SYMPOSIUM PAPERS AND AUTHORS

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THE ROLE OF RIPENING IN THE AFFAIRS OF MAN

Max E Patterson
Washington State University, Pullman, Washington

The purposes of this symposium are to focus attention on the subject of ripening, to consider the nature of the ripening process, to reveal developments concerning morphology and mechanisms, and to promote thinking covering control of ripening.

Hopefully a framework will evolve which will give new perspective and dimension to the subject. The speakers were chosen with these objectives in mind. The intent of this paper is to provide background information, promote questions, interject personal viewpoints and direct attention to the succeeding topics.

A need for a precise postharvest nomenclature

There is no desire to limit our thinking, but rather to interpret ripening in its broadest aspects. Some immediate questions follow. What organs are appropriately referred to as capable of ripening? Is it valid to refer to a potato, seed, or flower as being ripe or should this term be limited to the changes that occur only in fruits? Should there be ripening classifications for each type of organ?

It is apparent that attention must be given to the meaning of ripening and related terms associated with ripening and postharvest horticulture, because meanings can and do vary (11, 19, 33, 37).

A dictionary may define ripe as: 1) grain or fruit that has grown to maturity and is fit for food; 2) the condition to which wine is brought by care and by keeping; or 3) the state of being fully developed or matured.

In examining the ripening process from physiological, biochemical, cellular and subcellular viewpoints, should there not be a distinction between maturation and ripening? Certainly there is no need to confound ripening with growth processes or fermentation reactions. Science can not accept ripening as a convenience term. It requires clarification of the nature of ripening based on endogenous changes that occur in ripening organs and not subjective opinions. A proper distinction from maturation is needed. The time of senescent onset needs to be established, but based on bioenergetics not synthetic events. If ripening and other postharvest changes are to be studied scientifically it is essential to have an exact and precise nomenclature.

Although horticulturists' concerns are vested in horticultural commodities, ripening is not so restricted. To embrace all aspects of ripening, we cannot exclude the ripening that occurs in any botanical fruit, dry or fleshy. The definition must cover any ripening commodity and yet be specific for definite physical and physiological transitions.

In this paper ripening is regarded as a process, composed of a complex of interrelated, but separate physiological events resulting from biochemical and biophysical changes, occurring at the cellular, subcellular, and molecular level (5, 13, 27, 31). It is restricted to plant ovaries and accessory tissues.

The relevance of ripening to man's past, present and future

Ripening is a phenomenon so commonplace in nature that its importance has been ignored.

The past

Historically, a civilization is based upon its food production and its agricultural development (6, 24). The rise of civilization has been credited to particular crops, fertile flood plains and other agencies. The ripening process is more likely a candidate for this credit by indirectly focusing men's minds on the possibilities of a continuous food supply by planting seed. Succulent fleshy fruits in season are highly attractive to all primates and were often picked and carried to his habitat by early man.

The ripening process is visualized as a catalyst that disclosed discarded seeds from ripe fruits could regenerate new plants and fruit where they had been cast. How many times was this act repeated before its impact registered on an early thinker? How many times was this act repeated before seeds were deliberately planted?

Archeological evidence of fruits and seeds in association with man's remains (24) substantiate the hypothesis that civilization's advancement was contingent on the ripening phenomenon.

The present

The ripening process is a major contributor to our total food supply and our economy. Foods otherwise inedible become highly desirable upon ripening.

Harvesting, handling, storage, transportation and distribution of fruits constitutes a large segment of a perishable marketing system that touches every consumer and provides gainful employment to significant percentages of the working force in this country. In part the success of this system depends upon the ability to predict or modify the ripening process during marketing and deliver a product ready for consumption.

Developments in handling, storing, transportation and marketing necessitate recognition of the physiology of the commodity which dictates the environmental requirements. These in turn, govern the engineering developments and technological practices.

The future

Ripening fruits may yield knowledge concerning the control of senescence (3, 34).

While plant physiology often follows the course set in animal physiology, this is not so in studies of organ storage and senescence. Horticulturists working in postharvest physiology have accumulated much information applicable to longevity studies in animal physiology.

Every act of man in acquiring food, clothing or shelter is transacted for prolonging life span by maintaining himself as an organism. The primary purpose of the medical profession is to relieve pain and prolong life. Yet even though man's average life span has nearly tripled it has not been due to control of the aging but rather has been achieved by control of natural enemies, the conquest of ravaging diseases and better nutrition. Not one day has been added to man's life span by directly arresting his senescent decline.

A variety of organisms are being used in the search for an understanding of senescence. If there is no clear line of demarcation indicating the onset of senescence then growth, development, maturation and starvation can confound senescence studies.

Fruits displaying a climacteric phase offer ideal experimental material for fundamental research in senescence. The respiratory climacteric clearly separates growth and development from senescence and death, thereby providing a finite distinction between the metabolism of life and the metabolism of death. Certain regulator compounds alter the inception of the climacteric, hence the longevity of the organ, thus illustrating that life spans need not be finite (20, 29).

In detached plant organs, particularly fruits, environmental control is utilized to prolong life (16). Control of the gaseous environment further reduces respiration to the point of just supplying energy for maintaining cellular integrity.

Organ banks for human parts are being liberated from the realm of science fiction. The experience with controlled environments for excised plant organs should apply to storage of animal organs.

The nature of ripening

The ripening process consists of both covert and overt changes. The covert changes are interpreted as internal changes or mechanisms responsible for the overt changes. It is no longer enough to consider only one or the other in a commodity study.

Covert changes

The basic units of interest in a ripening fruit are the cell and the organelles of the cell. These morphological structures undergo physical changes and contain the machinery that drives the reactions of living and dying.

The sequence of disorganization of the organelles is reflected by the enzyme systems contained within the organelles. This view of ripening is visible through the electron microscope. Although promising, the difficulties are many.

Ripening is a time of high anabolic and catabolic activity. A loss of total energy occurs as respirable substrates are converted into simpler molecules, heat and phosphate bond energy. Bond energy is used for various physiological activities and maintaining integrity of the cell (2, 14).

Once it is initiated, ripening is analagous to embarking on Death's journey. Seldom is a ripened fruit seen to die of old age because of invasion by pathogens.

Respiratory metabolism is a pivotal process in the postharvest life of all commodities and occupies a dominant role in the ripening process (15). In climacteric fruits ripening proceeds concomitantly with a rise in respiration (4).

Fruits such as cantaloupes, tomatoes, apples, pears, plums, peaches, bananas, avocados and others exhibit this characteristic. It has been reported that citrus, grapes, cherries and pineapples, do not have a climacteric (3, 31).

Recently, however, oranges and grapefruit were reported to display climacteric-like patterns when a sufficient chronological series of samples were picked and analyzed (1). Other ripening changes followed the climacteric, but the climacteric occurred well before commercial harvests.

Pineapples (10) and cranberries (9) also have been shown to produce a small respiratory rise prior to commercial harvest.

In order to resolve conflicting opinions regarding the ubiquity of the climacteric, more precise measurements covering broader chronological periods and measurement over longer durations are needed.

This raises the question of how to properly interpret the seasonal respiratory drifts which display minor rises as commercial harvest approaches, but in which the postharvest rise is minor or nonexistant.

Overt changes

The overt changes such as color, odor, sweetness, acidity, astringency and texture which occur during ripening are readily detected by the senses of sight, smell, taste and touch.

Detailed descriptions of the ontogeny of the overt ripening pattern for many individual commodities is lacking. These data may have been considered too commonplace to merit recording.

Likewise, information concerning the path of ripening through fruit tissues is limited. In what tissues is the stimulus to ripen first overtly expressed and in what tissue does it appear last? What is the role of the vascular tissue? What is the influence of the new sporophyte?

The path of ripening through the tropical pawpaw and banana have been described. In these fruits, ripening first appears in the placental tissues, then proceeds through the outer pulp to the peel. The distal or stylar end of the banana ripens slightly ahead of the proximal stem end (36).

Descriptions of the overt changes and pathway of tissue ripening for other fruits are needed. These clues indicate that covert biochemical and biophysical changes have taken place. Knowledge and appreciation of the overt changes taking place in a particular organ can lead to productive research or prevent unnecessary analytical studies and unwarranted conclusions. They serve to establish effective parameters for detailed research.

For example, in searching for an enzyme to degrade chlorophyll, one selects an organ displaying a dramatic loss of green color. If a ripe fruit remains green, obviously chlorophyll degradation is minor and the active participation of a degradative chlorophyllase *in vivo* is also minor.

Color

Color changes in most fruits are the most obvious symptoms of ripening. These changes are primarily due to destruction of the chlorophylls and the synthesis of anthocyanin and carotenoid pigments. The other flavonoid compounds contribute little to color except when oxidized during browning reactions (32).

According to Looney (21) and Kvaale (18) increased chlorophyllase enzyme activity and loss of chlorophyll in apples and bananas occurs simultaneously with the climacteric rise.

In some deciduous fruits containing anthocyanin in the skin, synthesis is under photo control in which both light quality and light intensity are required (28). In other fruits and those colored throughout the tissue, direct photo energy is unnecessary.

Synthesis in each case is temperature controlled. In Italian prunes, cranberries and apples, anthocyanin synthesis is greatest at moderate temperatures and progressively declines with both increasing or decreasing temperatures (23).

Knowledge of anthocyanin synthesis has been utilized in preserving quality of Northwest Italian prunes. Prolonged periods of temperatures exceeding 90°F at harvest time retard coloration. However other ripening processes continue so that fruit harvested on the basis of color may be too soft to successfully market.

When harvest is based upon criteria other than color, the moderate temperatures encountered in the market channels will properly color poorly colored fruit.

Texture

Textural changes are overt changes that occur during maturation and ripening resulting from changes in the physical and chemical structure of the cellular components. The marked changes in the pectic constituents during ripening may have detracted from other changes that affect texture. The kind and size of cells, the size of intercellular spaces and vacuoles, and the presence of inclusions are textural factors not directly associated with ripening. Environment and cultural practices that affect cell morphology during growth can also alter fruit texture.

Softening due to hydrolysis of cell wall polysaccharides constitutes the principal ripening change that affects texture of succulent fruits. Unpublished work in our laboratory has shown striking increases in polygalacturonase activity in Bartlett pears ripened out of cold storage.

In some fruits lignin synthesis may adversely affect texture. Pressure testing merely indicates the firmness or softness of the tissue, but it does not separate textural changes due to growth from changes due to ripening.

Flavor

Flavor is a perception of a combination of sweetness, acidity, and astringency in conjunction with the odorous volatiles.

Ripening usually increases sugars and sweetness derived from degradation of polysaccharide substrates. Although starch conversions are particularly striking in banana, starch is nearly gone when ripening begins in the pome fruits (13). Further study is needed to determine the role of cellulose and hemicellulose in sugar formation.

In some fruits sucrose accumulates, while in others reducing sugars accumulate. Sugars differ in their degree of sweetness. A soluble solids determination does not differentiate between various sugars or their individual contribution to the relative sweetness. Once polysaccharide reserves are depleted, the sugar content declines through respiration. Temperature is a controlling factor for this loss.

Organic acids which contribute to tartness generally decline during ripening. Acids derived from the respiration of simple carbohydrates in turn are lost through respiration.

The various fractions of odorous volatiles provide much of the characteristic flavor of a given kind or variety of fruit. In climacteric fruits the peak of evolution of volatiles roughly coincides with the respiratory peak.

Astringency produces a generally undesirable sensation in the mouth when unripe fruits such as persimmons, bananas or cider apples are eaten. Fortunately, astringent materials decline on ripening. In small amounts and in combination with the other flavor factors, these materials may impart a desirable flavor.

Limits of ripening

The ripening process is not limited to mature, fully developed, fruits on the tree. It occurs whether the fruit is attached or detached from the parent plant. Admittedly when an immature fruit is excised and ripened it does not have the edible quality of a ripened mature fruit. This is not surprising, however, because it lacks needed substrates. This does not deny that ripening has taken place.

Pratt and colleagues have described ethylene induced ripening changes in cantaloupe within 23 days of anthesis and in tomatoes within 17 days of pollination (22, 25, 26). Hansen has described ripening of immature pears treated with ethylene and suggests that ethylene aborts development but allows ripening to proceed normally (12).

In mid-July of 1968, a number of small Starkerimson apple fruitlets ceased to enlarge and developed an intense red prigmentation. Eventually they dropped or shrivelled on the tree. Green fruits on the same blossom clusters continued to develop normally. No growth regulators had been applied. Fruits in each category were harvested July 16 and placed in respiration chambers at 20°C. Within 17 days the colored fruitlets exhibited a typical climacteric rise which peaked on the 22nd day. An analysis for ethylene revealed appreciable amounts at the climacteric peak. The green gruits did not exhibit a respiratory rise nor the presence of ethylene during the same time period.

Control of ripening

As practical horticulturists, we endeavor to control ripening in order to market at optimum edibility. Acceleration and retardation of ripening to meet the needs of supply and demand, distance to market and harvest conditions are realistic goals. With the spectre of starvation omnipresent, we urgently need controls to prevent the wastage that continuously erodes man's total food supply.

Environmental control

Most of our modern practices are technical improvements of age old environmental control practices of temperature, relative humidity and atmosphere modification. Understanding the biological principles supporting these practices is a modern contribution and has provided the basis for dramatic technological improvements. A change in harvest practices or an educational program on ripening at the retail store may be in order, as air freight and controlled atmospheres are introduced into the marketing system.

Parent plant control

In some instances the parent plant may exercise strong control in preventing ripening. In contrast, in some varieties of summer apples, the tree appears to have no control over ripening whatsoever. Fruitlets, green fruit, crisp red fruit and senescent mealy fruit may all occur at the same time on the same fruiting cluster.

Proliferation of cells from ripening tissue of peach mesocarp and vesicle stalks of mature lemon have been obtained in tissue culture (17, 30). These cultures demonstrate that ripe tissues removed from the influence of surrounding tissue still retain their youthful capacities for growth and development.

Chemical control

Chemical control of ripening processes is totally new if natural sources of ethylene are excluded. Even here advancements have been more fortuitous than intentional as new plant regulators became available for testing.

Hopefully, assimilation of information on the nature and mechanisms of ripening processes will foster new control methods and practices.

Conclusion

Do we know enough or have we thought enough about ripening as a fundamental process?

Protein synthesized during the climacteric rise is predominately enzyme protein composing synthetases, hydrolases and oxidases. These enzymes catalyze degradation reactions simultaneously with synthetic reactions.

The increase in RNA in climacteric fruits was anticipated as fundamental to the surge in protein synthesis.

It is as logical that DNA controls the ontogeny of death of an organ as well as programs the life of an organ (35). Evidence suggests that organs adjacent to an intact fruit as well as cells surrounding a given cell exert a marked influence upon genetic control.

Do these occurrences reveal that regulators from particular organs and tissues compete to trigger a code for living or dying?

In working with ripening have we limited the breadth of our outlook? Should we not search for a unified theory of life and death?

Is there still more for us to see in the overt ripening taking place before our eyes? Is nature revealing hidden but fundamental truths if we but take the time to observe and ponder the message?

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MORPHOLOGICAL CHANGES IN RIPENING FRUIT

Arthur R. Spurr
University of California, Davis

Many events in the ripening of fruit have received intensive study, yet we have little information on some of the structural changes involved. It is of value to summarize the current morphological information in the field of fruit ripening so that it may be considered in relation to the other contributions to this symposium. Not only is attention given here to structure during ripening, but some biochemical and physiological data in which structural changes are strongly implicated are considered. Our attention is limited to studies on the ripening of fleshy horticultural fruits; however, the phenomena considered may apply to other types of fruits or to other plant parts and tissues.

Most fleshy fruits are relatively large organs, an attribute that has received detailed attention by Forward (8) in her review of respiration of bulky organs. Such fruits also are often highly parenchymatous, with cells sometimes 0.5 mm or more in diameter and with walls that may be less than 1 μ thick. The vacuoles in these cells are frequently very large, with the protoplasm and included organelles, such as the mitochondria, nucleus, and plastids, confined to a thin peripheral layer. In *Capsicum annuum*, the pepper, this layer may be as thin as 500 A in some areas (Fig. 1), which includes the plasma membrane and the tonoplast with an interspace between them containing the ground substance of the cytoplasm. Fleshy fruits usually have a weakly developed but extensive vascular system, and their tissues are permeated by a system of intercellular spaces. The skin of the fruit is a protective layer well adapted, in many instances, for the retardation of water loss, even after removal of the fruit from the plant.

In addition to the rather distinctive structural features of the fruit, there are events in its early life that may have a bearing on structural changes during ripening. By the time ripening begins, a fruit has passed through complex developmental stages, some of which are