Practices & Research ON HORTICULTURE

Volume - 3

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Dr. Veena Pani Srivastava

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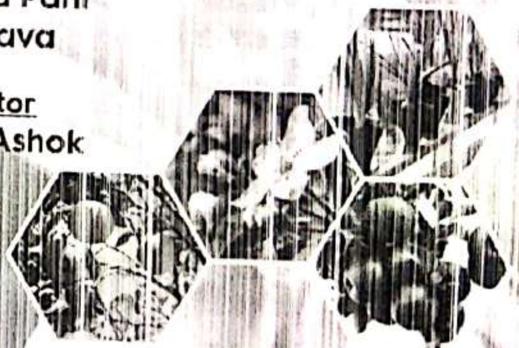

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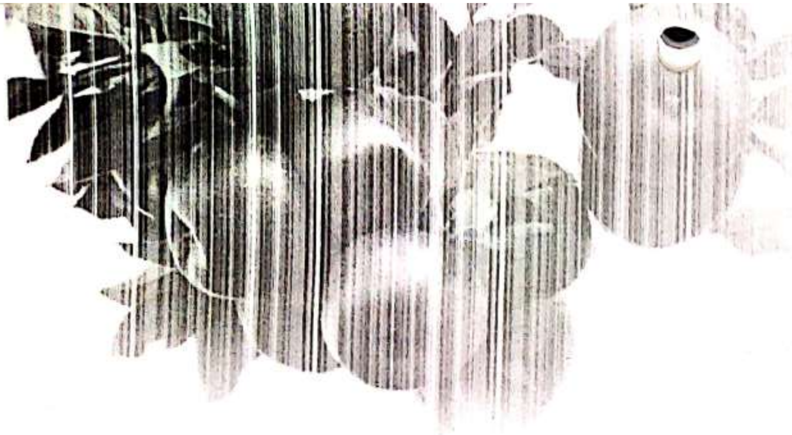

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Chapter - 1

Advanced Packaging Systems in Fruits and Its Processed Products

H.D. Chandore and Sanchita Ghosh

Abstract

Packaging is an important operation of the post harvest management of fruits and their processed products. The packaging industry now changing their pattern with respect to different kinds of innovations. Various advanced packaging systems like antimicrobial packaging, smart and intelligent packaging, thermally sensitive packaging, modified atmospheric packaging etc are playing major role in horticultural industry. However, still we are lacking to meet export standards in advanced level of packaging as compared to the foreign countries. Enormous quantity of produced are being wasted every year during transport due to improper packaging. An attempt has been made to make detail review on the traditional as well as advanced packaging systems in this chapter.

Keywords: packaging, antimicrobial packaging ethylene, CO_2, O_2 scavengers, smart and intelligent packaging, robotics, automation etc.

1. Introduction

In fruits and vegetable production, India secures second rank followed by china [1]. Although India produces good amount of fruits and vegetables, still huge quantity is being wasted every year due to improper post harvest handling which included appropriate packaging of the material. Post harvest management of fruits and vegetables is important pillars of Indian economy as Indian food industry facing post-harvest losses (35-40%) due to improper handling, storage, packaging and other post-harvest management practices which is worth of Rs. 2.40 lakh crore [2, 3]. The Ministry of Food Processing Industries (MFPI) estimated losses of 23 million ton of grain, 12 million of fruits and 21 million tons of vegetables of wroth Rs. 4.4 billion USD with total food loss of 10.6 billion USD in the year2017-2018 of India [4] (NAAS Report, 2019). These losses could be overcome by adopting modern technologies and process among which appropriate packaging is most important [5]. As per the reports of ASSOCHAM & EY, India, packaging is the fifth largest sector in India's total economy, which will reach its growth of 73.6 billion dollar by 2020 [6]. The packaging industry in India is rapidly growing reach 32 billion dollar in 2015 to 73 billion dollar in 2020 as per FICCI and Tata Strategic Management Group (TSMG) report of 2016. The Indian packaging industry contributed 4% of global packaging industry but, per capita consumption is very low (4.3kg) as compare to Germany (42kgs and Taiwan (19kg) [2]. Trends shows that Indian packaging industry is moving from use of rigid plastic materials to flexible packaging as these products are visually appealing, cheaper and durable. Some new technologies like IML (In Mould Labeling) MAP, nano-structured multilayered films etc. are gaining importance gradually due to excellent packing quality.

2. Benefits of packaging

Packaging has various benefits in overall production and marketing of produce. It not only deals with the reduction of postharvest losses but also effective for increasing consumers acceptability. Following are the benefits or importance of packaging.

i) It assembles the produce in convenient way so that it may reach consumer without any damage to the products. It protects internal products from tampering or any other kinds of damage such as hitting, shocking, vibration, compression, wetting, bruising, etc, It also acts as barrier for oxygen, water or its vapors, dust etc., that are the agents of microbial contamination

ii) It helps to maintain internal and external quality of produce including its freshness by avoiding contact with the external environment

iii) It prevents physiological, biochemical and any other environmental changes

iv) It reduces transport cost by pallatization method as compared to bulky transport system

v) It helps in maintaining quality standards as per export norms and regulations

vi) It is helpful to sellers, buyers, and consumers by providing all necessary information on their packing material

vii) It acts helps to maintain nutritional components at optimum level and hygienic condition

viii) It helps in transport and shipping by increasing it durability and handling capability

ix) Some advanced packaging tools like use of barcode, QR code, anti-theft devices such as dye packs, RFID tags, electronic article surveillance tags, that can be activated or detected by devices at exit points which useful to prevent retail loss

3. Packaging methods/types/levels

Generally, there are four methods/types/levels of packaging, which are as follows:

i) Primary packaging or sales packaging

Primary packaging is the first level of packaging where the product is in immediate contact with the thin layer. It means the first layer of product and any other secondary layer existed in that, e.g. a beverage can, candy wrapped in a pouch, mesh bag for fruit etc. The main function of this layer is to protect the product from external environment. This product directly goes in the hands of consumer hence also known as sales packaging. Mostly these kinds of packaging can wrap the product for end level consumer. Labelling and other cap fitting operation can be carried out for the sales the unit [7].

ii) Secondary packaging or group packaging or display packaging

The major function of this level of packaging is not only the product protection but also better visibility of the product at retails store while displaying. These kinds of packaging allow better handling of bulk product. These kinds of packaging is mostly used for used for beverage, food and cosmetic sector for display of primary packs in group in shelves or racks hence also referred as display packaging. The most common examples of these types of packaging are cardboard cartoons, the card cereal box that holds the inner bag, cardboard boxes and cardboard/plastic crates [7].

iii) Tertiary packaging or transport or distributive packaging

Tertiary packaging intended for mostly transporting of the bulk packages in container. This kinds of packaging is generally not available directly to the consumer but can be used by retailer, wholesaler for displaying in their shop. These kinds of packaging handle large amount of packages during handling, storage and transportation. Mostly for export point of view, this kind of packaging minimises handling, transporting losses at individual level due to less exposure of individual unit to the environment [7]. It contains number of secondary packages, which are packed and stacked together in warehouses and later transferred in large shipping containers for further transport. These kinds of package are also termed as distribution package as these kinds of packaging generally used for distribution of large number of products in different area. Example of tertiary packaging includes brown cardboard boxes, big corrugated cardboard box, wood pallets, wrapped pallet and shrink-wrap.

iv) Unit load

These kinds of packaging are generally loading or unloading of big containers or consignments. It also called as Unit because most of the secondary or tertiary materials have repacked on the palette using stretch film.

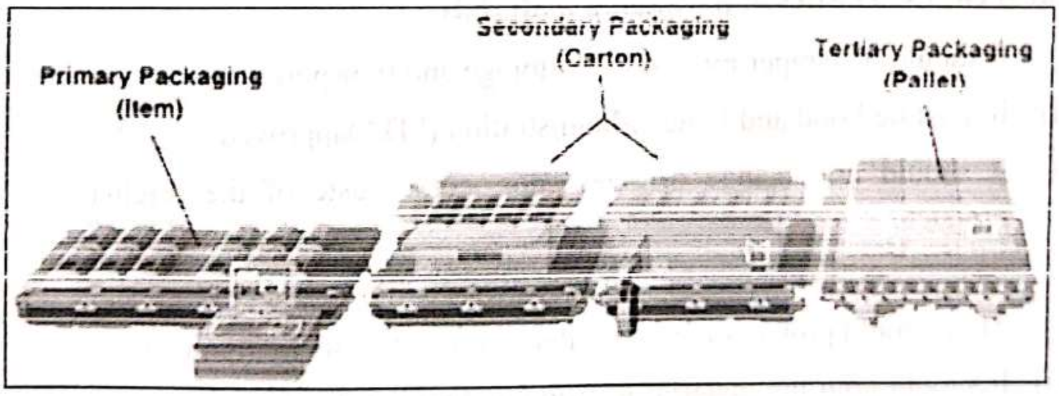
v) Other types

a) Consumer packaging

The packaging, which ended with consumer distribution or sales, called as consumer packaging. Major aim of this packaging is to display or sales the product for consumer. This packaging starts from the commercial sales point and arrives at consumer level.

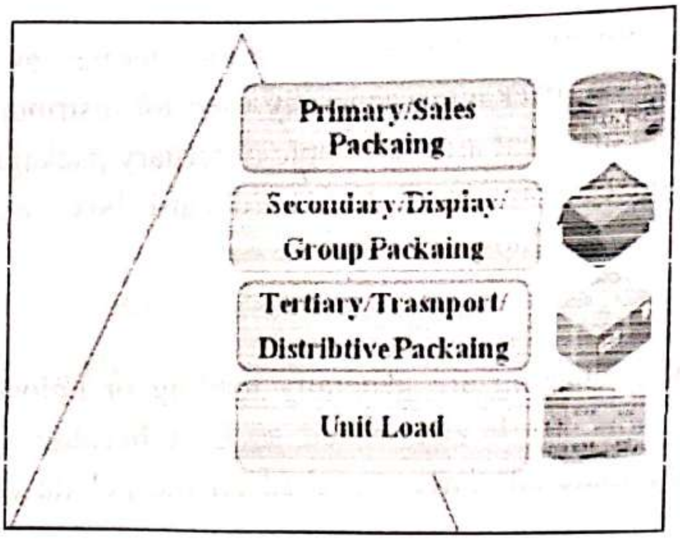
b) Industrial packaging

The packaging, which used to deliver goods from producer to consumer with all intermediate level of packaging i.e. transporting of packaging. Major aims of this packaging are to handover or transfer the goods from production level to next or consumer level.



Methods/types/levels of packaging

Source: https://www.theodorou.gr/en/products/products-smartcode.html



4. Sustainable packaging

With advancement of technologies, there is numerous packaging materials which are not environment friendly. Traditional packaging materials in one side has eco-friendly biodegradable but they required large quantity of raw materials for its production such as paper industry, wooden board industry etc which deplete forestation. But in another side use of plastic, and other artificial materials not only saves these resources in one hand but non biodegradable nature of the materials causes severe threat to environment. Therefore, it is quite difficult to understand the selection of proper materials for packaging industry. So answer is four R approaches that is Reuse, Recycle, Reduce, Resale approach with integrated packaging in optimized for environment sustenance [8].

5. Ideal characteristics of packaging materials

i) It should be tamper proof during storage and transport

ii) It must be Food and Drug Administration (FDA)approved

iii) It should not change original odor, color, taste of the original product.

iv) It should not make any kinds of reaction on the packed product.

v) They should protect internal product from external environment

vi) It should hold the material in firm condition without any excessive gap

vii) It should have adequate compulsive strength, capacity to bear pressure during palletilization, handling and transport system.

viii) It should be non toxic to product, handlers, and if possible environment

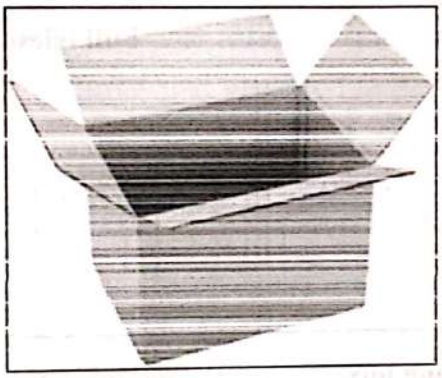
ix) It should be recyclable or reusable for sustainable environment

x) For processed product, they should be grease proof, temperature and light resistant with ability to protect from microbial contamination, leak proof etc.

6. Packing containers for fruits

a) Corrugated fiberboard boxes

Corrugated fiberboard boxes also referred as CFB boxes are most widely used in packaging industry. In USA more than 90%, products packed in CFB boxes [9]. It has various types, shapes and forms but mostly square and rectangular boxes are preferred. Now most of the CFB boxes are thinly wrapped with plastic film, which increase their durability, moisture and dust resistant ability. Several layers in the box act as a shock absorber due to flow of air inside the box, which gives protection against vibration, jerks and shocks.



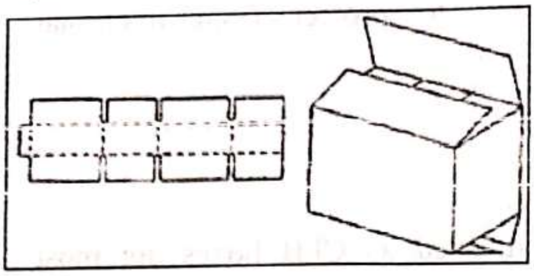
i) Advantages of corrugated boxes

- They are easily available in market at various sizes and shapes
- They are environment friendly and biodegradable in nature
- The boxes can be reused and recycled again if in well condition
- They can easily stackable or palletized
- It has tear resistant qualities, pilfer proof, and this is helpful in keeping the packaging intact
- They can be easily punched, printed, packed with tape etc.

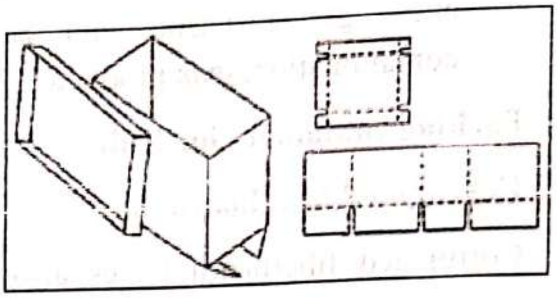
ii) Disadvantages of corrugated boxes

- Deformed or damaged with excessive pressure during handling and transportation with high temperature and pressure
- This is not good option for heavy items due low mechanical stress ability
- They also not good option for liquid products

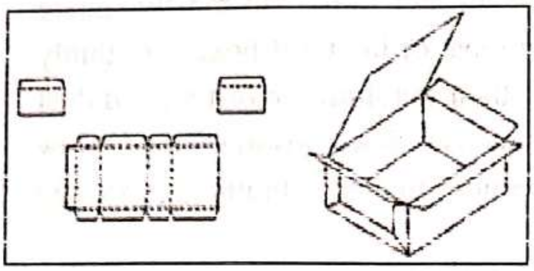
iii) Designs of CFB boxes



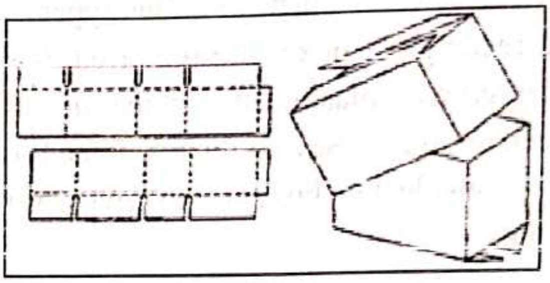
One piece box



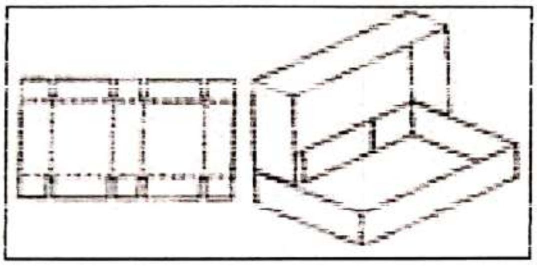
Two piece box with cover



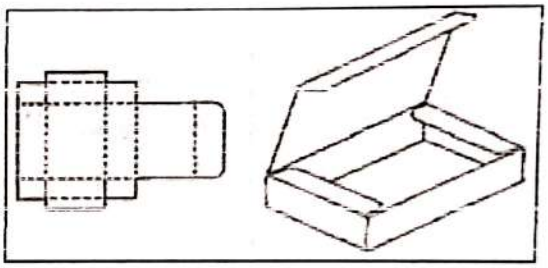
Bliss Style Box



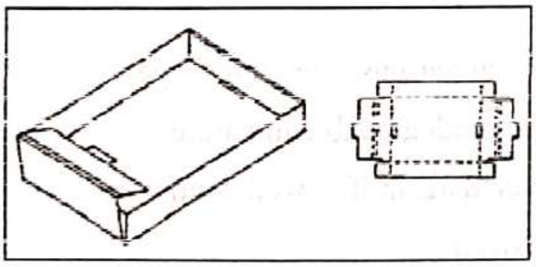
Full telescoping box



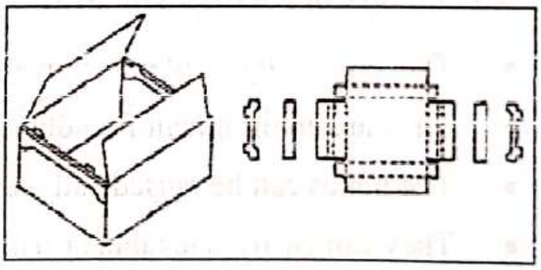
One-piece telescoping box



One-piece tuck-in cover box

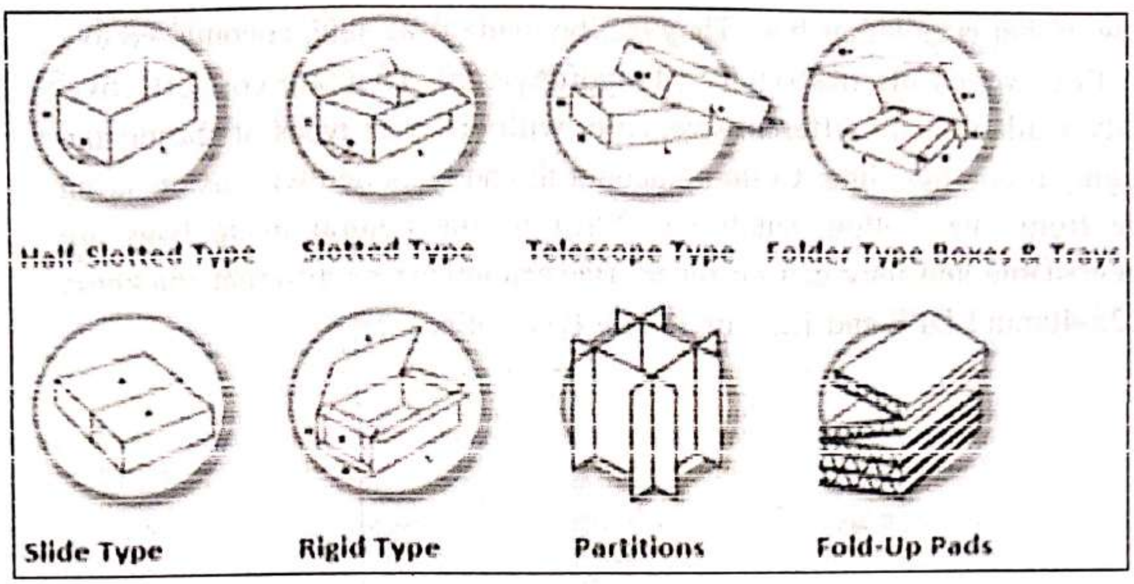


Self-Locking tray



Inter Locking tray

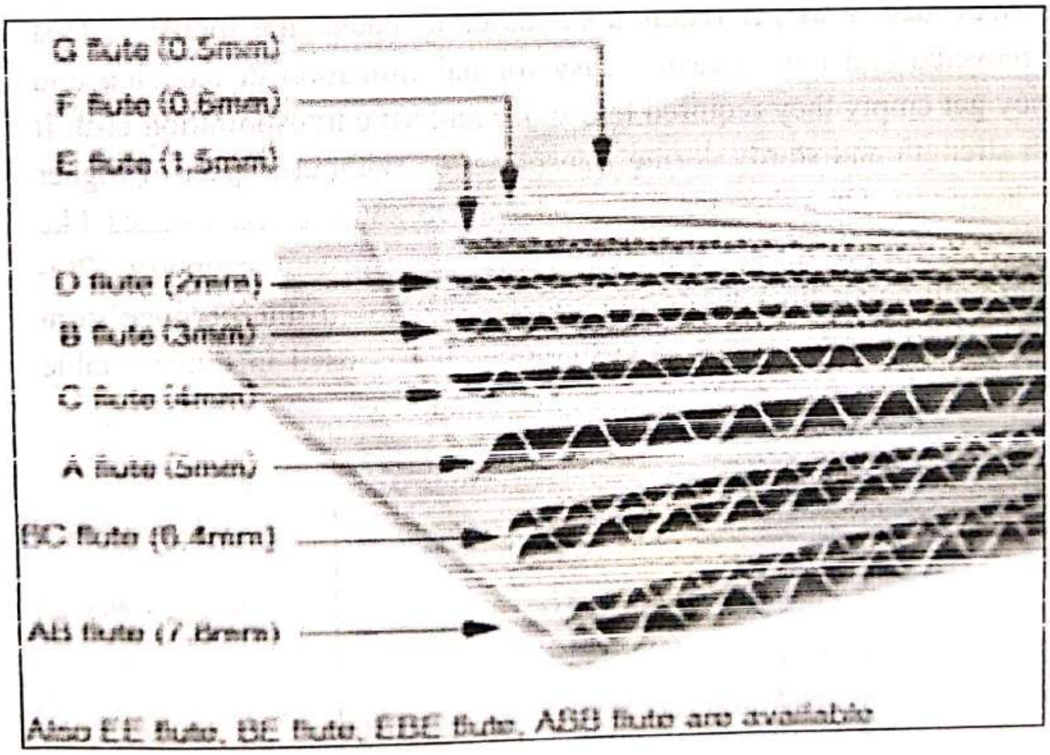
Source: McGregor, B. 1987. Tropical Products Transport Handbook. USDA, Office of Transportation, Agricultural Handbook Number 668.



(**Source:** https://www.ipack.com/solutions/packaging-101-the-corrugated-box/)

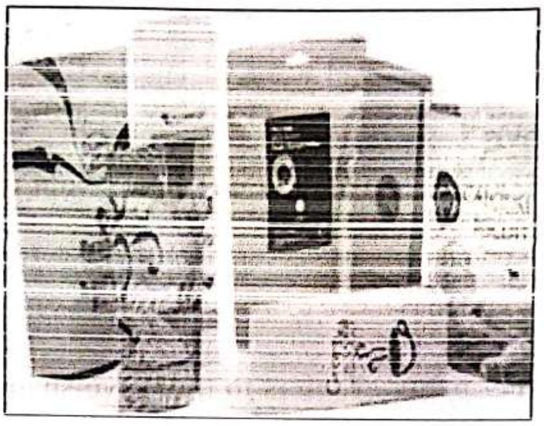
iv) Corrugation flute

This type of packaging material refers to the wave shapes or ridges that are pressed into a sheet material that has been softened by steam. The flutes serves as cushioning materials to give more strength to the box. It has various types according the thickness A (4.7mm), B (3mm), C (3.6mm), D (2mm), E (1.5mm), F (0.6mm) F, (0.5mm) flute (www.pinterest.com).



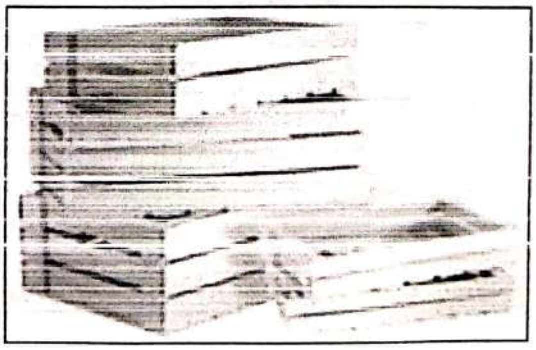
b) Sacks/bags

Sacks are the flexible materials used for shipping various products. If the weight of the packaging material is more than 10kg it called as sacks and below of that is called as bag. They can be made from jute, coconut, cotton, flan, flex, woven plastics (HDPE, Polypropylene). They are cost effective, readily available with different size, color with different types of tearing and snagging properties. Jute, Cotton, coconut thread bags tied with using string made from jute, cotton, sutali etc. Most of the natural made bags are biodegradable and they can be reuse. Bags might be of different thickness like 25-40mm LDPE and 12.5mm HDPE types of bags.



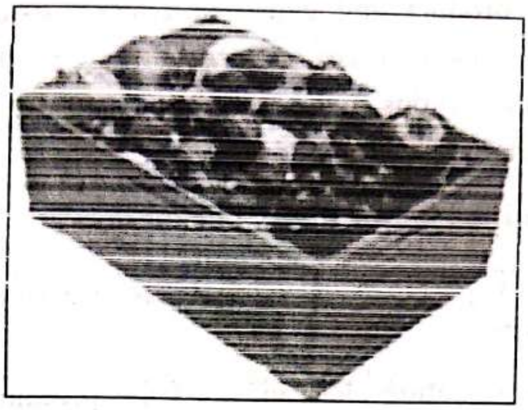
c) Wooden containers

Use of wood as packaging materials has very long history. These are easily available in market and environment friendly. They are available in various sizes, design as per required standards of packaging industry. This can be recycled and reused easily, easy for palletilization or stacking and when they get empty they required less space and save transportation cost. It has high strength and sturdy during transportation without impact of higher relative humidity. On other side, they also have some disadvantages like bulky, heavy weighted, lack of water or dust resistant property. Pre-packaging with paper liner gives additional protection to the produce form internal bruising injury or acts as cushioning. It has used in tomato, table grapes, mangoes, oranges, citrus fruit, pomegranate etc.



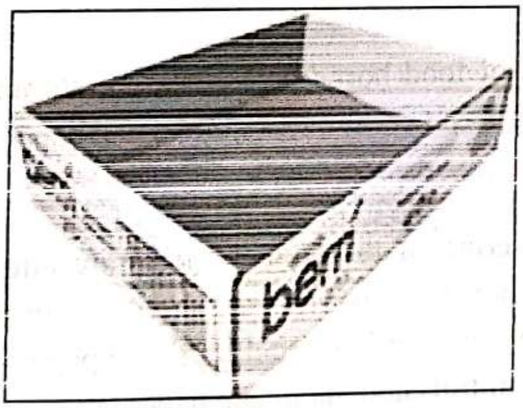
d) Use of plastics-plastic crate, plastic corrugated boxes

Plastic crates majorly used in local fruit markets. Plastic crates generally made from high quality HDPE or polypropylene materials. It has high strength and palletization. Now the crate has being replaced with the wooden crates and wire crates due to high resistant capacity for moisture and ultraviolet degradation.



e) Paper and paperboard container

Apart from Corrugated fiberboard box (CFB) some other paperboard containers are also made from Kraft paper, bleached Kraft paper, virgin paper mostly made from bamboo or grass material or crop residues etc. This biodegradable waste like, paddy, wheat straw, maize husk, baggase etc can be recycled into various types of paper and paperboard. One of the best examples is use of cotton stalk for the Kraft pulp preparation developed by CIRCOT, Mumbai [10], use of banana stalk or stem for preparation of various paper etc are among them.



f) Modified atmospheric packaging (MAP)

Fruits are highly perishable in nature, where most of the nutritional composition starts getting depleting after harvesting. MAP can maintain all optimum conditions inside the package thereby extending shelf life by reducing deterioration [11]. Modified atmosphere packaging (MAP) is a procedure, which involves replacing air inside a package with a predetermined mixture of gases (CO_2, O_2, or N_2) prior to sealing it. In these packaging the products has packed in plastic film bags and kept in a packaging container especially in CFB box. The modified atmospheric condition is maintained by increasing CO_2concentration (bacteriostatic and fungistatic and antimicrobial [12]) and N_2 level (neutral in action and acts as cushioning material to prevent packs collapse) and decreasing O_2 level (which breakdown the product and cause rancidity) inside the package. Along with these three gases traces of carbon monoxide, nitrous oxide, and ozone, argon, and ethanol vapor and sulphur dioxide are also used. The optimum temperature range during storage is 0-5 °C [13].

g) Advantages of modified atmospheric packaging

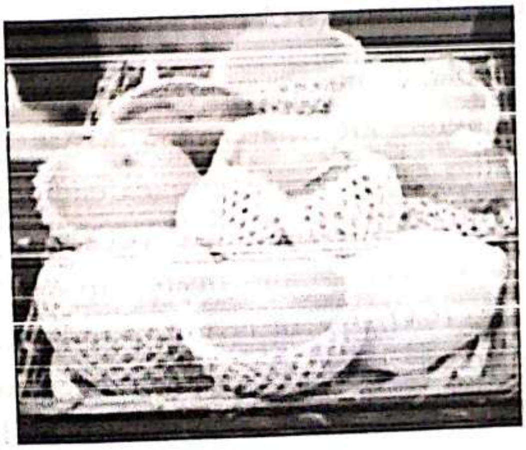
- It increases shelf life allowing lesser frequency of loading of retail display in shelves
- It improved presentation-clear view of the product and all round visibility
- It acts as a hygienic stackable pack, sealed and free from product drip and odor
- Reduction in production and storage costs due to better utilization of labor, space and equipment

h) Disadvantages modified atmospheric packaging

- Capital cost of gas packaging machinery is high
- Increased pack volume increases transport costs and retail display space
- Potential growth of food borne pathogens due to non-maintenance of required storage temperature by retailers and consumers.

i) Wrapping

Wrapping means covering of the fruit immediately after harvest with any type of materials. It increases post harvest shelf life. In market various types of wrapping materials are available such as tissue papers, waxed paper, cellophane paper, aluminum foils and alkathene paper etc.



Following are the benefits of wrapping

- It reduce the risk of post harvest losses and contamination
- It prevents moisture loss and shriveling of the products
- It increases consumer acceptance quality
- It reduces bruises, scratches, pest and diseases attack
- It reduce damages during transit, storage etc.
- It makes fruit more attractive

7. Packing containers for processed fruit products

Processed products have different properties compared to the fresh product hence requires special care and practices during operations. Mostly in India, metal containers used for packaging of processed products and wide mouthed glass bottles for semi-processed product (Jam, Jellies, pickles etc). Liquid products or beverage like RTS, Syrups, etc., are generally packed in glass bottles.

i) Tin containers

Tin cans are highly costly hence, now being replaced by aluminum can or chromium coated steel plate also referred as TFS (Tin free Steel). Some FDA approved coating such as Fruit enamel, C-enamel, citrus enamel and beverage can enamel coatings are preferred for protection of respective food materials from corrosion.

i) Aluminum cans

Aluminum cans are now a day's replacing the tin containers due to low cost and easily availability. It is lightweight, recyclable and less corrosive with the food materials. It extensively used for packaging of various fruit products.

iii) Collapsible tubes

Collapsible tubes made from the metal or plastic materials are most flexible item for packaging. Most of the products are made from aluminum metal but thermoplastic materials are also used as these are cost effective than aluminum. Collapsible tubes made form thermoplastic are having some demerits such as collapsibility, permeability for moisture, oil and acts as a plasticizers. It also creates some gaseous composition inside the container if kept for longer period.

iv) Glass containers

Glass materials are being used as packaging material since ancient times. It is easily available, reliable, non-corrosive, non-permeable and transparent materials. Most of the products like jam, jelly, marmalade, pickles, juices, and squashes are packed in these containers. Beverage product already started to use glass material in large quantity for its processing. These materials are recyclable hence environment friendly.

v) Plastic containers

Plastic containers are made from various types of polymers and petroleum product. Different types of plastic materials are LDPE, HDPE, Polypropylene, Poly Vinyl Chloride, Polystyrene, Biodegradable and photodegradable plastic. They are lightweight, easily available with different size, shape, color. But these containers are not eco-friendly.

vi) Coextruded films

Coextruded film has made by multilayer extrusion process. In juices and beverage three to five, layer films are used. Other examples are Stand-up pouches made of polyester/foil/poly and tetra packs made form paper/ foil/ poly are used in packing of juices.

vii) Dried or dehydrated products packing

Mostly plastic materials (LDPE/HDPE) are used for packaging of dried and dehydrated products, e.g. dehydrated slices of ripe papaya in 250 gauge PE bags, Banana chips in 300 HDPE or 400 gauge LDPE, Potato in HDPE with titanium oxide and brown light absorbing pigment or in metalized PE bags (AT + PE) [14] etc. Powdered dehydrated products like fruit juice powders, soups, custard powders etc are packed in heat sealable laminate containing aluminum foil layer etc. In some packages calcium oxide or silica gel has also used in package to absorb excess moisture.

viii) Accelerated freeze dried (AFD) food packaging or frozen products packing

Accelerated freeze-dried (AFD) products must be saved from oxidative changes like enzymatic browning, flavor and odor changes, loss of ascorbic acid etc due to oxygen hence required O_2 tight packaging and plastic wrappers for palletization of frozen food. Some examples are packing of deep freezed mango pulp paper aluminum- polyethylene laminated pouches [12], PE bags for black currant, plastic cartoons for frozen cut onion etc.

8. Labeling

Labeling and printing in the product is nowadays not only essential for the branding but also for disseminating information's regarding dos and don'ts during the handling, transporting and using of the product to handlers, retailers, wholesalers, consumers. Following information provided on the containers.

- Common name of the product
- Brand name of the product
- Net weight, count or volume of the product
- Name, address contact details or helpline numbers of the company, packers, shipper
- Country or region of making or origin
- Size, grade
- Recommended storage condition such as temperature, RH, etc.
- Special handling and storage instructions
- Nutritional status of the products
- Manufacturing, packaging and expiry date of the products
- Names of the added preservatives, approved waxes or pesticides etc used in the product
- Quality standards and approved agencies logo such as BSI, ISI, ISSO, Agmark, FDA etc.

9. Cushioning materials

Cushioning materials used to give protection to the products from brushing injuries. It absorbs extra pressure, shock, dissipate heat of respiration reduce spoilage, retains products original colour, flavour, taste etc. Dry grass, paddy straw, leaves,, saw dust, paper shreds, cassia leaves, bamboo mat board boxes, moulded pulp tray, honey comb portion, cell pack, air bag, bubble films, rubberized fibre cushioning, coconut fibre or its mat, plastic foam cushioning materials, polystyrene, polyurethane, air cellular cushioning, foam, polyethylene foam, quicksilver laminates, tissue paper, buffered tissue paper etc are wide examples of cushioning materials.

10. New technologies in packaging

i) Thermally sensitive packaging

Thermally sensitive packaging is those kind so packaging materials, which have the capability to change the properties with respect to temperature changes. Side Chain Crystalisable (SCC) Polymers technology has the ability to effectively and reversibly melt with increase temperature fostering gas transmission through them. The SCC polymer becomes molten fluid, which has high gas permeability properties. This permeability can have the capacity to change or maintain carbon dioxide and oxygen ration which is the main determining factor for all kinds of biochemical action. The oxygen level maintained at very low level without going in anaerobic condition. The SCC materials has manufactured by Landec Corp Menlo Park California [16].

ii) Ethylene scavengers

Ethylene is the ripening hormone naturally produced in plant, which causes rapid deterioration, decomposition thereby retards shelf life [17]. The most widely used ethylene-scavenging packaging technology is based on a sachet that contains either potassium permanganate or activated carbon with a metal catalyst. Several ethylene-removing plastic film-based products consisting of PE impregnated with finely dispersed minerals like clays, zeolites and carbon have been developed.

Examples of ethylene scavengers: 1-methylcylcopropene, Silver thiosulfate (STS), Aminoethoxy vinyl glycine (AVG), aluminium alumina, vermiculite, silica gel, with potassium permanganate, activated charcoal etc.

iii) CO_2 scavengers

Role of CO_2 especially in Modified Atmospheric Packaging of foods is very important to increased shelf life. Nevertheless, sometimes-excess CO_2 leads to detrimental factors for the quality of product. For that reason, CO_2 scavengers are advantageous to maintain equilibrium in quality. General mechanism behind this is CO_2 absorption in food packages are chemical reactions and physical absorptions. These scavengers are use along with the layer of various packaging materials. High level of CO_2 (10-80%) inhibits microbial growth and extends shelf life [18].

Examples of CO_2 scavengers: Ferrous carbonate, mixture of ascorbic acid and sodium bicarbonate, Ageless G, Topaan C, Vitalon VMA etc.

iv) Oxygen scavengers

Oxygen not only accelerates spoilage but also causes rancidity, off flavor, color change and nutrient loss among other degradation [19]. Therefore, oxygen scavengers can absorb this excess oxygen and delays oxidation. By using this technology sachet packed with oxygen absorbent where the scavenging materials is usually finely divided iron oxide. E.g.

Ferrous compounds, catechol, ascorbic acid, glucose oxides, unsaturated hydrocarbons and polyamides etc.

v) Humidity regulators

Moisture content in the product leads to growth of microbes. These materials acts as water vapor barrier. Some times when packaging materials sealed and airtight it leads to suffocation, condensation due to evaporation of fruits and vegetables. Mostly this problem arises when product is stored in low temperature like freezing etc. The use of humidity control concept in packaging minimizes all such problems eliminate high water content without drying the fruit materials. Moisture sensitive humidity of the tray is control by using following factors like presence of sodium chloride. Overwrap materials by using two layer technology external layer eliminates water films and internal layer allow only water vapor permeability (not water) called as duplex technology. Another sandwiched packaging technology consists of two sheet of polyvinyl alcohol (PVA) film selling along its edge within which propylene glycol humidifying agent is sandwich for water resistant. Multilayer packing technology consist of layer of PVOH or cellulosic fiber like paper sandwiched between PE films, sheet made of aluminum metalized film with nonwoven fabric on the reverse side are also available in market [20].

vi) Use of robots in packaging and automation

Advanced level of packaging includes use of robots. Mostly robots have found to use in welding and assembly lines [21, 22, and 23]. Use of robots can be a help in ever increasing labour crisis worldwide. They has faster operation, with less error low contamination, high carrying, packaging and processing capacity, to work day and night without asking payment are the main pros. FANUC robotics, Inc. has developed several models of pick-and-place articulated robot for the food industry that meet stringent hygiene requirements [24]. KUKA Robotics is one of the world's leading manufacturers of industrial robots [25]. Many food-processing plants are constantly automating their final product with the palletizing robot due to the demand for increased productivity [21]. The sophisticated control system with a built-in palletizing function makes it possible to load and unload the objects with high precision and accuracy. To increase safety, liability, reducing cost of operations, consistent working etc most of the food and packaging industries now wanted automation system. Some disadvantages are high maintenance cost, requirement of technical expertise lack of availability of spare parts, etc. PLC (Programmable Logic Controller)-based architecture has been commonly used in the food industry [21, 26]. They also articulated with peripheral devices such as sensors, actuators, and drive to minimise static operations.

vii) Smart or Intelligent packaging

Technological innovation is now in that stage where we can control most of the inbuilt environment of packaging by using smart and intelligent packaging. Some sensors have developed which can sense the condition of various parameter according to which we can make precautions of practices in future [27, 28]. The smart or intelligent packaging also use artificial intelligence concept where users get in details information of the product quality during storage, packing, and transit. It helps to monitor the product at each level. Some innovations like self-heating cans or cooling cans [29], can automatically start heating or cooling the product. Several segregated compartments are prepared in the container, which gives exothermic or endothermic reactions to maintain optimum condition of environment. Radio Frequency Identification chips (RFID), Time-temperature indicators (TTI) are also available in the market which used to track the package or consignment in transit, storage or retailer shops [19] These labels are called as smart label. Electronic article surveillance for checking counter shoplifting, security holograms, microwave technology, shock detectors for etc developed for smart packaging.

11. Summary

A specific packaging practice not only minimizes huge post harvest losses but also has tremendous scope to enhance export of quality produce and to gain foreign exchanges. The recent advances in packaging sector helps to minimizes post harvest losses, increasing quality of the consumer product by proper supply chain management. The automatic and robotic packaging, smart and intelligent or active packaging will help to increase the efficiency for better transport, handling and sales in the market. Various innovation, technologies, and new kinds of packaging materials and methods are need to be popularizing to satisfy the consumer demands.

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Chapter - 3

Physiological and Biochemical Factors Governing Post Harvest Life of Flower Crops

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Chapter - 3

Physiological and Biochemical Factors Governing Post Harvest Life of Flower Crops

Sanchita Ghosh and H.D. Chandore

Abstract

The major challenges of post harvest technology of flower crops are to delay the senescence and promoting the longevity in order to fetch high market price as well as to make it more attractive in a flower vase for a longer period. Several physiological factors like water relations, membrane integrity, carbohydrate metabolism, nitrogen metabolism, pollination, plant growth regulators etc and biochemical factors like ethylene biosynthesis, changes in the antioxidant compounds governs the post harvest life of flowers. This book chapter makes an effort to give detail about the reason and the methods to overcome and to prolong the vase life.

Keywords: senescence, post-harvest technology, biochemical factors, metabolism

1. Introduction

Flower is a complex morphological unit and there are several physiological and biochemical factors which govern the post harvest life of flowers. Flower senescence is a distinguished stage indicating the terminal phase of developmental processes that leads to the end of its life span. Postharvest senescence is a major limitation to the marketing of many species of cut flowers and considerable effort have been devoted to develop postharvest treatments to extend the marketing period [1]. The implementation of appropriate storage and postharvest techniques will add value to the produce and will increase the farmer's income. The floral, more specifically petal senescence, is accompanied by dramatic changes in cellular morphology, biochemical processes and gene expression [2]. Several physiological factors like water relations, membrane integrity, carbohydrate metabolism, nitrogen metabolism, pollination, plant growth regulators etc and biochemical factors like ethylene biosynthesis, changes in the antioxidant compounds governs the post harvest life of flowers.

2. Senescence and post-harvest life of flowers

A flower's lifespan and post-production quality has determined by senescence, a genetically controlled stage of flower development that culminates in the wilting or abscission of the corolla [3]. Senescence is the integral part of the normal developmental cycle of plants and can be view on a cell, tissue, and organ or organization level. Senescence is a highly regulated final event of flower development that bears hallmarks of programmed cell death (PCD), resulting in colour changes, petal wilting, abscission of whole flower and flower parts with various physiological, biochemical and ultra-structural changes [4]. Peroxidase enzyme activity in senescing petals are directly associated with respiration and increased levels of free radicals and peroxides [5].

Recent studies evidenced that flower senescence includes controlled disassembly of cells of corolla probably by a mechanism homologous with apoptosis, vacuolar and necrotic PCD [6] and transport of nutrient to other parts of inflorescence. Flower senescence is the terminal phase of developmental processes that lead to the death of flower, which include, flower wilting, shedding of flower parts and fading of blossoms. Since it is a rapid process as compared to the senescence of other parts of the plant, it therefore provides excellent model system for the study of senescence. Petals are the main floral organs, which primarily determine the commercial longevity of flowers, and therefore, it becomes necessary to study the physiological, biochemical and genetic processes that occur during petal senescence [7]. During flower senescence, developmental and environmental stimuli enhance the up-regulation of catabolic processes causing breakdown and remobilization of cellular constituents.

3. Post-harvest changes associated with senescence

3.1 *Ultra-structural changes*

There is a three-stage theory of senescence in case of flowers like those in leaf. First is the initiation of senescence followed by degradation and disassembly, which lead to third stage of death [8], which is due to decline in rate of anabolic processes and increase in rate of certain catabolic processes [9]. Characteristics of the last phase involve ultra-structural disorganization of tissues or cells and increased fluid filled extra spaces which lead to halted down of metabolic activities in all tissues or organs of plant though some organelles are still slightly visible [10]. Delicacy of petal cells and their rapid collapse during senescence are a challenge to study ultra-structural changes during senescence and showed dramatic changes in ultra-structure [11]. Ultra-structural events during senescence include increase in vacuolar size, loss of organelles, eventual collapse of tonoplast [12] and nuclear fragmentation [13]. With reference to the chloroplast ultra-structure, a higher level of thylakoid disorganization (especially of granal membranes) observed during senescence [14]. The loss of non-cellulosic natural sugar and the increase in soluble pectin, uronic acid and cellulose [10] lead to alternation in cell wall and initiation and increase in the loss of membrane integrity resulting in phase changes due to decrease in membrane fluidity and increased permeability.

3.2 Colour fading and change in pigmentation

Chlorophyll is the most prominent photosynthetic pigment in higher plants and the decreasing trend in photosynthetic rate and pigment level is generally due to the involvement of oxygen radicals and singlet oxygen [15]. Chlorophyll breakdown becomes a mandatory phenomenon for the remobilization of nitrogen from chlorophyll-binding proteins to proceed during senescence. Colour fading and discoloration are major reasons for termination of vase life in many cut flowers and important factors in determining display quality of cut flowers. Major three types of pigments contributing to the colour of flowers are anthocyanins, carotenoids, and betalains. Anthocyanins are the largest and most diverse group of plant pigments derived from the phenylpropanoid pathway, ranging in colour from red to violet and blue. Anthocyanins give red colour under low pH and blue colour under high and neutral pH, reflecting a phenomenon termed as 'blueing' where a shift from red to blue occurs with ageing [16]. In flowers, degradation of anthocyanins during senescence possibly related to oxidative process. A significant increase in antioxidant activity correlated with the rate of anthocyanin degradation [17].

3.3 Lipid peroxidation and loss of membrane integrity

Flower senescence was found to be related to the enhanced lipid peroxidation which in turn decreases the membrane stability index. This increase in the lipid peroxidation is most probably initiates by increased lipoxygenase activity just prior to the increase in lipid peroxidation. Lipid peroxidation generates a range of reactive oxygen species, including singlet oxygen (1O2), the alkoxy radical (RO•), hydroxyl radical (OH•) and hydroperoxyl radical (HO2•-), and the peroxy radical (ROO•) which amplify the lipid peroxidation with further degradation of released fatty acids affecting membrane permeability [18]. All the enzymes required for phospholipid degradation are apparently present in membranes. The main phospholipid degrading enzymes, including

i) Phospholipase D

ii) Phospholipase C

iii) Lipolytic acyl hydrolase

iv) Lipoxygenase

Which degrade fatty acids were reported to be upregulated during petal senescence [19]. A marked deterioration of the plasma membrane and loss of water are associated with lipid peroxidation with the senescence of chrysanthemum and *Hemerocallis* petals [7]. Therefore, membrane degradation may be a central step in the process of senescence that leads to mass lipid degradation during senescence and collapse of the tonoplast, which results in executing the death sentence.

3.4 Loss of cellular protein

Proteins are the key molecules that play important roles in various structural and functional aspects of plants. Senescence in tepals, stamens and carpels results in an increase in total protease activity and a decrease in total protein content. In many species of plants, protein degradation and remobilization are mediated through protein ubiquitination and the action of specific proteases [13]. An increase in amino acid content in phloem exudates from flower opening to petal wilting in *Ipomoea, Hemerocallis* and *Sandersonia* petals observed due to protein degradation. Actinomycin D, an inhibitor of transcription, if given 4 h prior to opening, suppressed the onset of visible senescence, which occurred at about 9 h after flower opening by downregulation of senescence associated genes in petals of morning glory [20]. In proteasomes dependent degradation, proteasome system involved in degradation of specific proteins was apparently up-regulated during petal senescence in *Hemerocallis* [21] and daffodil [22]. Expression of a homologous gene encoding a RING zinc finger ankyrin repeat protein (MjXB3), a putative E3 ubiquitin ligase, in petals of senescing *Mirabilis jalapa* flowers highly increased during the onset of visible senescence like that in *Petunia* [23]. Silencing of this gene also resulted in extension of flower life. Petal senescence accompanied by bulk non-proteasomal protein degradation, mainly in vacuoles. This process of protein degradation shows accumulation of considerable amount of amino acids during senescence.

3.5 Change in water relations

Due to decrease in water absorption and increase in water loss through transpiration, the stem develops water deficit conditions results in wilting of flowers [24]. The decrease in water uptake by the stem is mainly due to plugging of xylem vessels caused by growth of microbes mainly bacteria in the vase water or on the dipped portion of stem. Exposure of cut flower to water stress condition, for a short periods lead to the earlier appearance of senescence. Water deficit conditions are reported to cause physiological disorders in the cut stems such as stem break in gerbera and bent neck of rose [25]. Adverse water relations are also reported to cause changes in hormonal balance [26]. During senescence, the rate of water flux through vessels, tracheids and fibres get reduced and tylose formation was often shown to result in reduced water and oxygen availability for the respiration causes imbalance in water relations of flowers. The rate of water uptake of a cut flower depends on hydraulic conductance of the water conduits in the stem, water potential difference between the vase water and the cut flower tissue [27]. (Van Doorn, 2001).

3.6 Membrane integrity

Membrane deterioration is an early and characteristic feature of plant senescence, which leads to several structural and functional changes including the vesiculation of vacuolar and cytosolic compartments etc. In daylily flowers, during senescence the degeneration of vacuolar membrane of epidermal cells was reported [28]. It was revealed that in ethylene- insensitive Alstroemeria flower, during senescence, the loss of membrane function was not related to lipoxygenase activity [29]. In the ethylene- sensitive category, lipoxygenase activity may promote senescence through oxidative membrane damage as seen in rose and carnation. However, in some ethylene- sensitive plants such as Phalaenopsis, the lipoxygenase seems not to play any apparent role. At the biochemical level, senescence is associated with changes in membrane fluidity and leakage of ions in several different flowers. The loss of differential membrane permeability in morning glory flower [30]. The important changes in the membrane include decrease in all classes of membrane phospholipids and increase in neutral lipids, mainly due to increased action of phospholipases and acyl hydrolases. Analysis of phospholipid levels in petals of ornamentals like Tradescantia during senescence has shown that the increase in membrane permeability is accompanied by a massive loss of phospholipids. The activity of phospholipase D declines during senescence whereas that of acyl hydrolase remains essentially constant [31].

3.7 Carbohydrate and other macromolecules

Sugars are an important energy source and structural components. Sugar accumulation is a mechanism to reduce petal water potential-promoting water influx-cell enlargement and flower opening. Petal senescence accompanied by a loss of dry matter. Sugars or carbohydrates increased the vase life of cut flowers by reducing the sensitivity to ethylene [32]. It has suggested that maintenance of osmotic pressure might be the reason for the delay in senescence. It was also reported that exogenous application of sugars increase vase life by delaying proteolysis, promoting protein and amide synthesis, maintaining osmotic potential, delaying membrane integrity and maintaining mitochondrial structure and function [33].

3.8 Protein metabolism

There is a decline in protein content during senescence. The senescence of both climacteric and non-climacteric flowers have been associated with a loss of protein. The protein content is reduced due to little de novo synthesis and considerable protein degradation. It is understood that free radicals attack amino acid residues of proteins causing conformational changes in proteins causing them to be recognized by specific proteases for degradation. In Dendrobium *cv.* Khao Sanan Treatment with ethylene resulted in an early increase in the transcript abundance of a senescence-associated cysteine peptidases in the petals, in an early rise in peptidase activity, and in a later decrease in water-insoluble protein levels which promote senescence [34].

3.9 Pollination regulation of flower senescence

Pollination regulates a syndrome of developmental responses that contributes to successful sexual reproduction in higher plants. Pollination-regulated developmental events collectively prepares flower for fertilization and embryogenesis while bringing about the loss of floral organs that have completed their function in pollen dispersal and reception. Components of this process include changes in flower pigmentation, senescence and abscission of floral organs, growth and development of ovary and in certain cases, pollination triggers ovule and female gametophyte development in anticipation of fertilization [35].

Pollination regulated development is initiated by the primary pollination event at the stigma surface, but because developmental processes occur in distal floral organs, the activity of inter organ signals that amplify and transmit the primary pollination signal to floral organs is implicated. Inter organ signalling and signal amplification involves the regulation of ethylene biosynthetic gene expression, inter organ transport of hormones and their precursors. This stimulation of ethylene biosynthetic pathway results in enhanced ethylene production and thereby triggering flower senescence in ethylene sensitive flower. In carnation (*Dianthus caryophyllus*) the senescence of flowers is characterized by the in rolling of the petal margins, this process is accelerated by ethylene and pollination [34]. Pollination of orchid flower induces a dramatic increase in ethylene production, which subsequently causes a rapid petal wilting, whereas the longevity of intact unpollinated flowers may reach as long as several months [36].

4. Role of plant growth regulators in accelerating and alleviating flower senescence

Plant Growth Regulators can delay as well as can also accelerate the senescence. PGRs, which promote senescence, include ethylene, abscisic acid, jasmonate, methyl jasmonate, salicylic acid and brassinosteroids; whereas cytokinins, auxins, gibberellins, polyamines cause retardation of senescence [37, 38]. Abscisic acid (ABA) has observed to enhance ethylene production and hastened petal wilting. It is reported that, If gynoecia are removed, induction of ethylene no longer occurs, therefore, it is proved that ABA acts as an inducer of ethylene only through gynoecium [39]. Stimulatory effect of GA_3in retarding senescence is reported in cut gladiolus spikes [40]. It has been reported that PAs have the positive effects on the post harvest life of flower crops like gerbera [41] and carnation [41]. In gladiolus cut spikes, improved vase life was reported after use of PAs by improving membrane stability [42].

4.1 Ethylene

Ethylene is the major promoter of flower senescence in ethylene sensitive flowers, coordinating senescence pathways and floral abscission [43], while in ethylene- insensitive flowers abscisic acid (ABA) has thought to be primary regulator. Visible sign of senescence in ethylene sensitive flower has accompanied by sudden, transient increase in respiration resulting in burst of endogenously produced ethylene, coordinating the senescence pathways and upregulation of genes for enzymes required for senescence [44]. Ethylene imposes considerable problems in the postharvest handling of ornamentals, which cause a range of effects, including early wilting of flowers, yellowing or necrosis (death) of leaves, and shattering of leaves, buds, petals and flowers. Ethylene has been reported to be a signal to induce senescence of flowers [36]. Petal senescence accompanied with the concentration and duration of ethylene exposure [45]. In petal tissue, ethylene found to be responsible for inducing various biochemical processes leading to programmed cell death, including the activation of senescence-related gene transcription [46]. Polyamines inhibit ethylene production by regulating the activity of 1-aminocyclopane-1-carboxylic acid synthase and oxidase [47], while ethylene alters the formation of PAs by reducing the activity of arginine decarboxylase and SAM decarboxylase. Both theory and practices revealed that there are several other factors like age-specific factors, external stress, and nutrient status, which combined cause changes in gene expression and activation of senescence-related biochemical and cellular processes [2]. In climacteric or ethylene sensitive flowers, senescence is accompanied by sudden, transient increase in ethylene production and respiration (e.g. Carnations, Petunia, Gypsophila, Orchids etc.), whereas in non-climacteric flowers (e.g. Gladiolus, Tulip, Iris etc.) generally there is no increase in ethylene production and respiration during senescence.Molecules block ethylene receptors such as cyclopropene (CP), 1 methylcyclopropene (1-MCP) and 3,3-dimethylcyclopropene (3, 3-DMCP) and that block ethylene biosynthesis α-(2-aminooxyvinyl Glycine) (AVG) and aminooxyacetic acid (AOA) have been used to prolong the vase life of ethylene-sensitive flowers [48].

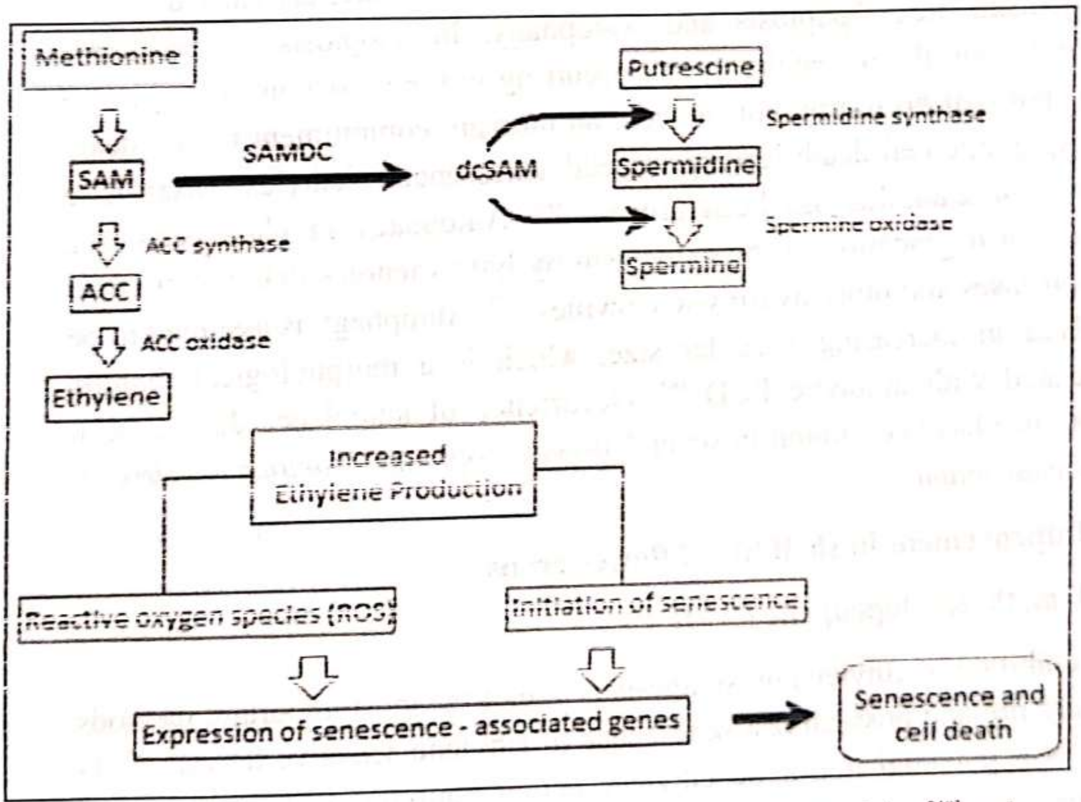

Fig 1: Polyamine and ethylene pathways and their interrelationships [49] and increasing ethylene production through key events regulating floral senescence in ethylene-sensitive species [2]

4.2 Abscisic acid

Abscisic acid while applied exogenously accelerates petal senescence in cut carnation and flower petals treated with ABA were more sensitive to exogenous ethylene [50]. Along with it, there was a decrease in total protein content and increased RNase activity. However, in attached system, the effects were reversible, probably because of closure of stomatal aperture [51].

Exogenous applications of ABA accelerate the symptoms of flower senescence in carnation, rose and daylily flowers [57]. Endogenous content of ABA increased during senescence in several flowers [22]. ABA presumably induces senescence independently of ethylene action, as the senescence of the flower known to be ethylene independent-Day lily [52].

5. Programmed cell death

Programmed Cell Death refers to any process by which cells eliminated as a part of a developmental or adaptive event in the life cycle of organisms [53]. Petal senescence is one type of programmed cell death (PCD) in plants which is a genetically controlled sequence of events during its final developmental stage. These changes include chromatin condensation, DNA fragmentation and nuclear fragmentation [54]. The changes are carried by two mechanisms i.e., Apoptosis and Autophagy. In apoptosis, the principle theory is that, the molecular events occurring in the mitochondria appear to be a "point of no return" for the cell, an ultimate commitment to cell death [55]. Apoptotic cell death leading to petal senescence in ethylene-insensitive flowers of gladiolus has been proven [56]. Autophagy in plant cells is a process of degradation of the cytoplasm by lytic vacuoles that contain acid phosphatases and other hydrolytic enzymes [57]. Autophagy is assumed to be involved in increasing vacuolar size, which is a morphological feature, associated with autolytic PCD [6]. Occurrence of autophagy during petal senescence has been found in several flower crops like *Ipomoea* [30], *Iris* [11] and *Dendrobium* [58].

6. Improvement in shelf life of flower crops

6.1 1-methylcyclopropene (1-MCP)

Inhibition of ethylene biosynthesis is one of the most important methods to retard pre-and post-harvest senescence of ethylene-sensitive flowers [4]. 1-MCP is a potential non-toxic ethylene action inhibitor, which has a high binding affinity to ethylene receptors suppressing the ethylene response pathway [59]. It used with cut flowers (carnations, roses, narcissus, orchids etc), potted flowers, and bedding, nursery and foliage plants to prevent premature wilting, leaf yellowing, premature opening and premature death [21, 22]. The gaseous nature of 1-MCPmakes application to cut flowers difficult during cold storage [60]. Therefore, consecutive research lead to development of liquid formulation of 1-MCP which can be applied as pre-harvest spray [61, 62].

6.2 Nitrous oxide

Nitrous Oxide is diffusible and ubiquitous gaseous bioactive molecule, which participates in the broad spectrum of path physiological and developmental processes of living organisms. It was reported that NO is a natural senescence-delaying plant growth regulating agent acting primarily, but not solely, by down regulating ethylene emission. A Study revealed that the vase life of flowers was extended by NO with an average extension of about 60% with the range being about 200% for gerbera to 10% for chrysanthemum compared with flowers kept in water, suggesting that NO appears to have widespread applicability to cut flowers and offers a simple technology to extend vase life [63]. Although NO has been reported to extend the vase life of cut flowers [64], the precise function of NO as signal molecule involved in cut flower senescence is still unknown.

6.3 Kinetin

Cytokinin increases flower longevity by reducing respiration rate [65]. Some studies indicated that, in rose petals kinetin increases net water uptake of expanding rose petals and delays wilting of petals especially when flowers are subjected to heat (28 °C) and low relative humidity (40-50%) [66]. They also reported that Kinetin had no effect on protein content of rose petals under these stressful conditions, but kinetin retarded the increase in RNase activity normally seen in rose flowers at the onset of senescence. Kinetin proposed to increase rose flower longevity by improving water balance and delaying senescence processes.

6.4 Polyamines

Polyamines (PA's) have been reported as effective anti-senescence agents that have ability to retard chlorophyll loss, membrane deterioration and increase in RNase and protease activities which help to slow the senescence process [66]. The major polyamines comprises putrescine, spermidine and spermine, which occur either naturally or as free bases or bound to phenolics or other low molecular weight compounds. It has been reported that PAs have the stimulative effects on enhancing vase life of different flower crops like gerbera [41] and carnation [67, 41] mainly by improving membrane stability. Exogenous application of spermidine has been found to transiently delay senescence of *Dianthus caryophyllus* and *Petunia hybrida* flowers, which has been implicated to be due to the ability of free spermidine to bind to the main intracellular constitutive molecules such as DNA and stabilizing their structures [68].

7. Summary

Senescence is the integral part of the normal developmental cycle of plants and can be view on a cell, tissue, and organ or organization level. Several physiological factors like water relations, membrane integrity, carbohydrate metabolism, nitrogen metabolism, pollination, plant growth regulators etc and biochemical factors like ethylene biosynthesis, changes in the antioxidant compounds governs the post harvest life of flowers. There are some PGRs, which can delay as well as can also accelerate the senescence. PGRs, which promote senescence, include ethylene, abscisic acid, jasmonate, methyl jasmonate, salicylic acid and brassinosteroids; whereas cytokinins, auxins, gibberellins, polyamines causes retardation of senescence. Further studies are needed to understand the mechanism as well as to find cost effective way to enhance the post harvest life of flower crops.

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