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Chapter 3

Processing and Preservation of Fresh-Cut Fruit and Vegetable Products

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.69763

Abstract

Fruits and vegetables are plant derived products which can be consumed in its raw form without undergoing processing or conversion. Fresh-cut fruits and vegetables (FFV) are products that have been cleaned, peeled, sliced, cubed or prepared for convenience or ready-to-eat consumption but remains in a living and respiring physiological condition. Methods of preserving FFV to retain its wholesomeness includes washing with hypochlorite, hydrogen peroxide, organic acids, warm water and ozone for disinestation and sanitization; use of antimicrobial edible films and coatings; and controlled atmosphere storage and modified atmosphere packaging of fruits and vegetables. Exposure of intact or FFV to abiotic stress and some processing methods, induces biosynthesis of phenolic compounds and antioxidant capacity of the produce. Conversely, loss of vitamins and other nutrients has been reported during processing and storage of FFV, hence the need for appropriate processing techniques to retain their nutritional and organoleptic properties. FFV are still faced with the challenge of quality retention and shelf life preservation mostly during transportation and handling, without impacting on the microbiological safety of the product. Hence, food processors are continually investigating processes of retaining the nutritional, organoleptic and shelf stability of FFV.

Keywords: fresh-cut fruits, fresh-cut vegetables, preservation, bioactive compounds, organoleptic properties

1. Introduction

According to the International Fresh-Cut Produce Association (IFPA), fresh-cut fruit and vegetable products (FFVP) are defined as fruits or vegetables that have been trimmed,
peeled or cut into a 100% usable product which has been packaged to offer consumers high nutrition and flavour while still maintaining its freshness [1, 2]. The importance of fresh-cut produce lies in its major characteristics of freshness, convenience, nutrient retention and sensory quality while providing extended shelf life [3, 4]. Fresh-cut fruits and vegetables (FFV) are products partially prepared and which require no additional preparation for their use. This makes it unavoidable that their overall quality diminishes during processing and storage. It is made more so, as the operations involved in preparing fresh-cut products damage the integrity of the cells, promotes contact between enzymes and substrates, increases the entry of microorganisms and creates stress conditions on the fresh-cut produce [4, 5].

According to Artes-Hernandez et al. [6] FFVP are also referred to as products prepared with slight peeling, cutting, shredding, trimming and sanitizing operations and which have been packed under semipermeable films and stored under refrigerated temperature. Fresh-cut products are also reported to contain similar nutrients and ingredients as whole products with the added advantage of short time preparation and the low prices at which they are been sold [7]. Fresh-cut products constitute a major rapidly growing food segment which is of interest to food processors and consumers. The fresh-cut industry is expanding more rapidly than other sectors of the fruit and vegetable market due to its supply of both the food service industry, retail outlet as well as its expanding production and access to new markets across the globe. The growth rate of the sector is reported to be in the region of billions of dollars in recent years with USA as the main producer and consumer while the UK and France follows after [6]. FFVP presently sold in markets across the globe includes: lettuce (cleaned, chopped, shredded), spinach/leafy greens (washed and trimmed), broccoli and cauliflower (florets), cabbage (shredded), carrots (baby, sticks, shredded), celery (sticks), onions (whole peeled, sliced, diced), potatoes and other roots (peeled, sliced), mushrooms (sliced), jicama/zucchini/cucumber (sliced, diced), garlic (fresh peeled, sliced) as well as tomato and pepper (sliced) [2].

Despite the fact that food processing methods extend the shelf life of fruit and vegetable products, processing of fresh-cut produce however reduces the shelf life of the commodity, rendering the product highly perishable as a result [6]. This biological changes may lead to flavour loss, cut-surface discolouration, decay, rapid softening, increased rate of vitamin loss, shrinkage as well as shorter shelf life of the fresh-cut produce. Interactions between intracellular and intercellular enzymes with substrates as well as increased water activity may also lead to flavour and textural changes upon processing [8]. A major effect of fresh-cut processing is stress on vegetable tissues with the resultant phytochemical accumulation and loss induced through reduced activity in key enzymes of secondary metabolic pathways. Fresh-cut processing also results in cell breakdown as well as the release of intracellular products such as oxidizing enzymes thereby quickening product decay [2].

Several factors are reported to affect the overall quality of fresh-cut produce. Among many of such factors is appearance [1, 9]. Appearance according to Kays [10] and Lante and Nicoletto [11] in combination with size, shape, form, colour as well as the absence of defects are factors which greatly affects the purchase of fresh-cut produce by consumers. All of these factors can
also be influenced by several pre-harvest factors. Available nutrients inherent in fruits and made available upon consumption includes antioxidant vitamins beta-carotene (pro-vitamin A), α-tocopherol (vitamin E) and ascorbic acid (vitamin C). Research has also shown that regular consumption of fruits and vegetables reduces risk of cancers, cardiovascular diseases and several inflammations [12, 13]. This apart from regular body exercise and genetics has made fruit and vegetable consumption one of the main factors that contributes to a healthy lifestyle. With studies showing the nutritional benefits of fruits and vegetables, consumption of FFVP therefore promotes health through increase in the supply of antioxidant and other phytochemical nutrients to the body.

2. Processing of FFV

FFV have been known to have a shorter shelf-life compared to intact fruit and vegetable products due mainly to processing. Several processes involved in the production of FFVP have been known to alter greatly the shelf stability of the cut-produce. There are traditional processing procedures involved in obtaining fresh-cut products and this procedures usually requires an order of unit operations such as peeling, trimming, shredding, dicing, cutting, washing/disinfecting, drying and packaging. Shelf life extension of the cut produce is therefore dependent on a combination of these unit operations as well as proper temperature management during storage, use of antibrowning agents, proper packing conditions as well as good manufacturing and handling practices [6, 7]. The unit operations required in the handling and processing of FFVP is shown in Figure 1.

Cutting

An essential aspect of processing of fresh-cut produce is cutting. Cutting helps divide whole harvested fruit and vegetable products into minute fractions before packaging. The effect of cutting however on the products is the wounding stress which the cut tissues are allowed to suffer thus accelerating the rate of spoilage and deterioration of the cut produce [14]. Cutting has been attributed to be the main factor responsible for the deterioration of FFV thereby enabling the product to experience a more rapid rate of deterioration than whole products [15]. Cutting increases respiration rate [16], induces deteriorative changes associated with plant tissue senescence and thus the consequential decrease in shelf life when compared to the unprocessed produce [4]. Cutting shape as well as the sharpness of the cutting blade has been attributed as some factors that affect the quality attributes of fresh-cut products [17, 18]. The works of Portela and Cantwell [19] showed that melon cylinders cut with a blunt blade demonstrated higher ethanol concentrations, off-odour scores, electrolyte leakage, and increased potential for ethylene secretion when compared to products processed with a sharp blade. It was also reported that use of sharp cutting implements reduces wound response, lignin accumulation, white blush, softening and microbial growth in fresh-cut carrots [18, 20–22]. Cutting-induced injury has been implicated as affecting the immediate visual quality of fresh-cut products and has also been known to have longer-term effects on metabolism with the concomitant quality changes that are detected at a later time. The actual cutting process
results in great tissue disruption as formerly sequestered enzymes and substrates mix are found to mix, hydrolytic enzymes released, while signalling-induced wounding responses may be initiated [23].

During the process of cutting, phenolic metabolism takes place: breakage of the plasma membrane with the resultant effect of inducing oxidative enzymatic reactions thus triggering browning of tissues and oxidation of polyphenols [14, 24]; and production of injury signals which induces the secretion of more secondary metabolites including phenolic antioxidants to heal the wound damage [14, 25, 26]. It has been reported that the content of phenolic acids increases in

Figure 1. Unit operations and maximum recommended temperatures for each step in the processing of fresh-cut fruit and vegetable produce. Adapted from Artes-Hernandez et al. [6].
fresh-cut products. This fact can be attributed to the cutting of fresh fruits and vegetables with knives thereby inducing the activity of polyphenol oxidase (PPO) in the cut fresh fruit and vegetables. FFV are thus easily susceptible to browning reaction as a result [27, 28]. Accumulation of phytochemicals can also be as a result of altered O\textsubscript{2} and CO\textsubscript{2} levels during packaging as well as the use of preservatives such as [14] ascorbic and citric acid [28–31].

Wounding as a result of cutting has been attributed as one of the basic source of stress experienced by fresh-cut produce. Some factors can however affect the wound response of the fresh-cut produce and these factors include stage of maturity, cultivar, storage, processing temperature, cutting method, water vapour pressure as well as O\textsubscript{2} and CO\textsubscript{2} levels [18, 19]. According to literature, wounding stress as a result of cutting of fruit and vegetables has been shown to increase the antioxidant activity as well as the polyphenolic content in fresh-cut produce such as carrots [32, 33], celery [34], lettuce [35], broccoli [36], mushroom, onions, and mangoes [37]. Consequences of wounding includes increase in respiration rate; production of ethylene; oxidative browning; water loss; and degradation of membrane lipids [4, 5]. This therefore increases the susceptibility of FFV to increased perishability than their source commodity [38].

**Sanitation and hygiene in processing facilities**

During the production process, cut fruits are exposed to environmental microbes in the processing facility. Reducing the level and rate of contamination will be dependent on the use of the appropriate disinfectants and sanitizers. One of such disinfectants of high use in the FFV industry is chlorine. The use of chlorine as a disinfectant is however of great concern and is presently prohibited in some European countries due to issues of public health [39]. Chlorine is generally used in the food industry due mostly to its low price and its wide application of antimicrobial effectiveness [39, 40]. However, under certain conditions, chlorine has been shown to be weak in reducing microbial loads [41] as it can easily be inactivated by organic matter [40, 42] and its action is highly pH reliant. Chlorine has also been shown to produce unhealthy by-products which are carcinogenic and mutagenic such as chloroform, trihalomethanes, chloramines and haloacetic acids, when reacting with organic molecules [43, 44]. Chlorine is also corrosive with its use banned in some European countries such as Belgium, Denmark, Germany and The Netherlands [40, 45–47]. Presently, alternative chemical compounds, biological methods and physical technologies which are more environmentally friendly and possess less risk to the health of workers and consumers have been developed to replace the use of chlorine [45–52].

Concentrations of 50–200 ppm and exposure time of 5 min of chlorine is commonly applied as hypochlorous acid and hypochlorite and as disinfectant in the FFV industry in order to enhance microbial safety of the produce [1, 49]. The exposure time of 5 min (depending on the microorganism) has been shown in literature as the maximum exposure time required as longer times of > 5–30 min did not result in increased removal of the pathogenic organisms [39, 53].

In the handling and processing of FFV, common practices are undertaken and needs to be taken note of. These practices consist of protection from damage as a result of poor handling and poor functioning of machinery, foreign body contamination, improper washing,
drying and unhygienic practices by personnel. Hence worker sanitation which is most often neglected in the fresh cut industry in collaboration with good manufacturing practices must be enforced by food processors. In accompanying this process, training of food handlers in food hygiene techniques must be undertaken [6].

Presently, new and alternative technologies for safety, improved quality and extended shelf life of processed fresh-cut products have been developed. Such technologies include: ozone (O₃), a strong oxidizing agent in destroying microorganisms which has also been suggested as an alternative to sanitizers due to its effectiveness at low concentrations, short contact times and in the breakdown of nontoxic products; chlorine dioxide (ClO₂), which is known for its efficacy against pathogenic spores, bacteria and viruses; organic acids and calcium (Ca) salts applied for maintenance of cell wall structure and firmness (Ca), inhibition of enzymatic and non-enzymatic browning as well as in the prevention of microbial growth at heights that did not affect flavour of the fresh-cut products with their efficacy against microbes higher for bacteria than molds; electrolyzed water employed due to its strong bactericidal effect against pathogens and spoilage microbes [6].

2.1. Inhibition of browning in FFV

It has been shown that fresh-cut process increases the metabolic activity mainly as a result of the enzymes polyphenol oxidase (PPO) causing discoloration and peroxidase (POD) causing enzymatic browning as well as de-compartmentalization of enzymes and substrates in tissues causing changes in flesh colour [54]. PPO can induce the browning occurrence by catalyzing the oxidation of phenol to o-quinones which are polymerized to produce brown pigments. Postharvest techniques maintaining the quality of fresh-cut fruit have been investigated by several researchers [55–57] including physical and chemical treatments. Many anti-browning agents or mixtures have been investigated like: calcium ascorbate with citric acid and N-acetyl-L-cysteine [58], citric acid [59], ascorbic acid with citric acid and calcium chloride [30], 4-hexylresorcinol with potassium sorbate and D-isoascorbic acid [60] and modelling of the effects anti-browning agents on colour change in fresh-cut [57].

In a study using mathematical modelling (Table 1), the effects of different anti-browning compounds (ascorbic acid, citric acid, L-cysteine and glutathione) at four concentrations of 0% as control, 0.5, 1.5 and 2.5% on \(L^*\)-value, hue angle, brown scores and brown pigments of fresh-cut mangoes were investigated by Techavuthiporn and Boonjaratthonghai [57] and they observed similar changing tendency of \(L^*\)-value and hue angle decreasing in time during storage at 10°C, while the brown scores and amount of brown pigment increased. They also observed that treatment with L-cysteine or glutathione was effective in suppressing tissue metabolism, PPO and POD activities, while citric acid significantly inhibited the growth of microorganisms.

2.2. Microbial safety of FFV

The unit operation employed in processing of FFV involves peeling, cutting, slicing and shredding; all of which cause disruption of surface cells, tissue and cytoplasm exposure, coupled with high water activity and low pH; thereby providing a breeding ground for growth of pathogenic
microorganisms such as *Escherichia coli* 0157:H7, *Salmonella* spp. and *Listeria monocytogenes*. Peeling and cutting of fruits and vegetables removes the protective epidermal layer, thus exposing the product to air and possible contamination by bacteria, molds and yeast. Contamination of produce can occur at any stage from production till consumption. During growth, harvest, transportation and further processing and handling, fresh-cut produce can be contaminated with pathogens from human, animal, or environmental sources [40, 61]. Since most FFV are consumed raw without any further treatment, consumption poses a potential health risk in cases where there is contamination. Several outbreak incidences have been documented by Centre for Disease Control and Prevention as reviewed by Ramos et al. [40]. Some of the sources of these microorganisms include soil, manure, silage, sewage, water, raw meat, and domestic animals. Presence of up to 10^4 of *Listeria monocytogenes* cells can cause food infection with up to 90 days incubation period. Symptoms of its infection includes flu-like symptoms, septicemia, encephalitis, still birth and abortion in pregnant women [62]. There are a number of factors affecting the microbial safety of FFV and which will be elucidated as follows:

<table>
<thead>
<tr>
<th>Treatments</th>
<th>( K_L \pm S.E. (\text{day}^{-1}) )</th>
<th>( K_H \pm S.E. (\text{day}^{-1}) )</th>
<th>( K_{BS} \pm S.E. (\text{day}^{-1}) )</th>
<th>( K_{BP} \pm S.E. (\text{day}^{-1}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0209 ± 0.0012</td>
<td>0.0158 ± 0.0008</td>
<td>0.2604 ± 0.0474</td>
<td>0.2616 ± 0.0187</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.0132 ± 0.0012</td>
<td>0.0132 ± 0.0002</td>
<td>0.3313 ± 0.0456</td>
<td>0.1566 ± 0.0231</td>
</tr>
<tr>
<td>0.5%</td>
<td>0.0088 ± 0.0007</td>
<td>0.0090 ± 0.0004</td>
<td>0.2146 ± 0.0777</td>
<td>0.1272 ± 0.0062</td>
</tr>
<tr>
<td>1.5%</td>
<td>0.0066 ± 0.0010</td>
<td>0.0079 ± 0.0003</td>
<td>0.2231 ± 0.0572</td>
<td>0.1186 ± 0.0129</td>
</tr>
<tr>
<td>2.5%</td>
<td>0.0014 ± 0.0026</td>
<td>0.0097 ± 0.0007</td>
<td>0.1443 ± 0.1053</td>
<td>0.1386 ± 0.0062</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.0213 ± 0.0023</td>
<td>0.0120 ± 0.0014</td>
<td>0.1859 ± 0.0466</td>
<td>0.1981 ± 0.0212</td>
</tr>
<tr>
<td>0.5%</td>
<td>0.0210 ± 0.0030</td>
<td>0.0098 ± 0.0012</td>
<td>0.1642 ± 0.0971</td>
<td>0.1559 ± 0.0177</td>
</tr>
<tr>
<td>1.5%</td>
<td>0.0164 ± 0.0026</td>
<td>0.0097 ± 0.0007</td>
<td>0.1443 ± 0.1053</td>
<td>0.1386 ± 0.0062</td>
</tr>
<tr>
<td>2.5%</td>
<td>0.0068 ± 0.0019</td>
<td>0.0095 ± 0.0011</td>
<td>0.1776 ± 0.0521</td>
<td>0.1427 ± 0.0263</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>0.0053 ± 0.0008</td>
<td>0.0077 ± 0.0011</td>
<td>0.1342 ± 0.0400</td>
<td>0.1188 ± 0.0127</td>
</tr>
<tr>
<td>0.5%</td>
<td>0.0025 ± 0.0002</td>
<td>0.0041 ± 0.0001</td>
<td>0.1385 ± 0.0213</td>
<td>0.0653 ± 0.0047</td>
</tr>
<tr>
<td>1.5%</td>
<td>0.0053 ± 0.0008</td>
<td>0.0077 ± 0.0011</td>
<td>0.1342 ± 0.0400</td>
<td>0.1188 ± 0.0127</td>
</tr>
<tr>
<td>2.5%</td>
<td>0.0025 ± 0.0002</td>
<td>0.0041 ± 0.0001</td>
<td>0.1385 ± 0.0213</td>
<td>0.0653 ± 0.0047</td>
</tr>
<tr>
<td>Glutathione</td>
<td>0.0180 ± 0.0010</td>
<td>0.0107 ± 0.0004</td>
<td>0.1866 ± 0.0487</td>
<td>0.1993 ± 0.0095</td>
</tr>
<tr>
<td>0.5%</td>
<td>0.0094 ± 0.0011</td>
<td>0.0089 ± 0.0004</td>
<td>0.1676 ± 0.0262</td>
<td>0.1311 ± 0.0115</td>
</tr>
<tr>
<td>1.5%</td>
<td>0.0061 ± 0.0007</td>
<td>0.0087 ± 0.0004</td>
<td>0.1329 ± 0.0229</td>
<td>0.0973 ± 0.0167</td>
</tr>
</tbody>
</table>

\( k_L \) and \( k_H \) represent the estimated rate constant of decreasing \( L^* \)-value and Hue angle in \( Q_t = Q_0 e^{-k\cdot t} \) (Eq. 1) and \( k_{BS} \) and \( k_{BP} \) represents the estimated rate constant of increasing brown colour (score) and brown pigment in \( Q_t = Q_0 e^{k\cdot t} \) (Eq. 2). \( Q_t \) = measured value of colour variables at time \( t \), \( Q_0 \) = initial value of colour variables at time zero, \( t \) the storage time (day), and \( k \) reaction rate constant (day\(^{-1}\)).

Adapted from Techavuthiporn and Boonyaritthonghai [57].

Table 1. The estimated parameters \( k \) and the standard error of estimates (S.E.) in Eqs. (1) and (2) for dipped fresh-cut mangoes at different concentration and anti-browning agents.
Product

Microbial growth and survival depend largely of the quality or type of fruit or vegetable in question. The quality factors of a product that may affect microbial growth include pH, water activity, respiration rate, type of packaging, competitive microflora and innate antimicrobials [61]. The pH of a product strongly influences microbial growth. Most vegetables are known to have a pH of ≥ 5 which supports the growth of pathogenic microorganisms. Despite the acidic pH of most fruits, organisms such as *E. coli* 0157:H7 and some *Salmonella* spp., still grow and survive in such acidic environment. *L. monocytogenes* was reported survive on cantaloupe & melon cubes [63] and apple slices at pH 3.42 [64]; *Salmonella* also survived on apple slices with 4.1 pH [65]. Fresh fruits and vegetables provide an ecological niche to some microorganisms; and these vary from one product to another depending on the type of product, climatic conditions, geographical location, harvesting, handling and transportation. Microflora of fruits and vegetables include bacteria such as *Erwinia herbicola, Enterobacter agglomerans, Xanthomonas, Leuconostoc mesenteroides, Lactobacillus* spp. *Flavobacterium*; and moulds like *Penicillium, Fusarium, and Aspergillus* [40]. An antagonistic behaviour of native microflora of fruits and vegetables against pathogenic microorganisms have been reported [61]. The growth of *L. monocytogenes* on lettuce was reduced by *Enterobacter* [66]. The natural microflora may help control growth of pathogenic microbes through (a) direct competition for nutrients and space; (b) production of antimicrobials [61].

Processing

Peeling and cutting increases respiration and ethylene secretion rate of fruits which in turn increases senescence, which makes more sugar available thereby allowing rapid microbial growth on fresh-cut fruits. Microbial contamination of fresh-cut produce can also be facilitated by cross-contamination of produce through: (a) transfer of organisms from surface of fruits onto FFV; (b) attachment of pathogens onto shredders and slicers which can be re-introduced, for instance *L. monocytogenes* has been recovered from the environment of vegetable processing plant [61]; (c) re-use of the same water for washing fruits and vegetables allows transfer of microorganisms from contaminated parts to uncontaminated parts. Packaging conditions also has influence on the growth of pathogenic microorganisms during storage. Modified atmosphere packaging which uses low oxygen and increased carbon dioxide for preservation of fresh-cut produce influences growth of pathogenic organisms. *E. coli* 0157:H7 grew on chicory slices when atmospheric CO$_2$ was increased to 30% when stored at 13 or 20°C. Storage temperature is one of the most important factor that affects the growth and survival of pathogenic microorganisms. Storage of FFV at temperatures ≤ 4°C reduces the growth of psychrotrophic organisms such as *L. monocytogenes* which can survive at low temperatures. However coliforms like *Salmonella* and *E. coli* 0157:H7 are unable to survive at low temperatures, but they proliferate at ambient temperatures. Fluctuations in storage temperatures should be avoided at all costs in order to maintain the safety of fresh-cut vegetables from microbial contamination.

2.2.1. Methods for detection of spoilage and pathogenic microorganisms on fruits and vegetables

2.2.1.1. Isolation using plate count method

Conventional method of bacterial enumeration works exquisitely on the recovery of viable bacteria from fruits and vegetables. Many food microbiology laboratories lack availability
and utilization of novel molecular-based technologies. Due to this, conventional methods such as standard plate count, selective or differential media for isolation and detection of bacteria samples; and further identification using biochemical methods such as gram stain and other commercially available profiling systems [67]. Non-selective media such as nutrient agar, tryptone soy agar can aid proliferation of bacteria cells at incubation time of 4 h before subsequent transfer to a selective media such as Mac Conkey agar for E. coli, and Salmonella-Shigella agar for Salmonella detection. Bacteria can be further separated into gram positive and negative using gram stain. The process of transfer from non-selective to selective media allows sub-lethally injured bacteria to recover in time for detection. Other biochemical tests used as follow up to conventional isolation of pathogenic bacteria include catalase test for identification of gram positive bacteria and oxidase test for gram negative bacteria [67].

2.2.1.2. Use of fluorogenic and chromogenic media

Another media used for isolation and detection of pathogenic bacteria is the use of fluorogenic and chromogenic culture media. Identification of bacteria on fluorogenic media is made possible through the incorporation of enzyme substrates (which consists of a sugar/amino acid-fluorogen complex) into a selective media which in turn speeds up biochemical confirmation of the bacterial identity [68]. For example, methylumbelliferyl is a fluorogen that has been incorporated in an array of media for detection of coliforms such as E. coli 0157:H7 which forms a blue fluorescence upon exposure to ultraviolet light. Rainbow agar, BCM O157:H7 (and other coliforms), CHROMagar, and Colilert are some examples of fluorogenic or chromogenic media for coliforms detection. The most commonly used culture reference methods for the detection of Listeria in foods are the ISO 11290 standards [69, 70]; FDA-BAM method to isolate Listeria spp. from vegetables [71].

2.2.1.3. Molecular-based methods

Nucleic acid-based systems designed for the detection of genomic DNA specific to particular microorganisms are capable of achieving rapidly and highly sensitive identification even when the target microbe is present in low numbers [72]. In order to achieve this, polymerase chain reaction (PCR) is quite useful. PCR is a molecular-based detection method. This method is focused on extraction of bacteria DNA; and it works best when there is enough bacterial cells from which is boosted by the enrichment step. Enzyme-linked immunosorben assay (ELISA) is another molecular-based method that works on the principle of antigen-antibody interaction. It is more sensitive and specific to a bacteria strain than standard plate count method. Steps in Antigen (food slurry/extract) is added to sample wells in a microtiter plate containing an antibody with specificity to the target molecule. ELISA has been successfully used to detect virulence determinants of pathogens such as Campylobacter, E. coli 0157:H7 and L. monocytogenes. Serotypes of Listeria spp. were categorized based on specific heat-stable somatic (O) and heat-labile flagellar (H) antigens [71]. ELISA procedure entails adding sample, washing, adding antibody complexes, adding detection reagents; and these steps are labour intensive. This has led to automation of the ELISA process, thus cutting back on time and labour. A good example is BioMerieux’s Vidas System in which the entire procedure is finished in 2 h after addition of overnight enrichment broth. This system is available for assays of pathogenic bacteria such as L. monocytogenes, Salmonella, E. coli, and Campylobacter [72].
2.2.1.4. Rapid methods of microbial pathogenic detection

Standard methods of isolation and identification of pathogenic microorganisms in foods are slower and time consuming which has led to demand of quicker methods; and for the past two decades the latter have been developed for both on-site and laboratory tests. A method can be characterized as rapid when it gives quicker results than the conventional method. Other factors that determine its effectiveness are sensitivity, standardization, reliability, accuracy, specificity, evaluation, ease of use, cost, validation, convenience and potential for automation. Most of the advances in development of rapid methods are in molecular-based methods and other areas including impedance and conductance, bacteriophages, biosensors, microscopy as well as in miniaturized, automated biochemical detection kits [72]. There are currently many diagnostic systems like RapID, Minitek, API, Biolog, MicroID, Crystal ID and VITEK systems which are commercially available for identifying different microorganisms [8].

3. Prevention of contamination, safety and hygiene practices during processing of FFV

The rate of contamination of FFVP after processing (cutting or wounding) is greater when compared to those of whole fruits and vegetables [73, 74]. This has been largely attributed to high moisture content in the fruits and vegetables as well as wound occurring in the tissues due to processing [75]. Wounding of tissue as a result of slicing, cutting or peeling releases the nutrients inherent in these products thereby enhancing microbial contamination and growth [76]. Upon growth of these microbes on the FFV surface, susceptibility to the formation of biofilms increases in the produce thus bringing about difficulty in the elimination of these microbes [77]. Microbial biofilms are thus able to attach, grow and spread to any surface with the cells associated with the biofilms possessing an advantage in growth and survival over planktonic cells. Growth and survival advantage over planktonic cells by biofilms has been attributed to the formation of exopolysaccharide matrix which surrounds the biofilms, thereby building a wall against the environment and protecting the biofilms from sanitizers [74, 78].

Pathogens of major concern in FFVP include *Listeria monocytogenes*, pathogenic *Escherichia coli* and *Salmonella* spp. A number of human pathogens have also been implicated in the contamination of FFV with a reported increase in recent years in the number of produce-linked foodborne occurrences. Agricultural practices during harvesting, human handling, quality of water and soil, contaminated equipment, processing methods, use of contaminated packaging materials as well as transportation and distribution have all been implicated in the contamination of fresh-cut produce [6, 79]. Similarly, microbial adhesion on conveyor belts, containers and food contact surfaces used along the food chain has been shown to lead to the formation of biofilms [41, 80]. Microbial contamination has also been shown to lead to internalization of pathogens into the fresh-cut fruit and vegetable produce. According to Golberg et al. [81], *E. coli* and *Salmonella typhimurium* are capable of penetrating the leaves of iceberg lettuce. The works of Seo and Frank [82] also showed that *E. coli* O157:H7 can penetrate between 20–100 μm below
the surface of lettuce leaves. Internalization of such pathogens has been reported to occur in the stomata, vasculature, cut edges and intercellular tissues [83] with elimination of such pathogens rather impossible thus hindering assurance of product safety and rendering processing and preservatory methods completely futile [83, 84].

Some of the biological methods employed in the reduction of pathogenic attack and spoilage of processed FFV include bacteriocins such as lactic acid bacteria which produces organic acids and bacteriocins that can act as antimicrobials [85]. For instance bacteriocin nisin is a natural preservative produced by *Lactococcus lactis* and is effective against mostly gram positive bacteria [86, 87]. Nisin acts on the cell membrane of the microbe thereby forming pores that result in cell death during the process [86, 88]. Other biological methods employed include the use of bacteriophages which has found application as disinfectants and preservatives. Other biological methods applied include the use of bacteriophages used in the destruction of bacteria. Bacteriophages are virus that infect bacteria thereby bringing about their death and destruction as a result. Its advantages in its application includes specificity in action; effectiveness [89]; availability and accessibility; and reduced effects on the organoleptic properties of the fresh-cut products [90].

Enzymes have also been employed in the control of pathogenic organisms in fresh-cut products. According to Simões et al. [91] and Thallinger et al. [92], enzymes are able to target directly the biofilms that interfere with their development process, speed up the formation of antimicrobials and even destroy mature biofilm. In attacking biofilms, enzymes mostly target the extracellular polymeric matrix which surrounds the biofilm cells and influences the shape of the biofilm structure as well as its resistance to shear forces [93]. Hence, enzymes can be used as an alternative to conventional chemical disinfectants in the removal of biofilms from fruits, leaves and other abiotic surfaces [39]. However, the use of enzymes requires prolonged contact times for effectiveness against biofilms and the fact that extracellular polymeric substances produced by biofilms are mostly heterogeneous confers some disadvantages on the use of enzymes [39]. Accordingly, use of enzymes alone as a biological control against pathogens in fresh produce does not guarantee total removal of biofilms. Lequette et al. [93] and Augustin et al. [94] therefore suggested the use of enzymes in combination with other treatments especially with antimicrobial agents.

**4. Storage of FFV**

**4.1. Modified atmosphere packaging (MAP) of fresh-cut produce**

One of the various ways of processing fresh-cut fruits and vegetable is the use of modified atmosphere packaging (MAP) of the produce. The process of MAP helps in altering the gaseous composition within a food packaging system. MAP relies greatly on the interface between the rate of respiration of the produce and the transfer of gases through the packaging material without any further alteration to the initial gas composition [95–98]. MAP can either be passive: which involves generation of MAP in a packaging material by reliance wholly on the natural process of respiration of the packaged produce as well as the permeability of
the packaging film material in bringing about the desired gas composition. MAP can also be active: involving the replacement of the gaseous composition in a packaged material through the introduction of gas scavengers or absorbers such as ethylene scavengers, oxygen and carbon (iv) oxide, thereby establishing the preferred gas mixture within the package [95, 98–100].

FFV have a short shelf life due to respiratory metabolism (Figure 2). Modified atmosphere packaging (MAP) has been used to reduce the rate of respiration and water loss leading to prolonged storage period. MAP comprising of low O\textsubscript{2} and elevated CO\textsubscript{2} atmospheres have been used to extend the shelf life and leading to high organoleptic characteristics of pear [101], apple [102], mango [55] and peach [103] fresh cut. The effects of low O\textsubscript{2} and CO\textsubscript{2}-enriched atmospheres associated to different packaging, traditional and compostable, on shelf life of fresh-cut nectarine slices stored (1°C) for 7 days by Maghenzani et al. [104] showed that low permeability of the film has a positive influence on weight loss and firmness as the less permeable film allowed a greater water retention, which caused a lower weight loss. They observed that MAP, acting on the respiratory metabolism, reduced respiratory metabolism with positive effect on colour, total soluble solids, titratable acidity, firmness and PPO activity, though efficacy differed among two cultivars of the fruit. Biodegradable films performs better than polyethylene film as a packaging material.

4.2. Freezing of FFVP

Freezing is a widely known and applied preservation process of various foods which offers the advantage of producing high-quality nutritious foods with prolonged shelf life. Freezing has also been described as one of the best methods used in preserving foods such as fruits and vegetables. Freezing of FFV will reduce the problem of spoilage experienced by the fresh-cut commodities. However, there is a perception by consumers that freezing reduces and affects negatively the nutritional composition of the fruits [106, 107]. A point of comparison is based on the fact that fresh produce could maintain its keeping quality in the consumer’s home for a number of days prior to consumption [108].

During freezing most of the liquid water constituent of the food materials is transformed into ice, thereby reducing water activity, which slows down the physical and biochemical changes.

Figure 2. Fresh foods such as fruit and vegetables are alive and continue to respire after harvest. Reducing the respiration rate and reducing the heat produced through efficient airflow inside ventilated packaging is important in maintaining product quality [105].
involved in the deterioration of foods as well as the growth and reproduction of spoilage microorganisms. Fruits and vegetables are composed of approximately 85–90% water which crystallizes during freezing. The freezing process prevents microbial growth, reduces water activity, and decreases chemical and enzymatic reactions [109]. According to Jaiswal et al. [110], decrease in temperature experienced during freezing impedes metabolic reactions taking place in the fruit and vegetable after harvesting. Freezing also reduces the rate of microbiological activities occurring in the FFV positively affecting the overall product quality. During the process of freezing, conversion of water to ice brings about various stress mechanism such as volumetric change of water converting into ice, spatial distribution of ice within the system as well as the size of the ice crystal [111, 112]. The effect of this stress mechanism is the deterioration of the frozen products by affecting the texture and structure of the cut fruit and vegetable.

It is well known that FFV undergo faster physiological deterioration, biochemical changes and microbial degradation [113] which may result in degradation of the colour, texture and flavour. However, the high water content of fresh-cut products adversely affects the textural quality of the products after thawing due to the formation of ice crystals and water solids within the cell structure. When thawing takes place the cellular structure of the fruit and vegetables is destroyed [114]. The reduction in the product water content results in improved freezing performance and ameliorated product quality including better preservation of structural and textural characteristics [115]. Thus, in order to preserve the structural and textural characteristics and improve freezing performance, the water content of the fruits and vegetables are reduced by dehydration before freezing. Frozen fruits and vegetables are mostly consumed cooked with majority of vegetables blanched prior to freezing. Blanching action prior to freezing has been reported to influence greatly the structure of the vegetable thus resulting in an initial loss of firmness due to disruption of the plasma lemma and an increase in the ease of cell separation accompanied by swelling of the cell wall [112, 116].

Several novel freezing practices are presently being investigated to overcome the problems of FFV and other food produce undergoing physical and chemical changes as a result of freezing. One of such novel methods which is presently being explored is dehydrofreezing [115]. During dehydrofreezing process, the food is first dried up to a needed moisture content level before the onset of freezing. Hence it is aptly described as a process of freezing relatively dehydrated foods [117]. For fresh-cut products, non-thermal dehydration techniques such as vacuum and air drying are mildly applied prior to freezing. When the method of drying the FFV is through osmosis then it is termed osmodehydrofreezing. Dehydrofreezing is particularly well suited for fresh-cut fruits and vegetables due to the fact that reducing the moisture content in the produce will allow for the formation and expansion of ice crystals without damaging the cellular structure of the product [115]. Theoretically, the dehydration treatment not only reduces the amount of water to be frozen but also makes cell structures less susceptible to breakdown by changing cell turgor pressure [118]. The reduced water content has the potential to reduce the freezing time, the initial freezing point and amount of ice formed within the product [117]. As a consequence, the damage to plant cells caused by ice crystal formation and the post-thawing quality degradation such as softening and loss of good textural attributes are alleviated. Reduction in moisture content before freezing also
leads to reduced freezing time since there is less water to freeze as well as a reduction in the amount of ice formed within the produce [119, 120]. According to Li and Sun [118], fruits and vegetables are said to exhibit better quality over those that are frozen without any form of reduction in moisture content.

Generally, the texture of a thawed frozen fresh-cut fruit and vegetable product is much softer than normal produce due to cell rupturing caused by expansion of the plant cells during freezing. Hence the recommendation that the moisture content of fresh-cut produce be reduced before freezing in order to mitigate the effect of freezing on the thawed product.

5. Nutritional content of FFV

FFV are derived from whole fruits by cutting them into desired shapes and sizes. However, peeling and cutting cause serious damage to vegetable tissues which leads to dissociation of cell components that brings about biochemical reactions such as accelerated oxidative browning and chlorophyll degradation. Other quality deterioration include water loss, development of off-flavours, stimulation of microbial growth and tissue softening which makes fresh-cut fruits have short shelf life [121–123]. Wounding stress as a result of cutting first causes the plasma membrane to break thereby inducing reaction of oxidative enzymes with existing phenolic compounds causing oxidation of the latter [24].

Nutritional value of fresh-cut fruits is usually a measure of vitamins A, B, C, E, polyphenolics and carotenoids; while that of vegetables include the previously mentioned vitamins, glucosinolates, carotenoids and polyphenolics through spectrophotometric and colorimetric methods [121]. Li et al. [123] tested the effect of cutting a whole pitaya fruit into slice, half and quarter slices on its nutritional quality. Their results revealed that the various cutting styles had little influence on vitamin C and soluble solids. However, total phenolic content, antioxidant activity, increased significantly with cutting wounding intensity up to first two days of storage before deterioration set in. Some nutritional contents of selected fruits and vegetables are highlighted in Table 2.

5.1. Sugars

Total sugar content of swede were not affected by storage temperature; while lower temperatures (0 & −2°C) increased the sugar content of turnip than higher temperatures as a result of glucose and fructose metabolism by enzymes at lower temperatures [124]. Benitez et al. [122] reported that soluble solid content of kiwi slices coated with aloe vera gel, alginate and chitosan did not significantly change up till day 8 of storage at 5°C.

5.2. Vitamin C

Vitamin C is the vitamin that usually degrades most rapidly and can be used as an index of freshness. Vitamin C is unstable in many vegetables such as asparagus [121]. There was no significant difference in the ascorbic acid contents of FC papaya stored at 10–20°C for 0 to 24 h
but a significant decrease was observed at storage temperature and time of 4°C, 48 h [125]. There was no significant influence of cutting style on vitamin C content of pitaya fruit when cut in slice, half and quarter shape [123]. Exposure of fresh-cut banana, pineapple and guava slices to ozone for 0–30 min drastically reduced vitamin C contents of the fruits by 12.21, 46.44 and 67.13% respectively [126]. Vitamin C content of kiwi slices coated with chitosan and alginate, stored at 5°C depreciated at storage time from day 1 to day 11. However, kiwi slices coated with aloe vera gel significantly increased from 44.99 to 47.99 mg/100 g at the same storage conditions [122].

5.3. Polyphenols

When wounding stress occurs, plants produce injury signals to induce the production of more secondary metabolites including phenolic antioxidants to defense and heal the wounding damage [24]. Wounding stress also activates phenylalanine ammonia lyase (PAL) - an enzyme responsible for synthesis of phenolic compounds in plant tissues. For example, carrots synthesize lignin along wound barriers [127]. Flavonoid contents of fresh-cut papaya significantly increased at storage conditions of 20°C at 24, 36 and 48 h when treated with 405 nm LED illumination [125]. Li et al. [123] reported a gradual increase in total polyphenol content of pitaya fruit with storage time of 4 days. An approximate increase of 63, 78 and 90% was reported for slice, half and quarter slice respectively at day 2; after which a decline was observed. Total polyphenols and flavonoids in fresh-cut pineapple, and banana increased as the fruit slices were exposed to ozone for up to 20 min; but a reverse trend was observed for guava slices. The reason for increase polyphenols by ozone treatment was attributed to activation of PAL [126]. Upon 1 day in storage, total phenolic content of untreated and fresh-cut apples treated with citric acid and UV light decreased by 50% Ref. [128].

<table>
<thead>
<tr>
<th>Fruits/veg</th>
<th>Sugars (°Brix)</th>
<th>Vitamin C (mg/100 g)</th>
<th>Polyphenols (mg/100 g)</th>
<th>Antioxidants (%DPPH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kiwi</td>
<td>12.6</td>
<td>44.99</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Apple</td>
<td>-</td>
<td>3.00</td>
<td>329.30–660.00</td>
<td>12</td>
</tr>
<tr>
<td>Pitaya</td>
<td>0.15 g/kg</td>
<td>0.16 g/kg</td>
<td>0.6–1.2 g/kg</td>
<td>40–70</td>
</tr>
<tr>
<td>Papaya</td>
<td>11.82</td>
<td>13.3–18.0</td>
<td>1.9–3.5</td>
<td>-</td>
</tr>
<tr>
<td>Pineapple</td>
<td>-</td>
<td>76.27–142.41</td>
<td>42.28–46.70</td>
<td>30–60</td>
</tr>
<tr>
<td>Banana</td>
<td>-</td>
<td>46.75–53.25</td>
<td>60.25–85.24</td>
<td>70–90</td>
</tr>
<tr>
<td>Guava</td>
<td>-</td>
<td>65.16–198.25</td>
<td>96.51–178.51</td>
<td>-</td>
</tr>
<tr>
<td>Radicchio leaves</td>
<td>-</td>
<td>-</td>
<td>143–360</td>
<td>-</td>
</tr>
<tr>
<td>Swede</td>
<td>535.2 g/kg</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Turnip</td>
<td>468.9 g/kg</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Source [1, 122–124, 127].

Table 2. Nutritional content of some fruits and vegetables.
5.4. Antioxidants

Kim et al. [6] observed no significant difference in antioxidant capacity of fresh-cut papaya stored at 4–20°C for 0 to 48 h. Antioxidant capacity of fresh-cut pineapple and banana increased when exposed to ozone for 20 min and declined upon further treatment; while that of fresh-cut guava reduced with ozone treatment and increased when ozone exposure time was increased to 30 min [126]. A drastic loss of antioxidant potential of fresh-cut apples was observed in both untreated and fruit pieces treated with pulsed light and gellan-gum coatings during the first week of storage [129].

6. Emerging/future trends in processing and preservation of FFV

Fruits and vegetables remain important health food with low in fat, sodium and calories and high concentrations of vitamins, minerals and phytochemicals especially antioxidants protecting body cells against free radicals [130, 131]. Emerging technologies to fresh-cut fruits and vegetables to inactivate bacteria and viruses are focusing on modified atmosphere packaging process. The microbial inactivation effect of this technologies has to be further assessed. The number of studies is still low in the area of emerging technologies such as low-pressure application to reduce microbial populations in FFV. Very few studies have focused on viral inactivation during MAP processes. More evidence is needed that MAP process can contribute to reduce or eliminate specific foodborne pathogens to reduce the risk for foodborne infection associated with FFV when consumed as such or when used further in the food supply chain as ingredients.

Packaging material, including low density polyethylene (LDPE), laminated aluminium foil (LAF), high density polyethylene (HDPE), polypropylene (PP), polyethylene (PE), is an essential component of the FFV, assuring the safe handling and delivery of such food products from the point of production to the end user. Technological developments in smart packaging offer new prospects to reduce losses, maintain quality, add value and extend shelf-life of agricultural produce [105]. More novel and emerging packaging technologies are therefore still needed in the way we handle and package FFV to meet the increasing consumer demand for consistent supply of high quality, wholesome and nutritious products.

Smart, active and intelligent packaging with food spoilage indicator label (Green = fresh; orange = warning) are beginning to emerge in FFV industry. We are also beginning to see freshness and leakage indicators are commercially available for monitoring food [132]. Recent advances in biotechnology, nanotechnology, nano-sensors and material science offer new opportunity to develop new packaging materials and design for the fresh-cut fruit and vegetable industry. Incorporation of nano-sensors in the packaging material could capture and analyze environmental signals and adjust stress response treatments on fresh fruits and vegetables. Evidently, recent developments and applications of nanotechnology could lead to the development of antimicrobial packaging in response to spoilage. As stated by Opara and Mdithsha [105], the application of emerging technologies in packaging design offers new prospects for advanced quality monitoring using electronic devices that monitor and report
real time information on nutritional quality and safety of food. This and other areas of packaging design remain a challenge for food, chemical and mechanical engineers.

7. Conclusion

FFV are increasing in demand due to its less processing and high nutritional content. However, the impact of processing and storage conditions should be taken into consideration by consumers due to the fact that nutritional quality of the produce can change as a result of storage and due largely to biochemical and enzymatic reactions. While conventional food processing method extends the shelf life and wholesomeness of fruits and vegetables, fresh-cut processing renders the products highly perishable and undesirable by the consumers. Suitable technology and techniques for preservation, retention of wholesomeness and consumer desirability of fresh-cut products are therefore required to meet the present day growing consumer demands.

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