

## **EFFECT OF DRYING AND PRETREATMENT ON ANTHOCYANINS, FLAVENOIDS AND ASCORBIC ACID CONTENT OF BLACK CARROT (*DAUCUS CARROTA L.*)**

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### **Abstract**

This study was conducted to investigate the influence of drying air temperature of 40-60°C and pre-treatments such as hot water blanching, citric acid, KMS, and calcium chloride treatments on the total anthocyanins, flavonoids and ascorbic acid content of black carrot. An untreated dried sample was used as control. The result obtained shows optimum retention of anthocyanin at drying air temperature of 60°C in combination with Calcium chloride and 3 min blanching pre-treatments and for the control sample. Higher ascorbic acid retention was observed at lower drying air temperature of 40°C for all the pre-treated samples and the control, except for blanched sample where higher ascorbic acid was observed at 50°C. This confirmed the sensitivity of black carrot ascorbic acid to the higher drying air temperature of 50 and 60°C. Optimum retention of flavonoids content was also recorded at 50°C for control and pre-treated samples.

**Key words:** *Black carrot, pre-treatment, anthocyanin, flavonoids, ascorbic acid, Hot air drying.*

### **INTRODUCTION**

Carrot is one of the important root vegetables rich in many bioactive compounds such as carotenoid, flavonoids, citric acid and dietary fibre and many other component having significant health-promoting properties. The consumption of carrot is increasing steadily because of its recognition as good source of natural antioxidants having the anticancer activity [1]. Black or purple colour carrots with high anthocyanin content have possible to be used in food, nutraceutical and pharmaceutical preparations. The red, purple and blue colour carrots have high potential as colorant in food because of their low toxicity [2]. Commercial anthocyanins are mostly derived from fruit and vegetables including red grapes, blackberry, black carrot, red cabbage, radish and berry [3]. Carrot is an excellent source of beta carotenes which is reported to prevent cancer, vitamin A and potassium deficiency, and contain cholesterol lowering pectin, vitamin C, thiamine, vitamin B<sub>6</sub>, folic acid and magnesium. The dried form of carrot can be used in dehydrated soups and in form of powder in pastries and sauces [4]. Blanching is one of the most widely practice pre-treatment method during drying of fruit and vegetable because of it is ability to inactivate enzymes, lead to change in structure and shortening of the drying time. Calcium chloride can be used to reduce or prevent browning, and was reported to act by blocking the amino group thereby restraining them from entering into browning reaction [5]. The main target of drying is to reduce the moisture content thereby increasing the life time of products by limiting enzymatic and oxidative degradation. In addition to reducing the amount of water, the percentage of active principles is increased in relation to the total mass [6]. Black Carrot is a seasonal fruit which is highly perishable with short keeping quality especially in its fresh nature. However, its unique because of it high phytochemical content particularly the anthocyanin and other phenolic acids, that play an important role in reducing the disease risk. This phytochemical diversity present a major challenges to the area of functional food and nutraceutical research and development. This research was undertaken to investigate the effect of drying and various pre-treatment including hot water blanching and chemicals pre-treatment on the anthocyanin, flavonoids and Ascorbic acid content of dried black carrot.

## MATERIAL AND METHODS

### Sample preparation

The fresh and ripe Black carrots (*Daucus carota* L.), were obtained from Lovely Professional University farm. The roots were selected according to the uniformity of color. Samples were manually peeled using knife and shredded uniformly.

### Blanching

The sample blanching was done using a modified procedure of Goncalves et al., [7]. Samples were immersed in a thermostatic water bath ( $\pm 1$ ) at 98°C temperature, after reaching the pre-established time of 3 min. The ratio of sample weight to water volume was 150 g of fresh sample/300 mL water. During the isothermal heat treatment, the temperature was maintained by means of thermometer. After blanching, samples were cooled in iced water bath for 2 min. Excess water was removed before any further analysis. The experiment was replicated twice, and unblanched sample was taken as control.

### Chemical pre-treatment

Chemical treatments were done by soaking the samples in different chemical solutions reported from literatures [8] [9] [10] with modification as follows: (a) calcium chloride solution 1:2 (w/v) of 1 g  $\text{CaCl}_2$  to 1L water ratio for 15 min at room temperature. (b) 3.5 g citric acids in 1 L water for 15 min at room temperature (c) KMS solution 2:1L (w/v) water for 15 min at room temperature. All treatments were done in duplicate, and samples were drained before drying.

### Drying

The black carrot sample was subjected to drying in laboratory scale tray dryer (Labfit India Pvt. Ltd, Ahmadabad). The dryer is having five set of trays, equipped with fan and the temperature regulator. Drying experiment was conducted at 40, 50 and 60°C and samples were taken out of the dryer and weighed on digital balance at interval of 15 minutes for 1.5 hrs followed by 30 minutes interval until moisture content of 4-8 % was obtained when no further change in moisture content was observed. The weight of the sample was 150 g per trail, and each weighing process lasted for about 2-3 minutes.

### Anthocyanins assay

This test was performed according to the procedure given by Srivastava and Kumar [11]. A 10 g sample was blended with 10 ml of ethanolic HCl and then transferred to a 100 ml volumetric flask and the volume was made up to the mark. The sample extract was then stored in a refrigerator overnight at 4°C, filtered through whatman No. 1 filter paper and the optical density was recorded at 535 nm.

$$\text{Total O.D. / 100 g} = \frac{\text{OD} \times \text{Vol made up} \times 100}{\text{wt of sample taken}}$$

$$\text{Total anthocyanin content (mg /100 g of black carrot)} = \frac{\text{Total O.D./100g}}{98.2}$$

### Total flavonoids

The total flavonoid for the sample was estimated according to Chang *et al.*, [12]. The method is based on the formation of a complex flavonoids-aluminium having the absorptivity maximum at 415 nm, after remained react at room temperature for 30 min. The sample was extracted using 80% methanol using pestle and mortar, 0.5 mL of each extract (1:10 g/mL) in methanol was separately mixed with 1.5 mL of methanol solution, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. It remained at room temperature for 30 min, and the absorbance of the mixture was measured at 415 nm with UV-Visible Spectrophotometer (Sytronic 104 Model). The standard curve was prepared by using quercetin solutions at different concentration of 12.5 to 100 µg /ml in methanol. The concentration of flavonoids in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent/g of sample.

### Ascorbic acid assay

#### Standard curve

The requisite volume of the standard ascorbic acid solution 1, 2, 3, 4, and 5 ml was pipette into a test tubes and volume was made to 5 ml with the 2%  $\text{HPO}_3$ . 10 ml dye was added with a rapid delivery pipette, shake and reading was taken with 15 to 20 seconds with the Spectrophotometer at 518 nm using

a suitable filter. A 5 ml of  $\text{HPO}_3$  solution and 10 ml of water was used as blank. The graph of absorbance against concentration was plotted to obtain the concentration of the sample [13].

### Sample preparation

A 50 to 100 g of the sample was weighed, grinded using pestle and mortar with equal amount of  $\text{HPO}_3$  (6%  $\text{HPO}_3$  for dried sample). The volume of macerate was made to 100 ml.

### Sample analysis

A 5 ml sample extract (or less made to 5 ml with 2%  $\text{HPO}_3$ ) was taken in a test tube, 10 ml dye was added, shake and reading was taken immediately with 15 to 20 seconds at 518 nm.

$$\text{mg of ascorbic acid/100 g sample} = \frac{\text{Ascorbic acid content} \times \text{Vol make up} \times 100}{\text{ml of solution taken} \times 1000 \times \text{wt of sample taken}}$$

## RESULTS AND DISCUSSIONS

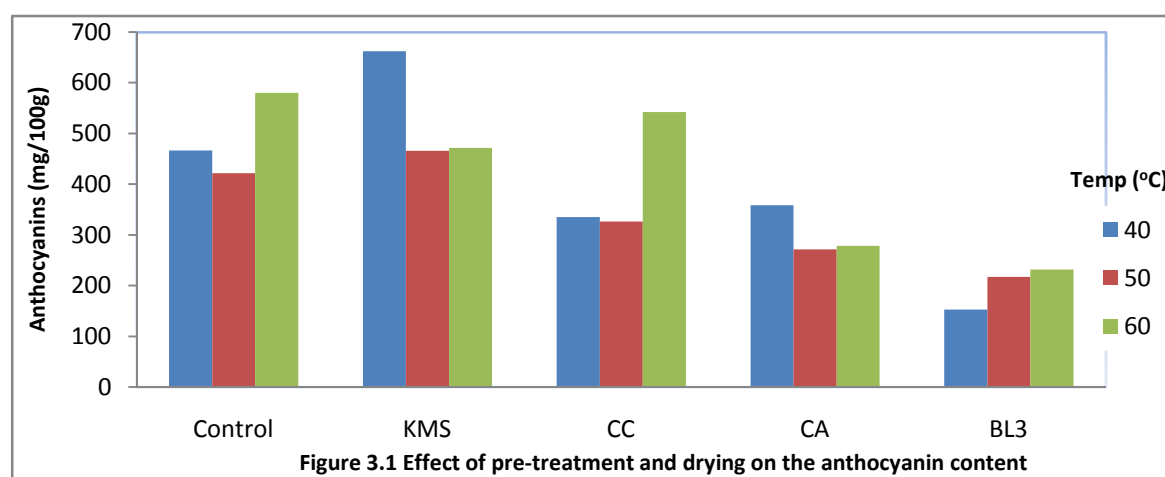
### 3.1 Effect on anthocyanin content

Many researchers investigated the degradation of anthocyanin pigment during thermal processing, which dramatically affect the colour and nutritional properties [14]. Anthocyanins like other polyphenols degrade enzymatically in the presence of polyphenol oxidises and glycosidase, that catalyses hydrolysis of anthocyanin to yield free sugar and a-glycone [15]. In this study, higher anthocyanin was recorded at higher temperature of  $60^\circ\text{C}$  for most of the samples including the control, calcium chloride treated, and 3 minutes blanched sample respectively. Except for the KMS and citric acid treated sample where maximum value was obtained at the lower temperature of  $40^\circ\text{C}$ . Although the anthocyanin was reported to degrade with higher temperature, the drying air temperature of  $60^\circ\text{C}$  was reported as the optimum for retention of most phenolic compounds [16] [10]. The anthocyanin from black carrot was reported to be relatively stable to heat and pH change compared to anthocyanins from other sources because of di-acylation of anthocyanin structure [14].

**Table 3.1** Effect pre-treatment and drying temperature on anthocyanins

Sample	Anthocyanin content (mg/100g)		
	$60^\circ\text{C}$	$50^\circ\text{C}$	$40^\circ\text{C}$
Control	$580.14 \pm 1.3$	$421.33 \pm 3.96$	$466.65 \pm 1.1$
KMS	$471.74 \pm 16.92$	$465.89 \pm 7.92$	$661.84 \pm 10.58$
CA	$278.51 \pm 7.2$	$271.64 \pm 6.12$	$358.71 \pm 14.76$
CC	$542.02 \pm 1.54$	$326.07 \pm 11.81$	$335.29 \pm 4.68$
BL3	$231.67 \pm 2.88$	$217.41 \pm 2.5$	$153.0 \pm 4.32$

Potassium metabisulphite (KMS), citric acid (CA), Calcium chloride(CC), and Blanched for 3 min at  $98^\circ\text{C}$  BL(3).The data presented are mean of the duplicate determination  $\pm$  standard deviation



The lowest anthocyanin content of  $231.67 \pm 2.88$  mg/100g was recorded for 3 min blanched sample dried at  $60^\circ\text{C}$ . A value of  $153.0 \pm 4.32$  mg/100g was also recorded at  $40^\circ\text{C}$  as compared to the control

sample with  $580.14 \pm 1.3$  at  $60^\circ\text{C}$  and  $466.65 \pm 1.1$  mg/100g at  $40^\circ\text{C}$ . Despite the fact that some authors reported that inclusion of mild heat treatment (at approximately  $50^\circ\text{C}$ ) can inactivate the degradation enzymes, the lower value recorded for the blanched sample was probability due to high leaching of the pigment observed during blanching. Wahyuningsih, recorded a decreased in anthocyanin content of red turi (*Sesbania grandiflora* L. (Pers) flower which was ascribed to the leaching of anthocyanin in the blanching media [17]. Heating was also reported to encourage cellular fluids, containing phytochemicals to diffuse from the plant cell to the water media. Thus, the phytochemical content after blanching is the net result of combined increased in extractivity, degradation and leaching [18].

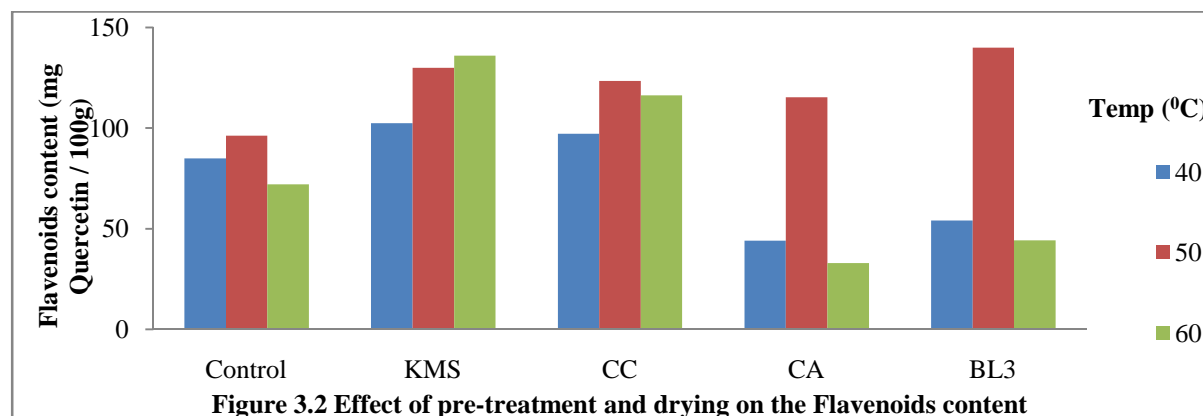
### 3.2 Effect on flavonoids content

The flavonoids content of all pretreated and control samples estimated from the standard plot of Quercetin ( $y = 10.19x + 0.00$ ,  $R^2 = 0.996$ ) were shown in Table 3.2. Higher flavonoids content was recorded at drying air temperature of  $50^\circ\text{C}$  for control and pretreated samples, except for the KMS blanched where higher content was recorded at  $60^\circ\text{C}$ . KMS, and calcium chloride treatment exhibited higher flavonoids content of 136 and 116.29 mg QE/ 100 g at  $60^\circ\text{C}$  and 219 and 115.33 mg QE/ 100 g at  $50^\circ\text{C}$ . The sample blanched at  $98^\circ\text{C}$  for 3 min has also exhibited higher flavonoids content at  $50^\circ\text{C}$ . In general, all the samples including the control exhibited higher retention of flavonoids at  $50^\circ\text{C}$ , and better retention when treated with the KMS and calcium chloride.

**Table 3.2.** Effect of pre-treatment and temperature on flavonoids content

Sample	Flavonoids content (mg/100g)		
	$60^\circ\text{C}$	$50^\circ\text{C}$	$40^\circ\text{C}$
Control	$72.13 \pm 4.48$	$96.17 \pm 3.43$	$85.01 \pm 3.43$
KMS	$136.06 \pm 1.53$	$219.14 \pm 1.53$	$96.6 \pm 4.17$
CA	$32.92 \pm 9.92$	$44.16 \pm 4.37$	$44.0 \pm 2.3$
CC	$116.29 \pm 4.86$	$115.33 \pm 6.55$	$97.15 \pm 7.01$
BL3	$44.16 \pm 4.37$	$139.9 \pm 1.7$	$54.1 \pm 12.26$

Potassium metabisulphite (Kms), citric acid (CA), Calcium chloride(CC), and Blanched for 3 min at  $98^\circ\text{C}$  BL(3). All data presented are mean of the duplicate determination  $\pm$  standard deviation



### 3.3 Effect on ascorbic acid content

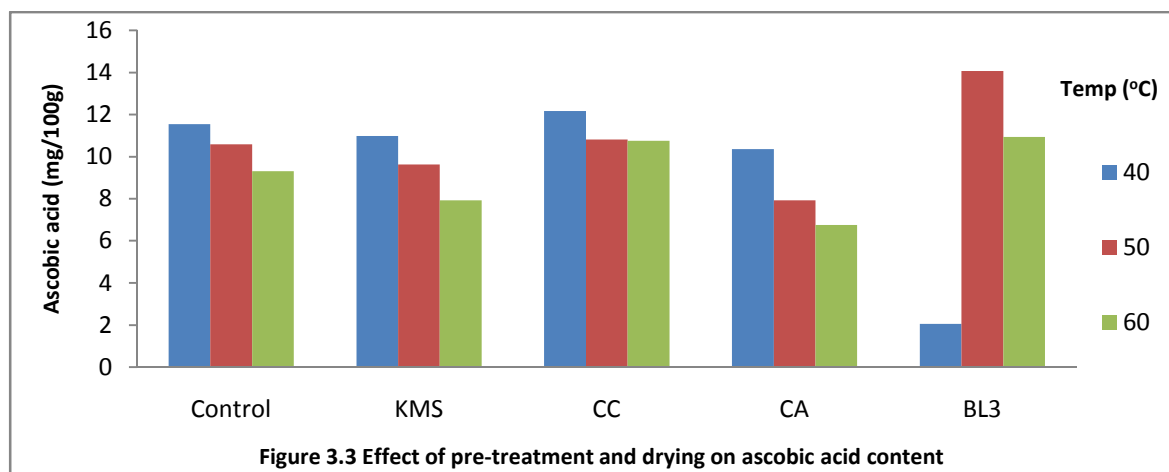
In the literature, ascorbic acid was mostly stated to be heat sensitive, water soluble and prone to degradation under the influence of many factors including enzymes, temperature and leaching. The ascorbic acid content of all pretreated and control samples dried at  $40$ - $60^\circ\text{C}$  were estimated from the standard ascorbic acid plot ( $y = -0.102x + 1.054$ ,  $R^2 = 0.994$ ) and the data was presented in table 3.3. It was found that total vitamin C, was higher at lower drying temperature of  $40^\circ\text{C}$  as expected. The same result was previously obtained dried chillies [10], red chillies drying [19] respectively. As the drying air temperature increases from  $40$ - $60^\circ\text{C}$ , decreased in ascorbic acid was observed (Table 3.3.) except in the case of blanched sample were the optimum drying temperature for the ascorbic acid retention was observed at  $50^\circ\text{C}$  and  $60^\circ\text{C}$ . Lin *et al.*, and Suttur *et al.*, have found that Vitamin C, and  $\beta$ -carotene degradation takes place at higher temperature [20] [21].

**Table 3.3** Effect of pre-treatment and drying temperature on ascorbic acid content

Sample	Ascorbic acid content (mg/100g)		
	60°C	50°C	40°C
Control	11.54±0.01	9.31±0.22	10.58±1.8
KMS	7.93±0.19	9.62±0.16	10.98±0.1
CA	6.75±0.28	7.92±0.62	10.36±0.02
CC	10.75±0.01	10.82±0.26	12.16±0.06
BL3	10.93±0.06	14.21±0.04	2.06±0.19

The data presented in the table are mean of the duplicate determination ± standard deviation

The ascorbic acid content was higher at 40°C for control, KMS, citric acid and calcium chloride treated samples. The higher ascorbic acid content was also reported from dried aonla shred treated with KMS [9]. Rajkumar and Sreenarayanan, reported higher ascorbic acid when drying red onion at 50°C and 0.4% sulphitation level [22]. However, higher ascorbic acid content of 14.21±0.04 mg/100g more than the control was observed at 50°C for samples blanched at 98°C for 3 minutes. The hot water blanched (98°C for 3 min) sample dried at 50°C have exhibited highest amount of ascorbic acid content of 14.21±0.04. Earlier findings with Sweet Pepper [23], dried dill[24], confirmed the effect of blanching on vitamin C content of many fruit and vegetables. Galoburda *et al.*, stated that the conditions of blanching are very important to achieve less loss in the ascorbic acid content.



**Figure 3.3** Effect of pre-treatment and drying on ascorbic acid content

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