments in beef burger at -18 °C	97
<b>5. SUMMARY</b>	99
<b>6. REFERENCES</b>	107
<b>7. ARABIC SUMMARY</b>	-

**LIST OF TABLES**

<b>Table No.</b>	<b>Title</b>	<b>Page</b>
1	Chemical composition of purple carrot pomace, orange and tomato peels (on wet weight basis)	57
2	Mineral content of purple carrot pomace, orange and tomato peels	58
3	Bioactive compounds of purple carrot pomace, orange and tomato peels	60
4	Identification of anthocyanin from purple carrot pomace by HPLC	61
5	Identification of lycopene from tomato peels by HPLC	63
6	Effect of pH on retention of anthocyanin pigment extracted from purple carrot pomace	65
7	Effect of different temperature on the retention % of anthocyanin pigment extracted from purple carrot pomace	67
8	Effect of light on stability of natural anthocyanin pigment	68
9	Effect of pH on retention of $\beta$ -carotene pigment extracted from orange peels	70
10	Effect of different temperature degrees on $\beta$ -carotene pigment stability under different pH values	71
11	Effect of time exposed to heat on $\beta$ -carotene stability at different pH values at 100°C	73
12	Effect of light on stability of natural $\beta$ - carotene pigment	74
13	Effect of pH on retention of lycopene pigment extracted from tomato peels	75
14	Effect of different temperature degrees on lycopene pigment stability under different pH values	76
15	Effect of time exposed to heat on lycopene stability at different pH values at 100°C	78
16	Effect of light on stability of natural lycopene pigment	79
17	Effect of crude $\beta$ -carotene extract addition on sunflower oil oxidative stability	81
18	Effect of crude lycopene extract addition on sunflower oil oxidative stability	82
19	Sensory evaluation of guava jam with natural anthocyanin pigment	84
20	Sensory evaluation of ice cream with natural $\beta$ –carotene pigment	85

## ***List of Tables***

---

<b>Table No.</b>	<b>Title</b>	<b>Page</b>
21	Sensory evaluation of beef burger with natural lycopene pigment	86
22	Color measurements of guava jam	89
23	Color measurements of ice cream	91
24	Color measurements of beef burger	94
25	Effect of storage on color intensity of guava jam	95
26	Effect of storage on stability of $\beta$ - carotene pigment in ice cream at -18 °C	96
27	Effect of storage on stability of lycopene pigment in beef burger at -18 °C	97



## LIST OF FIGURES

<b>Fig. No.</b>	<b>Title</b>	<b>Page</b>
1	General anthocyanin structure	17
2	Structural transformations of anthocyanin in aqueous medium with different pH	18
3	Chemical changes of anthocyanin during processing and storage of foods	19
4	$\beta$ -carotene structure	26
5	Carotenoids classifications	27
6	Mechanisms of carotenoid oxidation and initial products	31
7	Lycopene structure	35
8	Identification of anthocyanin pigment compounds extracted from purple carrot pomace	61
9	Identification of $\beta$ - carotene pigment compounds extracted from orange peels by HPLC.	62
10	Identification of lycopene pigment compounds extracted from tomato peels	64

### **6. SUMMARY**

Fruit and vegetable wastes and their by-products are remaining in great amounts during industrial processing and hence represent a serious problem, as they exert harmful impact on the environment. So, they need to be managed or they can be exploited. This study was performed to investigate the possibility of utilization of some food processing wastes to produce some natural pigments and using of these pigments for coloring some food products instead of artificial colors to avoid the bad effects on human health and to reduce pollution in the production lines during food manufacturing stages. In this investigation three raw materials were used, as food processing wastes, namely purple carrot pomace (as a source of anthocyanin pigment), Tomato peels (as a source of lycopene pigment), and orange peels (as a source of  $\beta$ -carotene pigment). This can be illustrated by the results that could be summarized as follows:

- **Chemical composition of purple carrot pomace, orange and tomato peels.**

The results showed that purple carrot pomace had approximately the highest total carbohydrate content recorded 77.71 % followed by tomato and orange peels (72.24 and 68.11%, respectively). As well as, tomato peels contained the highest amount of crude fiber (37.36%) followed by purple carrot pomace and orange peels (11.93 and 10.73%, respectively). As for, the mineral content of raw materials, it could be concluded that, purple carrot pomace and

## ***Summary***

---

tomato peels are considered as a good source of potassium and calcium (2340 and 1660 mg/100g, respectively, whereas, calcium is the predominant mineral in orange peels (1140 and 1030 mg/100g, respectively). For bioactive compounds and antioxidant activity in raw materials. The results show that orange peels and purple carrot pomace had the highest content of total phenolic (16.12 and 10.08 mg /g, respectively) and antioxidant activity (88.65 and 86.48 %, respectively). Meanwhile, tomato peels had the lowest content of total phenolic (3.75 mg/g), flavonoids content (0.97 mg/g) and antioxidant activity (38.31%).

- **Extraction of natural pigments.**

Anthocyanin pigment was extracted from the purple carrot by 4% citric acid, which is a good source of anthocyanin (130.54 mg / 100 g on dry weight basis).  $\beta$ - carotene was extracted from the orange peels by acetone, and the amount of  $\beta$ -carotene extracted was 14.15 mg / 100 g dry weight. Also, Lycopene was extracted from tomato peels with acetone and the extracted amount was 3.75 mg / 100 g on dry weight.

- **Identification of natural pigments by HPLC.**

Anthocyanin spectrometry and separation showed that there are two main anthocyanin extracted from purple carrot pomace, cyanidin 3-glucoside (62.27  $\mu$ g/ml) and delphinidin (39.46  $\mu$ g/ml).  $\beta$ -carotene, extracted from orange peels, was also identified and contained an important compound,  $\beta$ -carotene. Lycopene extracted

## ***Summary***

---

from tomato peels has also been identified and spectral measurements and separation show that there are two major lycopene compounds, lycopene and  $\beta$ -carotene ( 2.28 and 1.80  $\mu\text{g/ml}$ , respectively)

- **Study the factors affecting the stability of natural pigments.**
- **Stability of anthocyanin pigment extracted from purple carrot pomace.**

The effect of different pH values on the stability of the anthocyanin pigment using nine pH values (2, 3, 4, 5, 6, 7, 8, 9, 10) was studied. The results showed that the stability of the anthocyanin pigment was more stable at pH values (2 to 5), while the highest decrease occurred at pH 7. The effect of different temperature degrees (40°C, 60°C, 80°C, 100°C) at different time intervals (30, 60, 90, 120 min.) on the stability of the anthocyanin pigment was studied. The anthocyanin extract was more stable at 40 and 60 ° C for 30 to 120 minutes, while at 80 and 100 ° C for 30 to 120 minutes, the anthocyanin content decreased. It was observed that the rate of degradation of the anthocyanin pigment increased with the increase of the temperature of heating and the exposure period to these degrees, which leads to the faster deterioration of color during heating. The effect of light at different time intervals (1, 2, 3, 4, 5, 6 hours) on pigment stability and the light is a factor affecting anthocyanin pigment stability. The obtained results showed that anthocyanin decompose slowly in the dark recorded 5.13 % and degrade with high rate under lighting conditions recorded (7.05 %) for 6 hours, which accelerates the degradation of anthocyanin.

- **Stability of  $\beta$ -carotene pigment extracted from orange peels.**

The effect of different pH values (2, 3, 4, 5, 6, 7, 8, 9, 10) on the stability of  $\beta$ -carotene has been studied. The obtained results showed that the rate of degradation of the pigment increased with a decrease in the pH values. This indicates that the alkaline media pH 9 was highly effective in maintaining pigment stability. The effect of using different temperature degrees (30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C and 100°C) on the stability of the  $\beta$ -carotene pigment at different pH values (8, 10) has been studied. It was observed that the degradation of the pigment gradually increases with increasing temperature degree. The decrease in the rate of pigment degradation was even higher at higher pH values.

The effect of heat exposure time (30, 60, 90, 120 min.) at different pH values (8, 10) on the stability of  $\beta$ -carotene pigment was studied. It was observed that by increasing both the temperature and the exposure time, the pigment degradation was increased at the lower pH values. The effect of light at different time intervals (1, 2, 3, 4, 5, 6 hours) on pigment stability was studied. The results show that the pigment decomposes slowly in the dark reached to 6.25% and the degradation increases significantly under lighting conditions reached to 9.72% due to the effect of light on the acceleration of the pigment demolition.

- **Stability of lycopene pigment extracted from tomato peels.**

The effect of different pH values (2, 3, 4, 5, 6, 7, 8, 9, 10) on pigment stability was studied. The obtained results showed that lycopene was less stable in acid medium and more stable in alkaline medium. The effect of using different temperature degrees at (30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C, 100°C) on pigment stability under different pH values (8, 10). It was observed that the decrease in acidity increases the degradation of the pigment gradually with increasing temperature, and by studying the effect of heat exposure time (30, 60, 90, 120 min.) at different pH values (8, 10) on the stability of lycopene pigment.

It was observed that, when the pigment exposure time increased, there was an increase in the rate of degradation of lycopene at the lowest pH value and high temperature degree. The lowest degradation was recorded at low and medium temperature degrees and high pH values. The effect of light at different time intervals (1, 2, 3, 4, 5, 6 hours) on pigment stability was studied. It was concluded that lycopene slowly decomposes in the dark (5.64%) and strongly decomposes under light conditions (6.85%).

- **Study the stability of the pigment  $\beta$ -carotene and lycopene using Rancimat.**

The effect of crude  $\beta$ -carotene extract (100, 200, 300 ppm) on the oxidative stability of sunflower oil was studied. It was found that by adding different levels of pigment extracted from orange peels to

## ***Summary***

---

sunflower oil and comparing its oxidative stability compared to using BHT. It was found that the crude extract of  $\beta$ -carotene acts as a first antioxidant when it was present in high concentrations. The effect of the addition of crude lycopene extract (100, 200, 300 ppm) on the oxidative stability of sunflower oil. The results indicated that the crude lycopene extract at high concentrations acts as a first antioxidant, while lower concentrations of lycopene extract acted as an antioxidant and helped stabilize sunflower oil. Also, different concentrations of crude lycopene had antioxidant activity compared BHT.

### **• Sensory evaluation of natural colored products.**

Sensory evaluation of guava jam colored with natural anthocyanin pigment extract was evaluated in the following percentages (0%, 0.5%, 1.0%, and 1.5%). It was found that by adding the anthocyanin extract of 1.0% (the third treatment) scored the highest sensory evaluation of the jam and was favored by consumers. The addition of natural  $\beta$ -carotene to ice cream at 1.0% (4th treatment) was found to be widely accepted and favored by consumers compared with the artificial colored treatment and also the other treatments. As for, the sensory evaluation of beef burger stained with natural lycopene in the following percentages (0%, 0.5%, 1.0%, and 1.5%). In terms of color, taste and degree of overall acceptability compared with other treatments, as well as, added artificial color. Whereas, treatment (second treatment) without any addition was recorded to have lower scores in the sensory evaluation.

- **Color measurements for the products.**

The results showed that guava jam appeared in a distinctive bright red color due to the addition of anthocyanin. The simple change in the color of the guava jam produced by the use of the anthocyanin pigment indicates the suitability of these processes to produce high quality and palatable products. Also, there was a clear effect of adding  $\beta$ -carotene to the properties of ice cream color and giving a more yellow colored product than ice cream samples without adding the pigment, as well as, the use of lycopene pigment to produce meat burger led to a bright red color than the meat burger samples without Add lycopene pigment.

- **Effect of storage on the stability of natural pigments in jam, ice cream and burger.**

The addition of anthocyanin extract at room temperature and 4°C by 1% resulted in the production of guava jam, which was the most acceptable and recorded the highest sensory quality standards compared to other concentrations, followed by the treatment which the pigment was added by 0.5%. It was observed that the effect of storage on the color density of guava jam and the rate of low color density was higher in samples stored at room temperature compared to those stored at 4°C.

The effect of storage on the stability of  $\beta$ -carotene in ice cream at -18°C. A slight degradation of the pigment added was clearly observed during the ice-cream storage period for 21 days recorded



## *Summary*

---

4.69% during storage period under freezing conditions. The effect of storage on the stability of lycopene in meat burger stored at -18°C. From the obtained results it was observed that during storage for 12 weeks for the meat burger samples added the lycopene pigment deterioration of the added pigment recorded 11.49% during storage under freezing conditions. The main cause of the degradation of natural lycopene in foods is the oxidation process.

Finally, it could be clearly concluded through this study, that, it is practicable, available, economical and hence successful to the utilization of some food processing wastes to produce some natural pigments and using of these pigments for coloring some food products instead of artificial colors. This would lead to produce, high nutritional value, as well as, healthy products.