



# Practical Food and Research

*Rui M. S. Cruz*  
*Editor*

Food Science and Technology

NOVA

FOOD SCIENCE AND TECHNOLOGY

# PRACTICAL FOOD AND RESEARCH

RUI M. S. CRUZ

EDITOR



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**FOOD SCIENCE AND TECHNOLOGY**

# **PRACTICAL FOOD AND RESEARCH**

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## **PREFACE**

The field of food science deals with numerous processes and phenomenons. The development of processing treatments in order to stabilize foods and/or develop new food products is common in research. This book presents a realistic approach about the most concerning food issues in food processing, particularly in processing by heat, such as: enzymes, microorganisms, vitamins and physical properties. Focusing in their action mechanisms, research analyses, statistical analysis and the application of innovative technologies to reduce or replace the severity of heat treatment processing.

This book is intended as a reference book for food engineers and researchers. Students at undergraduate and postgraduate levels in food science will also benefit greatly from the contents of this book.

This book is the combined effort of several contributors from different countries, with expertise in food processing, that greatly improved the quality of the manuscript. The book has 16 chapters and contains 3 main parts, each dealing with important quality parameters in the processing of Fruits/Vegetables, Meat/Fish and Milk/Dairy Products. The 4th part is dedicated to application of Statistical Analysis in Food Science.

Rui M. S. Cruz

## **ACKNOWLEDGMENTS**

I would like to recognize and thank each one of the experts and researchers for their effort and making this book a reality with their contributions. I also wish to express my gratitude to the editorial staff at Nova Science for the opportunity and support.

I would like to dedicate this work to my dear Mother Deolinda Cruz, my dear Brothers Jaime Cruz and Cândido Cruz and to my dear Niece Laurinha Cruz and Nephews Jaiminho Cruz and Martim Cruz. A final and special dedicatory goes to my dear Father Jaime Mariano da Cruz, whose love and care will always perpetuate in my memory.

Rui M. S. Cruz

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## **PART I**

### **FRUITS AND VEGETABLES**



## *Chapter 1*

# COLOUR AND PIGMENTS

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## ABSTRACT

Plant pigments including anthocyanins, carotenoids, betalains and chlorophyll are principally responsible for the characteristic colour of fruits and vegetables and their products. These pigments have attracted attention due to their biological and health related functions. Apart from their role in human health these pigment contribute to colour which is one of the most important parameters of fruit and vegetable quality, greatly influencing consumer acceptance. This chapter provides fundamental information about these colour pigments, reviews the effects of various food processing operations on pigment stability and discusses proposed degradation mechanisms.

## 1.1 INTRODUCTION

Pigments present in fruits and vegetables are chemical compounds that absorb light in the visible wavelength range of the electromagnetic spectrum (380-750 nm). Natural pigments that produce colour are specific to chromophores [1, 2]. Plant compounds that are perceived by humans to have colour are generally referred to as 'pigments' [3]. Epidemiological studies suggest that consumption of foods containing natural colour pigments such as anthocyanins, beta-carotene, lycopene and other carotenoids may be effective against certain types of cancer [4]. These plant pigments may contribute to the prevention of degenerative processes, particularly lowering the incidence and mortality rate of cancer and cardio- and cerebrovascular diseases [5]. Interest in biological effects of natural pigments has increased

substantially due to their reported therapeutic effects including antioxidant capacity, inhibition of cancerous cells growth, inflammation and anti-obesity affects etc [6-8]. The dietary role of plant pigments along with their preventative role in various diseases is well documented [9-14]. The major classes of plant pigments such as chlorophylls, anthocyanins, carotenoids and betalains found in fruits and vegetables are influenced by various food processing operations. These may degrade due to various factors including; pH, light, oxygen, enzymes, ascorbic acid and thermal treatment [15-17]. Natural plant pigments imparting characteristic colour is a primary factor considered by the consumer in determining quality and can be correlated with both sensorial and nutritional quality attributes [18]. This chapter deals with the changes in fruits and vegetables pigments due to processing, degradation mechanisms and evaluation techniques.

### 1.1.1 Food colour

Pigments present in fruits and vegetables are natural substances in cells and tissues that impart colour. Natural pigments present in plants can be classified based by (a) the chemical structure of the chromophore; (b) their origin; (c) the structural characteristics of the natural pigments or (d) food additives [2]. Natural pigments of plant origin can be divided into four major groups; carotenoids, chlorophylls, anthocyanins and betalains. However, based on the chemical structure, plant pigments can be classed into five families i.e. tetrapyrroles (e.g. chlorophyll), carotenoids (e.g.  $\beta$ -carotene), flavonoids (e.g. anthocyanins), phenolic compounds (e.g. catechin) and N-heterocyclic compounds (e.g. betalains) [19].

#### 1.1.1.1 Anthocyanins

Anthocyanins (in Greek *anthos* means flower, and *kyanos* means blue) are the more important plant pigments visible to the human eye [20, 21]. Anthocyanins are also the largest family of coloured compounds. They are responsible for colour ranges from pink and salmon, through scarlet, violet to purple, and blue of a large variety of flowers, petals, leaves, fruits and vegetables and are a sub-group within the flavonoids characterized by a C-6-C-3-C-6-skeleton [22, 23]. Anthocyanins are bioactive compounds and are believed to provide a broad variety of health benefits such as prevention of heart disease, inhibition of carcinogenesis, and anti-inflammatory activity in the brain [13]. Anthocyanins occur ubiquitously in the plant kingdom and confer bright red or blue colouration on berries and other fruits and vegetables. Apart from imparting colour to plants, anthocyanins also have an array of health-promoting benefits, as they can protect against a variety of oxidants through various mechanisms.

Anthocyanins are found in various fruits and vegetables e.g. fresh red grapes, strawberries, blackberries, raspberries and their products. To date about 550 anthocyanins have been identified in nature [24]. Table 1.1 shows some examples of fruits containing anthocyanins. Since anthocyanins impart characteristic colour to fruits and vegetables they influence a key quality parameter in influencing consumer sensory acceptance [25]. Berry fruits are often processed into juice and juice concentrate or purees in order to reach a more widespread market. Anthocyanin content can be greatly influenced by various genetic (cultivar), environmental and agronomic factors. Anthocyanins are highly unstable and very susceptible to degradation [26]. Their stability is greatly affected by several factors such as

pH, storage temperature, chemical structure, concentration, light, oxygen, solvents, presence of enzymes, flavonoids, proteins and metallic ions [27]. Because of their possible beneficial effects, particular attention has to be given to the changes that anthocyanin pigments undergo during various food processing operations. Anthocyanins, as well as other phenolic compounds, are easily oxidized and, thus, susceptible to degradation reactions during processing (section 1.3.3).

**Table 1.1. Presence of anthocyanins in some fruits and vegetables.**

Fruits and vegetables	Major anthocyanins	Minor anthocyanins
Strawberry	Pelargonidin-3-glucoside	Cyanidin- 3-glucoside, pelargonidin-3-rutinoside
Blackberry	Cyanidin-3-glucoside	cyanidin-3-rutinoside, malvidin-3-glucoside
Raspberry	Cyanidin-3-glucoside	Pelargonidin- 3-glucoside, Pelargonidin-3-rutinoside
Sweet cherry	Cyanidin-3-rutinoside	Cyanidin-3-glucoside, Peonidin-3-rutinoside
Blackcurrant	Cyanidin-3-rutinoside	Cyanidin 3-glucoside, Delphinidin-3-glucoside
Bilberry	Delphinidin-3-galactoside	Peonidin-3-glucoside, Peonidin-3-galactoside
Red onion	Cyanidin-3-glucoside	Delphinidin 3-glucoside, Petunidin glucoside
Blood orange	Malvidin-3-glucoside	Malvidin-3-acetylglucoside

#### 1.1.1.2 Betalains

Betalains are water-soluble nitrogen-containing pigments [28], which comprise the red-violet betacyanins (from Latin *beta*, red beet and *kyanos*, blue colour) and the yellow betaxanthins (from Latin *beta*, red beet and Greek *xanthos*, yellow). Betacyanin structures show variations in their sugar (e.g., 5-O-D-Glucose) and acyl groups (e.g., feruloyl), whereas betaxanthins show conjugation with a wide range of amines (e.g., glutamine) and amino acids (e.g., tyrosine) in their structures [1]. Compared to other pigment classes such as the carotenoids, chlorophylls and anthocyanins, the betalains have been studied to a lesser degree [29]. To date, the betalains comprise of about 55 structures including the red-violet betacyanins and the yellow-orange betaxanthins [29]. The betalains in red beet (*Beta vulgaris* L.) consist of betanin, isobetanin, betanidin, isobetanidin, betaxanthins and some other yellow pigments [30, 31]. Table 1.2 lists some of the health related functions of betalains.

Betalains are the red and yellow plant pigments obtained from members of the order Centrospermae, the most food-significant of these being beetroot (*Beta vulgaris* L.). Betalains are divided into two groups, the betacyanins which are purplish-red in colour and the less common yellow-coloured betaxanthins. Betacyanins differ from other naturally occurring water-soluble plant pigments, especially anthocyanins, in that their colour is not significantly affected by pH changes in the range normally encountered in foodstuffs [37]. Betacyanins are relatively stable under food-processing conditions, although heating in the presence of air at neutral pH causes breakdown to brown compounds. Davidek et al. [38] reported that the most important betalain is betanin (or phytolaccanin), the  $\beta$ -D-glucopyranoside of betanidin, which

may be enzymically hydrolysed to the corresponding aglycon. In the presence of acids, it is transformed into its isomer, and further, to yellow betalamic acid products, containing an open ring system, and finally to brown products [39]. In alkaline medium, the red-violet pigment is decomposed into colourless products [40]. Vulgaxanthin I, the most important betaxanthin, and its amide vulgaxanthin II, are hydrolysed in acid medium to amines or amino acids bound to the dihydropyridine moiety. Like many other natural pigments, betalains are very sensitive to heat, light and oxidation, especially caused by peroxidases which is one of the major causes of discolouration of the pigment [41-44]. The stability of betalains during food processing is influenced by many factors, the most significant of which are temperature, pH,  $a_w$ ,  $M^{n+}$ ,  $O_2$ , and  $h\nu$  [45].

**Table 1.2. Some functions of betalains.**

Function	References
Antioxidant	[32]
Radical scavenging activities	[33, 34]
Oxidative stress-related disorders	[32]
Chemoprevention against lung and skin cancers	[35]
Inhibition of cell proliferation	[36]

### 1.1.1.3 Chlorophyll

Chlorophyll is a fat soluble tetrapyrrole pigment, occurring in chloroplasts of green plants, photosynthetic bacteria and algae [46, 47]. Plants are predominantly characterized by the presence of chlorophylls (*chloros* "green" and *phyllon* "leaf") that are crucial for photosynthetic activity. Chlorophyll absorbs light most strongly in the blue and red regions but poorly in the green region of the electromagnetic spectrum, hence the green colour of chlorophyll-containing tissues like plant leaves.

Chlorophylls, are susceptible to degradation during processing, resulting in colour changes in food [48]. The major chlorophylls in plants include chlorophyll a and chlorophyll b, which occur in an approximate ratio of 3:1 [49]. Chlorophyll a has a methyl group at the C-3 carbon, while a formyl group is bonded to the same carbon atom in chlorophyll b. In addition to structural differences between chlorophyll a and b, their thermal stabilities are also different. Chlorophyll a was reported to be thermally less stable than chlorophyll b [48, 50-54]. Chlorophyll retention has been used as a measure of quality in green vegetables [55].

Chlorophyll destruction can proceed as an acid-, base- or enzyme catalysed reaction. Weak acids liberate the Mg atom bound to the porphyrin ring to form pheophytins by substitution with hydrogen [19]. This results in a colour change from green to dull brown. Magnesium may be replaced by copper (as in copper chlorophyll additives, i.e. chemically modified chlorophylls) and by tin and zinc. Alkaline salts may also be produced, but these are very unstable [56]. Chlorophylls may also undergo photo-oxidation accompanied by the loss of colour. The rate of oxidation has been shown to be dependent upon water activity and the temperature and duration of blanching in dehydrated products. Lipoprotein-bound chlorophylls are somewhat protected against acids, but can be affected by cooking and processing. In alkaline media, the decomposition of chlorophylls is very rapid and they are not stable against the action of free-radicals, e.g. during lipoxxygenase- catalysed oxidation of

lipids, probably due to the effect of hydroperoxides. The allomerization reaction of chlorophylls takes place spontaneously in a polar medium and is metal-ion catalysed; allomeric 10-hydroxychlorophylls and 10-methoxylactones have been detected [57]. Chlorophylls also form Schiff bases, the colour maximum of which is pH dependent [58]. For example the major organic acids involved in the degradation of chlorophylls are acetic acid and 5-oxopyrrolidinecarboxylic acid [59]. Chlorophyll destruction occurs most readily at acidic pH, with little destruction occurring above pH 8 [56].

#### 1.1.1.4 Carotenoids

Carotenoids form one of the most important classes of plant pigments and play a crucial role in defining the quality parameters of fruit and vegetables. Carotenoids are fat soluble colour compounds that are associated with the lipidic fractions. This class of natural pigment occurs widely in nature. Like other plant pigments these also have some health related functions as shown in Table 3. Carotenoids are synthesized by plants and many microorganisms with more than 600 carotenoids isolated from natural sources [60-62]. Carotenoids are natural pigments present in chromoplasts of plants and some other photosynthetic organisms (e.g. algae). Chemically carotenoids are polyisoprenoid compounds and can be divided into two main groups: (a) carotenes and (b) xanthophylls [62]. Table 1.3 shows some of the functions for carotenoids. Apart from nutritional benefits, carotenoids also have photooxidative protection and photosynthesis functions [63]. Carotenoid content in fruits and vegetables depends on several factors such as, genetic variety, maturity, post-harvest storage, and processing and preparation parameters.

**Table 1.3. Some functions of carotenoids\*.**

<b>Carotenoids</b>	<b>Function</b>	<b>References</b>
$\beta$ -Carotene, $\alpha$ -carotene,	Provitamin A activity	[63]
$\beta$ -cryptoxanthin		
All carotenoids	Antioxidant	[64]
$\beta$ -Carotene, canthaxanthin,	Cell communication	[65]
cryptoxanthin		
$\beta$ -Carotene	Immune function enhancers	[66]
$\beta$ -Carotene		[64]
$\beta$ -Carotene, lycopene	UV skin protectant	[67]
Lutein, zeaxanthin	Macula protection	[68]

\*adapted from [70]

## 1.2 EVALUATION TECHNIQUES

Colour is a fundamental property of food products, playing a major role and is consequently one of the most common quality control test carried out in industry. Fruits and vegetables and their products exhibit a wide spectrum of colour. Presently, a number of approaches are available for colour measurement but three methods seem to be widely



adopted [18]. The first is a series of visual systems in which the object under consideration is compared with a series of visual standards. The second is a physical system in which a reflection or transmission spectrum is obtained and either used directly or converted into a tristimulus system. The third is a system of tristimulus colorimetry in which the signals from a sample by reflection or transmission are calculated directly into units related to human vision hence, restricted to the visible light region.

The colour of fruits and vegetables can be described using several colour coordinate systems [71-75] namely RGB (red, green and blue), Hunter  $L a b$  and CIE (Commission Internationale de l'Eclairage)  $L^* a^* b^*$ , CIE XYZ, CIE  $L^* u^* v^*$ , CIE  $Yxy$ , and CIE  $LCH$ . These colour systems differs in the symmetry of the colour space and in the coordinate system used to define points within that space [71]. The tristimulus methods based on CIE and Hunter colour system are of greatest importance to instrumental colour measurement of fruits and vegetables. According to the CIE concept, the human eye has three colour receptors—red, green and blue—and all colours are combinations of these. These instrumental measurements provide a consistent measurement of the true surface colour of the fruit and vegetables. However, current laboratory practices for instrumentally measuring colour changes of fruit have a limited viewing area ( $2.5 \text{ cm}^2$ ), and consequently are unable to capture and describe the entire fruit in a single measurement. Descriptions of heterogeneous fruit colour are only possible through measurement at multiple locations. Also errors are introduced due to choice of location, when tracking the quality changes of the same produce as they mature [76].

Colour can be expressed in variables that correspond to the colour perception of the average person. One system using this approach is the CIE-Lab system, with the  $L$ ,  $a$  and  $b$  values expressing the 'brightness', the 'green-red' and the 'blue-yellow' axis, respectively. The CIE-Lab system is frequently used as a versatile and reliable method to assess the colour of fruit and vegetables and any changes during storage and processing [77-81]. Kidmose and Hansen [82] reported a good relationship between instrumental colour, sensory yellowness and chlorophyll content in cooked and stored broccoli florets. These chlorophyll pigments degrade during storage, acid and heat treatments, resulting in green colour changes. Colour can also be rapidly analyzed by image analysis techniques, also known as computer vision systems (CVS). CVS can be employed for measurement of uneven colouration and also the other attributes of total appearance [83]. CVS is a computerized image analysis technique, which overcome the deficiencies of visual and instrumental techniques and offer an objective measure for colour and other physical factors [84, 85]. CVS finds applications in classification and quality evaluation of various fruits and vegetables such as apples [86-88], chicory [89], banana [90], pomegranate [91] and mango [76]. Mendoza and Aguilera [90] employed CVS for colour measurements and other appearance parameters and observed that they closely correlate to parameters obtained from visual assessment and colourimeters. They employed CVS to identify the seven ripening stages of bananas and calibrated the computer vision system to quantify colour changes during ripening using the  $L^*$ ,  $a^*$ ,  $b^*$  colour space. Kang et al. [76] employed CVS to characterise the colour change of a bicoloured mango fruit during storage (Figure 1.1).

Various dedicated commercial CVSs are available for a variety of industrial applications. These are especially recommended for measurement of colour assessments of samples with curved and irregular shapes such as whole fruits and vegetables. The knowledge of these effects, such as the variations of  $L^*$ ,  $a^*$ , and  $b^*$  for a particular shape of the sample, could be