Bhagyalakshmi Neelwarne *Editor*

Red Beet Biotechnology

Food and Pharmaceutical Applications

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Preface

Desire for living longer with good health has made people wise in shifting their food choices; and hence fruit, vegetables, pulses (legumes) and fish products occupy more space in their diet plates than starchy cereals, fries and red meats. Red beet - a vegetable, includes all sorts of colored beets belonging to the plant species *Beta vulgaris.* Anciently, beets were indeed considered as powerful food because of the rapid nourishment imparted to cattle and horse, and energy to humans to thrive in cold winter. However, their popularity declined with changing fashions in food, where more preference for starchy cereal products, red meats and fries dominated. Such fads, fuelled by the desperate requirement for sugar those days, made people concentrate their research efforts on white sugar beets, rather than the life elixir—the red beet. Recent health concerns and enormous developments made in research methodologies channelled more organized research towards finding the key health-deteriorating factors, with a main emphasis on oxidative damage. To combat such deleterious effects, researchers took a re-look at several food items consumed by humans, as well as animals sourced for human food. Stream-lined research efforts resulted in placing red beet among the top ten health-promoting vegetables because of its rich content of nutrients and enormous nutraceutical properties, which are expected to benefit children to build a strong foundation towards good health and elderly humans to tide-over geriatric problems.

Although recent research developments made on red beet have been chronicled in research journals and reports, a unified summary of this exciting crop was made available only in 2004, through an online publication by Nottingham. Research on red beet has increased rapidly in the last decade owing to the presence of brightly colored water-soluble pigments complemented by its richness of antioxidants, neuro-stimulators and strong anti-hypertensive and anti-cancer properties. This book has a special focus on biotechnological research done in red beet, the topics oriented towards the industrial development of food and pharmaceutical formulations. This treatise will hopefully substantiate its usefulness to all, particularly those in horticulture, corporate and academia, in keeping abreast with recent developments in red beet material per se, the science associated with it as well as processing technologies, all with projections towards future perspectives.

With an overview about the red beet crop and its chemical composition, Chap. 1 is a compilation of both biotechnological and other complementing breakthroughs made in science using red beet as a model plant. The presence of large quantities of bright pigments in colored beets have attracted plant physiologists to unravel the pigment biosynthesis pathways, as discussed in Chap. 2, whereas, the stability of beet pigments during their extraction process as well as in food/pharmaceutical products are important for their sustained applications as nutraceuticals. Characterizations of the stability of betalains, from their biosynthesis all the way to products that receive them, make up the content of Chap. 3. Indeed, red beet has always been a material of choice for pigment research, owing to the large cellular pigment-laden vacuoles that can be isolated from beet tuber quite easily. Once separated from the cell, a large body of biochemical information is discernible from such vacuoles. Chapter 4 is a compilation of research developments made in unravelling physiologies governing the transport of nutrients and metabolites into and out of vacuoles and the enzymes/energetics involved in such transport mechanisms.

Mitochondria are another set of subcellular oragnelles with a main function of orchestrating the energy balance of the cell. Mitochondria also involve themselves in biosynthesis of vitamins and lipids and generate reactive oxygen/nitrogen species. Respiration, the chief function in mitochondria, is much different in bulky tuberous underground organs such as beet roots. Chapter 5 provides interesting insights into the physiological events involved in respiratory metabolism and alternative pathways evolved in tubers. For such studies, red beet has served as a model system, especially because of its very long shelf life after harvest. In nature, plant organs that generate oxidative molecules are invariably equipped with powerful radical quenching mechanisms. In red beet, apart from several antioxidant enzymes, betalains serve as strong antioxidants. Therefore, understanding the kinetics of the radical-scavenging process rendered by the antioxidants of red beet has interested scientists. Chapter 6 deals with kinetics of radical scavenging by betalains in comparison with standard antioxidants. Betalains have shown stronger lipoperoxyl radical-scavenging and other anti-oxidative effects in both chemical and biological models.

The strong antioxidant effects are further translated into the larger effects such as anti-cancer (Chap. 7) and anti-diabetic properties of red beet (Chap. 8). Cancer and diabetes have been chronic problems in modern societies, probably due to higher stress faced by people in their ever-increasing daily challenges. Such stress has culminated in life-threatening health problems such as cancer, cardiac/circulatory diseases, diabetes and neurodegeneration. The chapters dealing with these aspects in the book describe, with appropriate and enormous number of examples, how red beet helps in preventing, and often reversing such pathologies.

The growing realization of the application of plant cell/organ cultures for propagation and breeding has provided great research impetus to horticultural biotechnologists. Densely pigmented nature of red beet has attracted scientists to opt for this plant material as a research model for generating basic information about cellular nutrition, their growth, pigment biosynthesis and morphogenesis towards organ formation. On the other hand, cell cultures have also paved ways for technological developments such as designing bioreactors for cell culture, product enhancement and fine-tuning recovery mechanisms. Chapter 9 provides a summary of the progress made in the culture of red beet cells and tissues from test-tube level to bioreactor level cultivation.

Genetic engineering techniques have resulted in the establishment of transformed cells/organs as well as whole plants, which would not have been existed through natural breeding process. One area which has emerged recently is the use of genetically transformed cultures, where "hairy root" cultures obtained after genetic transformation with *Agrobacterium rhizogenes* are of tremendous technological importance. Hairy roots per se can synthesize and consistently produce novel biochemicals akin to the root system of the plant from which they originate, but they can also be engineered to produce new proteins, including human therapeutic proteins. Red beet hairy roots have been extensively researched as model systems for engineering technology development on one hand and provide opportunity for new products on the other hand. Their high sensitivities to sugars and their ability to undergo re-engineering into double transformants have made them very remarkable candidates for basic research. Chapter 10 has interesting information on the recent upsurge of research conducted using red beet hairy roots. While Chap. 11 provides different bioreactor designs and several gadgets developed through ingenious methods, using red beet hairy roots as model systems, Chap. 12 encompasses research observations made on enzymes produced in red beet hairy root cultures, their characterization and presumptive applications. Product recovery from live materials, particularly from cultured cells and hairy roots, has been a challenging strategy where a great level of intricate cellular mechanisms and their monitoring without causing damage to cell and the product needs to be understood. Chapter 13 provides information on innate and active modes of product release and recovery, where safe and novel permeabilization techniques are discussed. Subsequently, applications of current and newer technologies for product concentration and separation for integration to form continuous processes are presented. Bioengineers have also been successful in applying mechanotronics for in situ product recovery and on-line product separations. Other advanced smart technologies for the extraction of betalains at industrial scales are explained in Chaps. 14 and 15.

Opportunities are enormous for benefiting from the innumerable health potentials of red beet. To reap the health benefits of red beet, it is essential to supplement current research with the new innovative biotechnologies, particularly chemogenomics for the in vivo identification of new biochemical pathways, combinatorial chemistry for pathway engineering and computational chemistry for newer ingredients/processing parameters. Such and other technologies, as well as legal requirements of new biotechnology products and their environmental/economic impacts are the components of Chap. 16.

I remain immensely grateful to 24 scientists from 9 countries for their great efforts and elegant preparation of the chapters with lucid illustrations, making this compendium attractive to readers. I am thankful to all reviewers who have contributed tremendously towards improving the quality of this treatise.

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Suggestions are solicited from all around the world, for the future improvement of the content and approach of this book.

Mysore, India Bhagyalakshmi Neelwarne

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Chapter 1
Red Beet: An Overview

Bhagyalakshmi Neelwarne and Sowbhagya B. Halagur

Abstract Research on red beet has increased rapidly in the last decade owing to the presence of brightly colored water-soluble pigments complemented by its richness of antioxidants, neuro-stimulators and strong anti-hypertensive and anti-cancer effects. Apart from their increasing traditional applications in food and pharmaceutical products, betalains have found newer applications such as for developing solar cells and anti-ageing formulations. While the visible presence of vacuolar pigments has made the tissues of red beet a model system for studying several vacuole-related cellular physiologies, the material is ideal for developing color-extraction techniques and related engineering aspects. Although its derivative variety – the sugar beet – has attracted great attention because of its capacity to accumulate high sugar content, similar emphasis has never been placed on red beet. The modern knowledge and molecular techniques of genomics, proteomics and metabolomics have not been applied efficiently for improving existing nutraceuticals within the plant or for identifying hitherto unexplored biomolecules. The present chapter provides an overview of recent biotechnological research developments on red beet, highlighting the important research areas worth addressing in the near future.

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1.1 Introduction

Beet is said to have got its name from the Greek letter *beta* owing to the presence of the swollen root resembling a Greek *B,* although it was the foliage beet variety, namely chard, that was domesticated by Greeks and Romans in 2000 BC. Natural diversity probably first occurred in the fleshy (swollen) taproot varieties, known to have existed in the second century AD, which probably were the first to serve as dietary sugar in winter times (McGrath et al. 2007). Italians made selections from wild beets of the Mediterranean seacoast, developing both red and white varieties, which became popular as the Roman beet. Beet was used as a vegetable boiled in stews, baked in tarts, roasted as a whole and cut into salads. Various other applications such as in drinks and for medicines emerged based on individual experience and their popularities.

1.1.1 Origin of the Genus Beta and Production of New Beet Cultivars

The plants of genus *Beta* have been traced to have originated in North Africa and spread through the Mediterranean Sea route, occupying the seashores of Asia and Europe. Many leafy types would have emerged while adapting to newer agro-climatic conditions where they were grazed by animals. Thus earliest usage of beets by humans was only the foliage. Storage tap roots probably started increasing their sizes and pigmentation while adapting to rich soils, and people selected the roots for food and found many uses for them. Thus Romans first cultivated beets to use their roots in food while the foliage was fed to domestic animals. Tribal people, who frequently changed their living locations widely in Europe, were probably responsible for spreading this plant in the continent. During the eighteenth century, the large-rooted beets known as Mangel wurzel, recognized as nutritious, were being fed to cattle presumably to reap high meat and milk yields. They were introduced into England in the 1770s for use as livestock feed after being developed from early fodder beets in Germany and Holland. Beet was then found throughout Europe, chiefly for hybridization purpose (Eastwood and Nyhlin 1995) to produce the vast range of colors and shapes found in table beets today. In those days, white beets were perhaps more common, but less desirable than red beets. More details of the history of origin and development of beet crops are described elsewhere (Nottingham 2004).

In European countries beet was first used as animal fodder in large scale and became a popular vegetable for human consumption in the sixteenth century. Analytical intervention by alchemists, and then by modern chemists of the nineteenth century, resulted in the discovery that beet root was a concentrated source of sugar. While sugar supply from cane industries of Britain was curtailed to other parts, Napoleon was the first to pronounce that beet be used as an alternative for sugar production, which not only catalyzed beet's popularity but also encouraged breeding and selection, resulting in many cultivars. Taxonomically, Beet is classified under Kingdom: Plantae; Order: Caryophyllales; Family: Chenopodiaceae; Genus: *Beta* and Species: *Beta vulgaris* by Linnaeus (C. Linnaeus, 1753, 1762, *Specia Plantarum* vol. 1 and 2).

Diversifying from red beets, modern sugar beets emerged after several series of selections, followed by repeated breeding and re-selection (Hanelt et al 2001) for enhancing sugar level from 6% to 20%. A large support for its commercialization emerged from the support by the King of Prussia in the eighteenth century. Andreas Marggraf (1749) first demonstrated in 1747 that sugar could be isolated from beet roots, which was almost similar to that produced from sugarcane at concentrations of 1.3–1.6%. From then on, beet was recognized for commercial sugar production. Beet cultivation in Europe reached a commercial scale in the nineteenth century following the development of the sugar beet in Germany as an alternative to tropical sugar cane. As a response to blockades of cane sugar by British during war, Napoleon opened schools in France specifically for studying sugar production at commercial scale. This encouragement came with an additional package of devoting 28,000 ha (69,200 acres) for growing the newly selected beet varieties, pushing the rapid growth of the European sugar beet industry (Zohary and Hopf 2000). In North America the sugar beet was introduced in 1830, where the first commercial production started at a farm in Alvarado, California in 1879 (Zohary and Hopf 2000). Meanwhile, in 1850, the sugar beet had been introduced also to Chile via German settlers. It now remains a widely cultivated commercial crop in Europe, America and Canada for table sugar production, accounting for 30% of the world's sugar.

Generally growing as a herbaceous biennial and sometimes as a perennial plant, *Beta vulgaris* is classified under the family Chenopodiaceae (Lange et al. 1999). However, in America this genus was recently classified under Amaranthaceae. Plants bearing red or purple tuberous root vegetable are known as beetroot or garden beet. Beetroot is a firm, clean, globe-shaped vegetable with no mucilaginous or watery tissues, and the tubers available in the market often contain freshly emerged young leaves. The characteristic feature of red beet beetroot is the presence of distinctive bright red, purple of yellowish–orange flesh. The sugar bearing varieties do not display many colors, although pigments are formed under stress and adverse conditions and can be induced when cultured in vitro (Pavoković et al. 2009). The two most common varieties of red beet are: (a) the Globe beetroot, having a round and smooth tuber with a dark red flesh; (b) the Egyptian beetroot, which is spherical with a reddish smooth surface. The Crapaudine beetroot is another variety of red beet with a slightly wrinkled, rough skin.

While many varieties of beetroot are available (Table 1.1), the traditional dark red globe beets are still favorites with growers. Among the dark red globe beetroots, the varieties Derwent Globe, Darkest Globe, and Detroit Dark Red are the most popular ones, while Rapid Red, Early Wonder, Early Egyptian, and Early Market are recommended for fast growth and maturation. Several globular varieties display unusual concentric circles of pink or red with white, similar to growth rings of tree trunks. Golden Beets are known for their non-bleeding qualities and the White Albina is said to be sweeter than the standard red varieties. Both are globular shaped

Table 1.1 List of some original and bred beet varieties

Genus, species and variety	History, appearance and use
Original varieties	
B.v. ssp. *vulgaris* convar. *cicla* (leaf beets)	The leaf beet group has a long history dating to the second millennium BC. These were used as medicinal plants in Ancient Greece and Medieval Europe. Their popularity declined in Europe following the introduction of spinach
B. v. ssp. *v.* convar. *cicla.* var. *cicla* (spinach beet)	This variety is widely cultivated for its leaves, which are usually cooked like spinach and popular around the world
B. v. ssp. *v.* convar. *cicla.* var. *flaviscens* (chard)	Chard, thought to have arisen from the spinach beet by mutation is also a leaf beet, having thick and fleshy midribs used as a vegetable. Some cultivars are also grown ornamentally for their colored midribs
B. v. ssp. *v.* convar. *vulgaris* var. *crassa* (Mangel wurzel)	This variety was developed in the eighteenth century for its tubers for use as a fodder crop
B. v. ssp. *v.*convar. *vulgaris* var. *altissima* (sugar beet)	The sugar beet is a major commercial crop owing to its high content of sucrose, which is processed to produce table sugar. Since the origin of this beet is from red beets, the genes for betalains remain redundant and often expressed under stress conditions and in vitro cultures
B. v. ssp. *v.*convar. *vulgaris* var. *vulgaris* (garden beet)	This is the red root vegetable that is most typically associated with the word “beet”. It is especially popular in Eastern Europe where it is the main ingredient of borscht
Bred varieties	
Albina Vereduna	A white variety
Burpee’s Golden	Beet with orange–red skin and yellow flesh
Chioggia, an open-pollinated variety originally grown in Italy	The concentric rings of its red and white roots are visually striking when sliced. As a heritage variety, Chioggia is largely unimproved and has relatively high concentrations of geosmin
Detroit Dark Red	Relatively low concentrations of geosmin, and is therefore a popular commercial cultivar in the United States
Lutz Greenleaf	A variety with a red root and green leaves and a reputation for maintaining its quality well in storage
India Beet	India beet is more nutritious than Western beet, and is not as sweet as Western beet
Red Ace	Principal variety of beet found in the United States, typical for its bright red root and red-veined green foliage
B. v. ssp. *v.* convar. *vulgaris* var. *altissima* (sugar beet) Many bred lines of sugar beet	The sugar beet is a major commercial crop due to its high concentrations of sucrose, which is extracted to produce table sugar. It was developed in Germany in the late eighteenth century after the roots of beets were found to contain sugar in 1747

B. v. Beta vulgaris

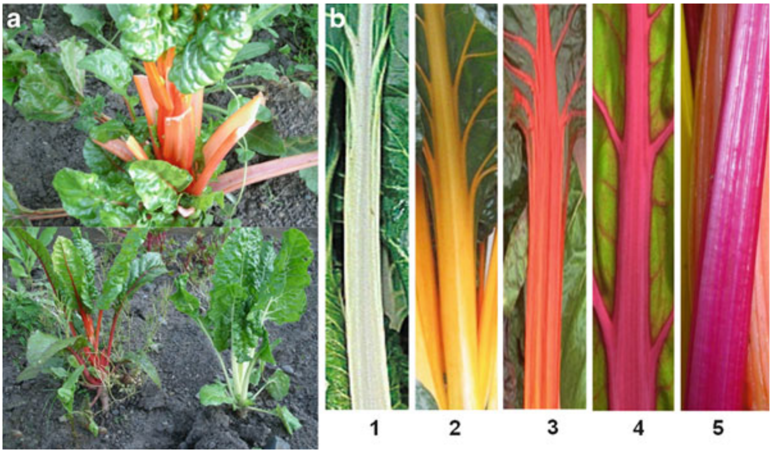


Fig. 1.1 Field-grown chards showing high diversity (**a**) and petioles of chards (**b**) showing the absence of pigments (***b***-*1*), dominant presence of betaxanthin (***b***-*2*), high betaxanthin + low betacyanin (***b***-*3*), low betaxanthin + high betacyanin (***b***-*4*), and dominance of betacyanin (***b***-*5*)

and produce tops that can be harvested and cooked like spinach. The other cultivated varieties include the leafy vegetables chard and spinach beet, as well as the root vegetable sugar beet, which is important in the production of table sugar; the var. Mangel wurzel is a fodder crop. Leaf beets, generally known as chard, display high diversity (Fig. 1.1a), where the petioles exhibit an array of pigments from zero pigment to intense red (Fig. 1.1b).

1.1.2 Crop and Production

Beetroot is grown all over the world in temperate areas, with main production in North America, Europe and USA. In Europe, the production of beetroot is mostly concentrated in the UK and France but Italy, Netherlands, Germany, Greece, Spain and Denmark also contribute significant productions. The total production of beet in different parts of the world is presented in Table 1.2. Although the season for beet is June to October, it is available throughout the year, owing mainly to improved horticultural practices. Among largely cultivated varieties, mainly three subspecies are typically recognized, with all cultivated varieties grouped under *Beta vulgaris* subsp. *vulgaris*, while *Beta vulgaris* subsp. *maritima*, commonly known as the sea beet, is the wild ancestor of these and is found throughout the Mediterranean, the Atlantic coast of Europe, the Near East, and India. A second wild subspecies, *Beta vulgaris* subsp. *adanensis*, occurs from Greece to Syria.

Economic satisfaction is realized in red beet cultivation since the annual crop yield ranges from 50 to 70 t per hectare. This crop is entering into the economic

Table 1.2 World-wide beetroot production in 2009

Country	Production (t)
Asia (total)	31,709,126
Europe (total)	158,852,215
Africa	8,063,010
South America (total)	1,096,863
North America (total)	2,743,690
Germany	25,919,000
China	7,178,960
Japan	3,649,000
Canada	657,700
Italy	3,307,700
United States of America	26,779,200
Poland	10,849,200
Russian Federation	24,892,000
World	227,158,114

Source: FAOSTAT, Sept. 2011

zone because of the presence of a high content of natural red pigments, betalains, which display intense hue properties and have many-fold health benefits, as discussed in other chapters.

1.2 Cytogenetic Analysis

Diploid (2X) red beet has 18 chromosomes. The first tetraploids having 36 chromosomes were obtained as early as 1940 by colchicine treatment. The occasionally formed auto-tripoids (3X = 27) in Europe were found more resistant to the fungus Cercospora, and have 10% higher yields than diploids (Skaracis 1994). When excised ovules pre-treated with colchicine were cultured in vitro, polyploidization and chromosome doubling were observed. After further re-culturing, ovaries formed embryos, which subsequently regenerated, showing an average of 8.1% spontaneous chromosome doubling (Hansen et al. 1994). In *Beta vulgaris,* DNA content (C-value) has been reported to be 714–758 million base pairs per haploid genome. However, variations from the above numbers have been recorded among sub-species (Bennett and Smith 1976; Armuganathan and Earle 1991). About 60% of the beet genome is constituted of highly repetitive DNA sequences (Flavell et al. 1974). Many families of short (140–160 nucleotide) repeats with high copy numbers (>10^5 per genome), not considering ribosomal RNA repeats, have been recorded (Schmidt and Heslop-Harrison 1996). Several sequences, like those of transposable elements, have also been reported (Schmidt et al. 1995; Staginnus et al. 2001). Each chromosome in beet has characteristic repetitive sequences. All together, the repetitive sequence diversity is very high in the genus *Beta*, which has proven advantageous for their characterisation (Desel et al. 2002).

Molecular analyzes of chromosomes are recognized as highly effective tools for understanding genomic evolution, meiotic recombination and chromosome stability (Heslop-Harrison and Schwarzacher 1993), where fluorescence in situ hybridization (FISH) and genomic in situ hybridization (GISH) have been widely applied for identifying chromosomes, and detecting inserts of T-DNA and fragment repeat distribution. In the genus *Beta*, the FISH technique was applied to visualize mitotic metaphase chromosomes of *B. procumbens*, using one family of highly repeated DNA and two *Procumbens*-specific repeats, where probes bound mainly to positions in the centrometric regions, although not always on all chromosomes (Schmidt and Heslop-Harrison 1996). Subsequently, GISH and FISH techniques with various probes were applied to screen foreign chromosomes or chromosome fragments for selecting nematode-resistant genotypes (Schmidt et al. 1997). The foreign chromosome material could be visibly detected, and the fragments were classified into discretely distinguishable groups (Mesbah et al. 2000). High-resolution FISH techniques for application in plants, with enhanced resolution, was also obtained using pachytene chromosomes and extended DNA probes, which allowed chromosome studies at the molecular level (Fransz et al. 1996) (Zhong et al. 1996a, b; Shen et al. 1987). Using FISH, monosomic addition of *B. procumbens* genes in *B. vulgaris* ($2n = 19$) could also be established (Mesbah et al. 2000).

1.2.1 Genetic Markers

Similar to many other crops, the major success in improving beets was accomplished by traditional breeding methods. However, great contributions have been made utilizing genetic markers for rapid characterization of beet germplasm (McGrath et al. 2007). Genome fingerprinting was used for the characterization of individual alien chromosomes of both *B. patellaris* and *B. procumbens* (Mesbah et al. 1997). Diversity analysis using molecular markers suggested the occurrence of a great diversity among plants of *B. vulgaris*, because it comprises chards (foliage beets), red beets and sugar beets. Moreover, a greater diversity was found in *B. vulgaris*, *sub-species maritima*, which comprises wild beets. Ruderal beets emerge as an introgression of wild beets with cultivated varieties. When molecular and morpho-physiological characteristics of ruderal beets were compared with wild beets and cultivated sugar beets from coastal regions of Italy using amplified fragment length polymorphism (AFLP) markers, the ruderal beets were genetically distinctly different from both wild sea beets and cultivars. Such differences were manifested even at physiological levels, displaying significant differences in root structure, growth rate and branching patterns (Saccomani et al. 2009).

The alleles for betalain pigment expression were found to be *loci Y* and *R* when dominant pigment is expressed. Recessive *rr-yy* plants do not synthesize betalains and their seedlings express some amount of betaxanthins, and tubers are pigmentless, a characteristic of sugar beets (McGrath et al 2007). In an effort to screen for diversity of betalain content, recurrent half-sib family selections were practiced for seven cycles, each time selecting for high pigment and solids. Several random amplification of polymorphic DNA (RAPD) markers were found linked to genes involved in betalain synthesis (Eagen and Goldman 1996). Thus, cultivars with high pigment levels (>310 mg/100 g fresh weight) have also been developed (Gabelman et al. 2002).

1.2.2 Male Sterility Genes

Male sterility has played a major role in crop improvements. In the mitochondrial genome of *B. vulgaris*, specific markers were found to be associated with male sterility (Ivanov et al. 2004), and hence such organelle-associated genetic characteristic is often termed a cytoplasmic trait. Cytoplasmic male sterility (CMS) results from disrupted microsporogenesis, leading to abortive pollens, first described by Owen (1942) in *Beta vulgaris* L. The interaction of recessive alleles of at least two nuclear fertility restorer genes (*Fr-fr*) with the cytoplasm of a specific S type, characterized by an altered structure and expression of the mitochondrial genome, has been found mainly responsible for CMS in *B. vulgaris*. This was confirmed by partial restoration of fertility in CMS plants after introduction of the dominant *Fr* alleles. CMS has often been found accompanied by changes in a set of plasmid-like genes in mitochondrial DNA (mtDNA) (Dudareva et al. 1988), where the structure of the mitochondrial genome remains unchanged, with changes taking place in its transcription profile (Kubo et al. 1999). When several 5′-degenerate primers, designed and selected after computation, were used for typing mtDNA of S-type (typical for cytoplasmic male sterility) and N (normal)-type cultivars, a number of N- or S-specific markers corresponded to transcribed mitochondrial genes. One of these was from the *orf215* region of the N-type mtDNA. When a physical map of the corresponding region from S-type mtDNA was constructed, a substantial difference was observed for the two genome types. One N-specific marker was found to contain a truncated copy of *atp9* copy and a rearranged *rps3* region. After nucleotide sequencing, PCR primers were designed that showed that both variations occur simultaneously in the *rps3* region of the mtDNA pool (Ivanov et al. 2004).

1.2.3 Current Gene Flow Problems in Beets

Since most of the domesticated crops retain their genetic compatibility with their ancestors, they are often able to hybridize with their wild relatives. Therefore one may expect gene flow from cultivated populations into the gene pool of wild populations, as well as among different cultivated populations inhabiting the same vicinity. While the inter-specific gene flow may lead to evolutionary change of the receiving population, the small populations that receive genes may also be endangered (Levin et al. 1996), because they lose their original gene constitution. This is true in the case of *B. vulgaris*, where the wide varieties easily cross with each other (McGinnis et al. 2010), although such out-crosses segregate fast because of the high genetic load on them. When wild beet populations growing in the vicinity of cultivated populations were analyzed by measuring isozyme allele frequencies of cultivated beet, one allele specific to sugar beets and another allele with a much higher frequency in Swiss chard and red beet than in sugar beet were found (Bartsch et al. 1999). Analyzed through microsatellite markers, clear genetic cleavage between wild individuals and their weedy relatives could be depicted (Levigne et al. 2002). Also, using a chloroplast genome-based genetic marker and a set of nuclear microsatellite loci, the occurrence gene flow from beet crop to wild beets was investigated (Arnaud et al. 2003). Although the results did not reveal a large degree of pollen dispersal from weed to wild beets, several pieces of evidence clearly indicated an escape of weedy lineages from cultivated fields via seed flow. Although gene flow from a crop to a wild taxon does not necessarily result in a decrease in the genetic diversity of the native plants, such a gene flow may lead to significant evolutionary changes in the recipient populations. Such events are of concern when the cultivated crops are genetically engineered and capable of flowering profusely. Probably for these reasons, sugar beet lines genetically modified for glyphosate herbicide were allowed to be planted in 2008, but were later withdrawn due to environmental and socio–economic impacts (McGinnis et al. 2010). Thus concerns with biotechnology–derived crops remain to be sorted out, particularly when wind cross-pollinated species, such as beets, Swiss chard and spinach, require greater stringency and isolation than most other insect-cross-pollinated crops. Such isolation from genetically engineered crops is also important to organic producers and in other markets with low or no tolerance for biotechnology-derived material.

1.3 Beet Nutrients

The composition of the various nutrients of red beet presented in Table 1.3 shows its rich mineral and vitamin content, with distinct differences between foliage and tuberous root. tuberous root. An unusual compound, 5,5,6,6-Tetrahydroxy-3,3-biindolyl was identified in red beetroot peel (Kujala et al (2001a)), although no further information on its nutritional benefits are identified.

1.3.1 Beet Foliage

The foliage of red beet makes a delicious green vegetable used in a similar manner to spinach, with higher concentrations of various nutrients than in roots. Beet foliage is an excellent source of carotenoids viz., beta carotene, lutein and zeaxanthin; and flavonoids, which are strong antioxidants and dietary sources for the biosynthesis of vitamin A in mammals. Beet greens are a source of folate, a component of vitamin B, which is needed in the body to release energy and donate organic carbon for various cellular functions. Folate is instrumental in the functioning of the nervous

Table 1.3 Major nutrients found in foliage and tubers of red beet (100 g raw)

	Nutrient	Units	Greens	Tubers
1.	Water	g	91.02	87.58
2.	Energy	kcal	22	43
3.	Energy	kJ	92	180
4.	Protein	g	2.20	1.61
5.	Total lipid (fat)	g	0.13	0.17
6.	Ash	g	2.33	1.08
7.	Carbohydrate, by difference	g	4.33	9.56
8.	Fiber, total dietary	g	3.7	2.8
9.	**Sugars, total**	**g**	**0.50**	**6.76**
Minerals				
10.	Calcium, Ca	mg	117	16
11.	**Iron, Fe**	**mg**	**2.57**	**0.80**
12.	**Magnesium, Mg**	**mg**	**70**	**23**
13.	Phosphorus, P	mg	41	40
14.	Potassium, K	mg	762	325
15.	Sodium, Na	mg	226	78
16.	Zinc, Zn	mg	0.38	0.35
17.	Copper, Cu	mg	0.191	0.075
18.	Manganese, Mn	mg	0.391	0.329
19.	**Selenium, Se**	**µg**	**0.9**	**0.7**
Vitamins				
20.	Vitamin C, total ascorbic acid	mg	30.0	4.9
21.	Thiamin	mg	0.100	0.031
22.	Riboflavin	mg	0.220	0.040
23.	Niacin	mg	0.400	0.334
24.	Pantothenic acid	mg	0.250	0.155
25.	Vitamin B-6	mg	0.106	0.067
26.	**Folate, total**	**µg**	**15**	**109**
27.	Folic acid	µg	0	0
28.	Folate, food	µg	15	109
29.	**Choline, total**	**mg**	**0.4**	**6.0**
30.	**Betaine**	**µg**	**0.00**	**128.7**
31.	Retinol	µg	0	2
32.	**Carotene, beta**	µg	**3,794**	0
33.	Carotene, alpha	µg	3	20
34.	Vitamin A, IU	IU	6,326	0
35.	Lycopene	µg	0	33
36.	**Lutein + zeaxanthin**	**µg**	**1,503**	0
37.	Vitamin E (alpha-tocopherol)	mg	1.50	0
38.	**Vitamin K (phylloquinone)**	**µg**	**400.0**	**0**
Lipids				
39.	Fatty acids, total saturated	g	0.020	0.027
40.	Fatty acids, total monounsaturated	g	0.026	0.032
41.	16:1 undifferentiated	g	0.000	0.000
42.	18:1 undifferentiated	g	0.026	0.032
43.	Fatty acids, total polyunsaturated	g	0.046	0.060

(continued)

Table 1.3 (continued)

	Nutrient	Units	Greens	Tubers
44.	18:2 undifferentiated	g	0.041	0.055
45.	18:3 undifferentiated	g	0.004	0.005
46.	**Phytosterols**	**mg**	**21**	**25**
Amino acids				
47.	Tryptophan	g	0.035	0.019
48.	Threonine	g	0.065	0.047
49.	Isoleucine	g	0.046	0.048
50.	Leucine	g	0.098	0.068
51.	Lysine	g	0.064	0.058
52.	Methionine	g	0.018	0.018
53.	Cystine	g	0.021	0.019
54.	Phenylalanine	g	0.058	0.046
55.	Tyrosine	g	0.052	0.038
56.	Valine	g	0.065	0.056
57.	Arginine	g	0.063	0.042
58.	Histidine	g	0.034	0.021
59.	Alanine	g	0.081	0.060
60.	Aspartic acid	g	0.129	0.116
61.	Glutamic acid	g	0.267	0.428
62.	Glycine	g	0.081	0.031
63.	Proline	g	0.052	0.042
64.	Serine	g	0.070	0.059

Some of the rich components indicated with bold letters are deficient among populations of developing countries

Compiled based on data from USDA National Nutrient Database (Release No. 24, 2011); Accessed on 20-10-2011: http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl

and immune systems and in the synthesis of red blood cells. Beet greens are also a good source of vitamin C, which is also a strong antioxidant, and hence an anti-ageing nutrient.

1.3.2 *Beet Tuber*

Raw beetroot is a good source of minerals. When cooked, based on the method, the nutrient composition can be markedly different. Boiled beetroot is particularly high in potassium, carbohydrate and protein content. Pickling boiled beetroot decreases the carbohydrate and protein content to a level below raw beetroot, with a corresponding decrease in energy value (Nottingham 2004). Beetroot is often recommended in calorie controlled diets because of its low calorific value. Apart from rich pigmentation, table beets are rich in vitamin B folate, which is essential for various cellular processes from carbon donation to normal tissue growth and cognitive functions. Dietary folate and its synthetic counterpart, folic acid, play important roles in cardiovascular diseases, cancer, in the prevention of neural tube defects in infants

(Scott et al. 2000) and exhibit antioxidant activity (Joshi et al. 2001; Asensi-Fabado and Munne-Bosch 2010). Under typical commercial processing conditions, 8% loss of vitamin C, 60% loss of color and 30% loss of dietary folate were observed. There was a significant 5% increase in the phenolic content of processed beets (Jiratanan and Liu 2004), which may be the reason for their unchanged antioxidant property even after processing.

1.4 Beet Pigments

The importance of red beet continues because of its high red pigment content, namely betalain, which display excellent hue values suitable for applications in food and pharmaceutical products. Although many plants accumulate betalains, only red beet and prickly pear (O*puntia ficus-indica*) are approved for food and pharmaceutical applications (Jackman and Smith 1996; Mabry et al. 1963). Use of beet extract to color food is approved by the US Food and Drug Administration (FDA). Crop yield of red beet ranges from 50 to 70 t/ha, with betanin content ranging from 40 to 200 mg betanin/100 g fresh (0.4–20 mg/g of dry) beet root; a level that not has been reached with any other betalain-producing crop. Table 1.4 lists important beet cultivars and their pigment contents.

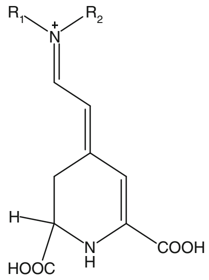
Table 1.4 Pigment content in tubers of different red beet cultivars

Cultivar	Source and accession no.	Betacyanin (mg/100 g)	Betaxanthin (mg/100 g)
Uniball	Burpee (Netherlands)	38.7	20.6
Slowbolt R-2289	Burpee (Denmark)	35.8	15.1
Red E 403	Burpee (Denmark)	34.9	19.3
Bordo 237	USSR	34.6	21.9
Detroit Nero RS	Burpee (Netherlands)	33.2	16.2
Detroit Dark Red MT	Burpee (USA)	32.0	11.5
Detroit Dark Red ST	Burpee (USA)	31.6	13.5
Podzimniaja 0474	USSR	31.3	15.0
Detriotsluis	Burpee (Netherlands)	30.7	17.3
Early Wonder	Burpee (USA)	30.4	13.2
Little Ball	Burpee (Netherlands)	30.3	11.1
Choghundur	P11631179 (India)	30.2	18.2
Iowa	IA	28.3	14.9
Gladiator	Burpee (USA)	27.9	12.0
Asmer Beethoven	Burpee (England)	27.8	10.8
Crveno	P1357355 (Yugoslavia)	27.1	15.2
Polsko	P1357351 (Yugoslavia)	26.9	16.7
Okragly Ciemnozerwony	P1285591 (Poland)	25.8	11.4
Spangsbjerg	P1269310 (Sweden)	25.2	16.7
Rubidus	Burpee (Netherlands)	23.5	9.6

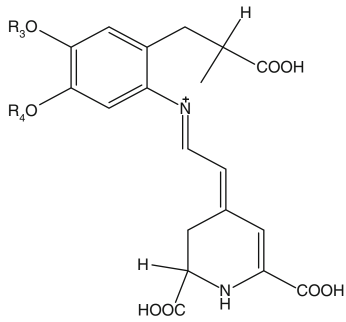
Source: Sapers and Hornstein, 1979, J.F.S. 1245–1248

Table 1.5 Betaxanthin and betacyanin

Betaxanthin



Betacyanin



	R1	R2	Botanical source
Vulgaxanthin-I	H	Glutamine	*Beta vulgaris*
Vulgaxanthin-II	H	Glutamic acid	*Beta vulgaris*
Indicaxanthin	Both groups together form proline		*Opuntia ficus-indica*
	R3	R4	
Betanin	β- glucose	H	*Beta vulgaris*

In addition to red beet, plants from 9 of 11 families of the Caryophyllales order contain betalains. The recent addition to the list of betalain families is Didieraceae, a small family from Madagascar. Evolutionarily, betalains are interesting because the presence of betalains or anthocyanis is mutually exclusive in Angiosperms, i.e., betalains and anthocyanins have never been reported in the same tissues of plants (Mabry and Dreiding 1968; Strack et al. 2003; Cai et al. 2005; Grotewold 2006). Betalains are also synthesized in a class of fungi: the mushrooms *Amanita*, *Hygrocybe*, and *Hygrosporu* (Delgado-Vargas et al. 2000).

1.4.1 Characterization of Beet Pigments

The visible red pigment of red beet has two major groups of water-soluble nitrogenous pigments: betacyanins, which display red to purple color, and the yellow water-soluble betaxanthins (Table 1.5). The ratio between the red and yellow pigments determines the hue of the pigment extract. The mechanism of *in planta* betalains production is proposed to occur as a defense response, since betalains accumulate when tissues are injured. For example, red beet leaves, which are normally not the major sites of pigment accumulation, showed betalain accumulation in wounded and infected areas, which also undergo oxidative bursts (Sepulveda-Jimenez et al. 2004). The high content of nutritious material in red beet root, along with the high sugar content (Table 1.3), probably calls for a strong defense mechanism in these tissues that is fulfilled by betalains. Unlike anthocyanins, which display a wide array of sugar moieties, betalains are condensed with either glucose (betacyanin) or one of eight amino acids in the case of betaxanthin (Table 1.5). The glucose of betacyanin (betanin) could also have other organic molecules attached at its R-marked positions, this causes color shifts. While the content of total betalains vary from 35 to 120 mg/100 g fresh weight, the content of betacyanin ranges between 0.04% and 0.21% and betaxanthin between 0.02% and 0.14%, depending on the variety (Nilsson 1970). However, under tissue culture conditions, wide variations in the ratios have been reported, sometimes with a higher content of betaxanthins in the Detroit dark red variety (Pavlov et al. 2002). The major pigment, betanin, has a tinctorial strength equal to or better than that of artificial dyes.

1.4.2 Attempts to Enhance Pigments in Beets

Since betalains are nitrogenous pigments, attempts to increase the synthesis of this pigment *in planta* comprised of the addition of either ammonium nitrate or ammonium sulfate to the soil, and/or foliar sprays of Fe and B (El-Tantawy and Eisa 2009). Despite its high betanin content, *B. vulgaris* root has several drawbacks. It has a limited pigment spectrum and adverse flavor due to geosmin (4,8a-dimethyldecalin-4a-ol) and various pyrazines. It contains a high nitrate level, which may form carcinogenic nitrosamines in the human body when ingested in large quantities. Common names of betalains are assigned in relation to plant source from where they were first isolated, and accordingly more than 50 betalain structures have been elucidated from various plants and fungi (Delgado-Vargas et al. 2000; Francis 1986). The chief pigment molecules of red beet are betacyanin or betanin and the betaxanthins, vulgaxanthin I and II (Table 1.5). The presence of other minor pigments and intermediary compounds presented in Tables 1.4 and 1.6 vary depending on the variety as well as the geographical status.

1.4.3 Betalains in Different Beet Varieties and Cultivars

The color of beetroot differs depending on the cultivars, varieties, growing conditions, age and size. The pigment content is influenced by the time of harvest; late harvest results in better color (Nilsson 1973). Pigment composition in five different varieties of red beet was studied by (Gasztonyi et al. 2001). A range of red–violet pigments betanin, isobetanin, betanidin and isobetanidin was observed, of which the chief pigment betanin ranged from 0.4–0.50 g/kg and yellow pigment vulgaxanthin I content ranged from 0.32 to 0.42 g/kg (Table 1.6). There was not much variation among the varieties.

Nilsson (1970) in Sweden studied the pattern of pigment accumulation in red beet from sowing to harvesting time, also recording the size of beets in three varieties viz., Banco, Egyptische-Platronde and Rubia. It was observed that pigment content was influenced both by sowing time and harvesting time. In all the varieties the amount of betacyanin and betaxanthin in June was lower than in September/October.

Table 1.6 Pigment content (g/kg) in Dutch (D) and Hungarian (H) varieties of red beet

	Betacyanins				Betaxanthins	
Variety	Betanin	Isobetanin	Betanidin	Isobetanidin	Vulgaxanthin 1	Vulgaxanthin II
Bonel (D)	0.50	0.27	0.04	0.01	0.42	0.06
Nero (D)	0.41	0.13	0.03	0.01	0.32	0.03
Favorit (H)	0.49	0.24	0.05	0.02	0.41	0.02
Rubin (H)	0.46	0.25	0.07	0.03	0.37	0.03
Detroit (H)	0.44	0.21	0.05	0.01	0.37	0.04

Gasztonyi et al. 2001

Table 1.7 Pigment content (mg/g dry weight) in peel and flesh of tubers of red beet cultivars

Cultivars		Vulgaxanthins I and II	Betanin	Isobetanin
Egyptische Platronde	Peel	1.4±0.3	3.8±0.4	1.2±0.2
	Flesh	1.5±0.2	2.9±0.2	0.03±0.02
Forono	Peel	1.8±0.1	7.6±0.1	3.1±0.1
	Flesh	4.0±0.2	5.2±0.2	0.4±0.03
Little Ball	Peel	4.3±0.4	7.5±0.5	2.1±0.2
	Flesh	1.9±0.1	3.6±0.2	0.02±0.01
Rubia	Peel	2.2±0.2	5.4±0.1	2.2±0.1
	Flesh	2.3±0.2	4.1±0.2	0.3±0.03

Kujala et al. 2002

Betacyanin content increased and reached a maximum in August and betaxanthin content continued to increase during the autumn, resulting in higher concentrations of yellow pigment in later harvests. The pigment content decreased with increasing beet diameter and the ratio between red and yellow content was affected by the beet diameter. Small beets had a higher pigment content than large beets. The pigment content in different cultivars of beet reported by different authors is presented in Tables 1.6 and 1.7.

1.4.4 Biotechnology for the Production of Betalains

Because the red beet tubers are loaded with pigments, this material has attracted the interest of many in the early years of plant tissue culture research for the production of secondary metabolites, since the presence of pigments is visible (Akita et al. 2000, 2001, 2002) and can be easily quantified by spectrophotometry. With the development of biotechnology, genetically transformed hairy root cultures find great use for the commercial production of red beet pigments (Pavlov et al. 2002; Georgiev et al. 2010a; Thimmaraju et al. 2003a, b, 2004, 2006; Pavoković et al. 2009). In addition to enhancing yields (e.g., up to 250% increase in betanin content even from sugar beets) (Pavoković et al. 2009), these technologies appear to provide better control over the quality of pigment, since the production is independent of environmental factors, which normally cause great variations in field-grown beets. However, there are reports of significant differences in the chemical composition of

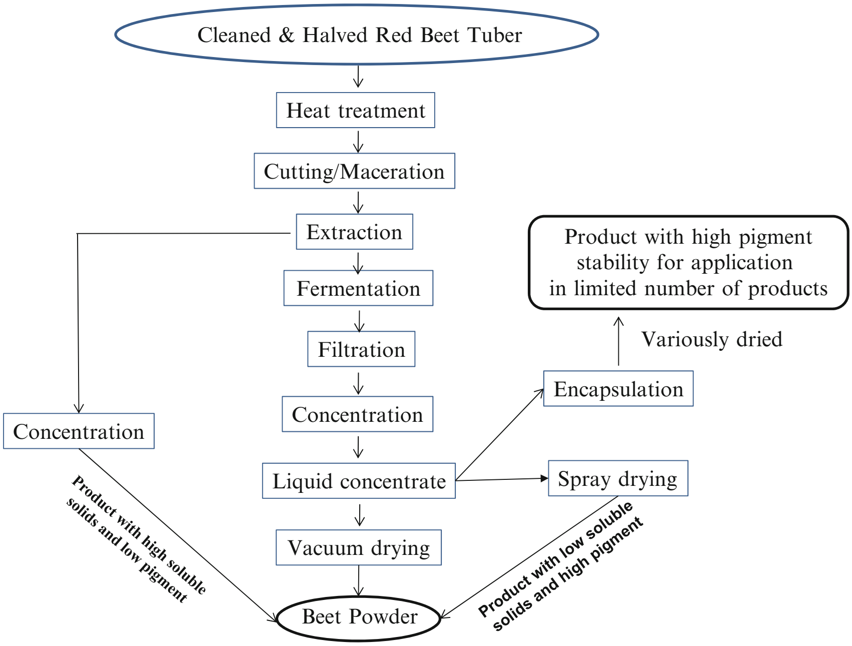


Fig. 1.2 Steps involved in the pigment extraction from red beetroots

the two sources. For example, rutin was present only in the extracts from hairy root cultures, whereas chlorogenic acid was found only in the extracts of field-grown plants (Georgiev et al. 2008). Also, the observed higher antioxidant activity of the hairy root extract compared with that of the field-grown beetroot extract (Georgiev et al. 2010b) was attributable to its increased concentrations (≈20-fold) of total phenol compounds, which may have a synergistic effect with the beetroot betalain pigments.

1.4.5 Extraction of Red Beetroot Pigments

Invariably, the roots of red beet have been utilized in all studies for pigment extraction, although there are rare occasions where beet leaves, stems and seeds were also researched (Gennari et al. 2011; Lee et al. 2009; Ninfali et al. 2007; Pyo et al. 2004; Križnik and Pavoković 2010). The steps involved in pigment extraction from red beet roots (Fig. 1.2) starts with chopping beet tubers into small pieces or quickly grinding them. Further steps are added to achieve the maximum yield of the betalain pigments, while keeping the losses to the minimum; the aim is to obtain a stable pigment extract with a long shelf life. In general, pigments are extracted with water, although methanol or ethanol solutions (20–50%) may be required for complete

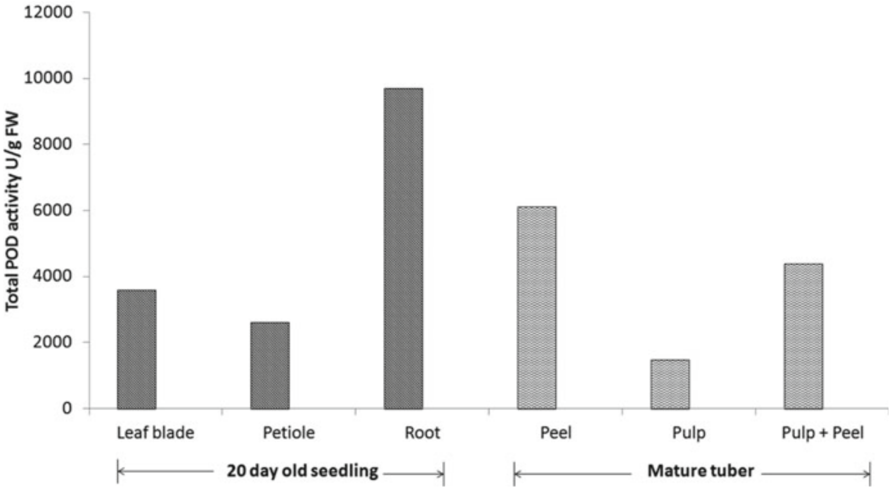


Fig. 1.3 Peroxidase activity in red beet (Figure plotted on the basis of data from Thimmaraju et al. 2005)

extraction (Delgado-Vargas et al. 2000). Aqueous extraction increases pigment stability and the pigment may be further stabilized by slight acidification of the extraction medium with ascorbic acid, which renders color constituents more stable and resistant to oxidation, both chemical and by endogenous polyphenol oxidases (PPOs) (Escribano et al. 2002; Strack et al. 2003). In the case of hairy root cultures of yellow beetroots, where tyrosinase activity was found to be high, addition of ascorbic acid was essential to avoid losses of betaxanthin and miraxanthin V, as well as to avoid the appearance of artefacts formed by degradation products (Strack et al. 2003).

Red beets have several endogenous enzymes such as β-glucosidases, PPOs and peroxidases, which if not properly inactivated by blanching may account for betalain degradation and color losses (Escribano et al. 2002; Lee and Smith 1979). The optimum pH for enzymatic degradation of both betacyanins and betaxanthins is reported as approximately 3.4 (Shih and Wiley 1981). Betacyanins are more susceptible than betaxanthins to degradation by peroxidases, whereas betaxanthins are more susceptible to oxidation by hydrogen peroxide (Wasserman et al. 1984). Generally, unpeeled whole beets are processed and more than 30% color is lost by removal of the peels. This is noteworthy because the greatest PPO activity, which is deleterious to both betacyanins and betaxanthins, is located in the peel, as is the case with peroxidases (Fig. 1.3). The presence of these enzymes at the peel part of beet tuber indicates their apparent participation in defence functions either by scavenging the peroxides or by oxidizing other molecules formed at the surface. Blanching before extraction inactivates the unfavorable enzymatic action. In theory, the oxidizing and hydrolysing activities of PPO action require monophenolic or diphenolic structures that are rarely found in betaxanthins and betacyanins and are formed only after prior hydrolysis by glucosidase activity. Hence for enzymatic betalain degradation, a concerted action of glucoside-cleaving enzymes, PPOs and peroxidases is