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Fresh-cut fruits and vegetables: Critical factors influencing microbiology and novel approaches to prevent microbial risks—A review

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Abstract: Fruits and vegetables comprise an essential part of human diet as they are the major source of dietary nutrients of great importance. Consumption of fruits has been found to counteract many of the chronic diseases, including cancers and cardiovascular diseases. Therefore, recommendations for a balanced diet must include the consumption of fresh fruits and vegetables. Consumers in developing countries have become more concerned about the nutritional and sensory aspects as well as the safety of the food they eat due to growing health awareness. At the same time, consumer demand for convenience products is increasing and so is the demand for fresh-cut fruits and vegetables. Fresh-cut market has expanded considerably in recent years. However, quality and safety of such products are an issue of concern as these products can act as vehicles for transmitting infectious diseases. Furthermore, fresh-cut produce is more susceptible to spoilage and can facilitate rapid growth of spoilage micro-organisms as well as the micro-organisms of public health significance. Nonetheless, keeping in consideration the vast scope of fresh-cut products, this article intends to thoroughly review information about microbiology and public health risks associated with them. Discussions regarding different approaches to extend the shelf life and to minimize the risk of infection associated with their consumption are also included.

Subjects: Food Microbiology; Food Packaging; Fruit & Vegetables; Preservation; Processing

Keywords: fresh-cut; fruits; microbiology; safety; antimicrobial coating

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The main subject area of our research group includes processing and preservation of fruits and vegetables, drying of agricultural commodities, minimal processing and application of novel techniques to improve their quality and shelf life.

PUBLIC INTEREST STATEMENT

Fruits and vegetables comprise an essential part of human diet. Fresh-cut products are gaining popularity among consumers due to their convenience. Therefore, it is of utmost importance to ensure quality and safety of such products and prevent them from acting as vehicles for transmitting infectious diseases. This paper reviews information regarding microbiology of fresh-cut produce which may be helpful from both industrial and consumer perspectives.

1. Introduction

As a result of growing education level among consumers from developing countries, they have become more concerned about the nutritional and sensory aspects, as well as the safety of the food they eat (da Costa, Deliza, Rosenthal, Hedderley, & Frewer, 2000). This has resulted in an increasing demand for healthy and nutritious products that requires a continuous assortment of new and more differentiated food products (Linneman, Meerdink, Meulenberg, & Jongen, 1999). Because of this, the most important area of research rated by a large majority of food companies is the development of healthy foods, especially foods followed closely by natural foods (Katz, 2000). Fruits and vegetables are the major dietary sources of nutrients of greater importance from the human nutritional point of view. These substances include anthocyanins and other phenolic compounds with antioxidant and free radical scavenging properties (Nichenametla, Taruscio, Barney, & Exon, 2006; Stintzing & Carle, 2004); carotenoids; tocopherols; and ascorbic acid due to their possible role in the prevention of several human diseases. Therefore, recommendations for a balanced diet must include the consumption of fresh fruits and vegetables, which is in fact a very important part of the diet of people around the world. Advances in agronomic, processing, distribution and marketing and preservation techniques have enabled the produce industry to supply nearly all types of high-quality fresh fruits and vegetables to those who desire and are willing to purchase them year around (Artés & Allende, 2005). In developed countries, there has been an ever-growing demand for convenience products and the same trend is followed for fresh-cut fruits and vegetables. However, microbial contamination of fresh-cut produce may pose threats to human health by causing various diseases like diarrhoea, abdominal cramps, vomiting as well as death. Over the past few decades, there has been a dramatic increase in outbreaks of food-borne illness caused primarily due to *Escherichia coli* O157:H7 and *Salmonella* by the consumption of fresh and minimally processed fruits (Harris et al., 2003). Food safety thus constitutes a growing concern for producers, public and the regulatory agencies (Bhagwat, Saftner, & Abbott, 2004). Therefore, critical steps like proper procedures of production and disinfection are to be closely monitored to ensure the safety of fresh-cut fruits and vegetables (Abadias, Canamas, Asensio, Anguera, & Vinas, 2006). This paper provides a review of the microbiology and quality associated with the fresh-cut produce.

2. Fresh-cut produce

According to International Fresh-cut Produce Association or IFPA (<http://www.creativew.com/sites/ifpa/about.html>), fresh-cut produce is defined as “any fruit or vegetable or combination thereof that has been physically altered from its original form, but remains in a fresh state”. There has been a rapid growth of fresh-cut produce industry worldwide in recent years to a multi-billion dollar sector, mainly because of increasing consumer demand for healthy, freshly prepared, convenient fruits and vegetables. Fresh-cut produce may consist of peeled, sliced, shredded, trimmed and/or washed fruits and vegetables (Francis et al., 2012). Conventional preservation methods like freezing, dehydration, curing or salting are never applied to fresh-cut produce because these foods are not heavily processed. Fresh-cut produce on the market in some developed countries include melons, cantaloupe, watermelon, mango, jackfruit, papaya, grapefruit, pineapple, fruit mixes, shredded leafy vegetables and salad mixes, vegetables for cooking like peeled baby carrots, baby corn, broccoli and cauliflower florets, cut celery stalks, shredded cabbage, cut asparagus, cut sweet potatoes and many more (James & Ngarmasak, 2010). Despite the benefits derived from eating raw fruits and vegetables, quality and safety are still an issue of concern as these foods have long been known to be vehicles for transmitting infectious diseases (Allende, Jacxsens, Devlieghere, Debevere, & Artes, 2002; Beuchat, 1996). The destruction of surface cells during processing (such as peeling, slicing and shredding) of fresh-cut produce exposes the cytoplasm and provides micro-organisms with a richer source of nutrients as compared to intact produce (Barry-Ryan, Pacussi, & O’Beirne, 2000). Besides this, the high water activity and approximately neutral (vegetables) or low acidic (many fruits) tissue pH facilitate rapid microbial growth (Parish et al., 2003). These conditions provide a perfect platform for a number of important human pathogens and spoilage micro-organisms to contaminate fresh-cut produce, which results in a faster deterioration of fresh-cut produce compared to whole fruit or vegetable. A number of physical and physiological changes affecting the viability and quality of the fresh-cut produce are a direct result of the wounding associated with processing (Brecht, 1995;

Saltveit, 1997). Flaccidity due to loss of water, changes in colour (especially browning at the cut surfaces) and microbial contamination (Brecht, 1995; Varoquaux & Wiley, 1994) deteriorate the appearance of fresh-cut produce. Wounding of plant tissues may also result in the acceleration of nutrient losses.

3. Critical factors affecting microbiology

Fresh and minimally processed fresh-cut products are naturally contaminated by micro-organisms because of a number of sources, including the farm environment, post-harvest handling and processing (Beuchat, 1996; Heard, 1999). Hence, the microflora linked with fresh-cut produce is diverse. Also, contamination with pathogens can occur at various points during growing, harvesting, processing and handling of the produce. The microflora associated with most vegetables is dominated by gram-negative bacteria, while dominant microflora associated with raw fruits mostly includes yeasts and moulds (Burnett & Beuchat, 2000; Tournas, 2005). The factors that influence the survival and/or growth of the micro-organisms on the fresh produce may include the type of organism, the commodity and environmental conditions in the field and thereafter, including storage conditions.

3.1. Physical environment

As fruits and vegetables frequently are in contact with soil, insects, animals or humans, they may be contaminated with pathogenic micro-organisms at any time from farm to table and the contamination may be through faecal material, human handling, harvesting equipment, processing, transportation and distribution (Sothornvit & Kiatchanapaibul, 2009). The protective character of the plant's natural barriers (cell walls and outer covering) may prevent the growth of micro-organisms on the uninjured outer surface of fresh fruits or vegetables. The physical environment surrounding the produce surface in the field is considered to be inhospitable for the growth and survival of bacteria due to lack of nutrients and free moisture, temperature and humidity fluctuations and ultraviolet light (Dickinson, 1986). However, environmental conditions can have great influence on bacterial populations. Survival and growth of bacterial populations may be promoted by the presence of free moisture on produce surface (Beattie & Lindow, 1995, 1999), whereas certain conditions, such as sunlight, particularly the shorter ultraviolet wavelengths, can damage bacterial cells (Sundin & Jacobs, 1999).

3.2. Processing

In fresh-cut produce production chain, there are several processing steps and in each of these steps, many points for potential microbial contamination may exist (Nguyenthe & Carlin, 1994). The protective barrier provided by the epidermis against development of microbes on the fruit surface is removed during processing (Martn-Belloso, Soliva-Fortuny, & Oms-Oliu, 2006). Thus, processing and storage lead to enhanced decay and loss of quality of fresh-cut fruits and vegetables (Busta et al., 2003). The damage of the tissues during processing operations such as cutting, shredding and slicing not only makes the fresh-cut produce more susceptible to microbial attack compared to intact produce, but also causes damage to fruit and vegetable tissues and cellular structure, leading to leakage of nutrients and cellular fluids (Heard, 2002). Therefore, minimal processing may increase microbial spoilage of fresh-cut produce due to transfer of microflora from surface to the fruit flesh, which acts as a complete medium for growth.

3.3. Packaging

Packaging is one of the important factors influencing the microbial quality of fresh-cut products. Fresh-cut products are mostly packaged under modified atmospheric conditions and stored under refrigeration which gives rise to a favourable environment and time for proliferation of spoilage micro-organisms and micro-organisms of public health significance (Francis, Thomas, & Beirne, 1999). The economic value of fresh-cut products is impaired by micro-organism proliferation because it may lead to decrease in product shelf life, through spoilage, and also pose a risk to public health by causing food-borne illnesses (Nguyenthe & Carlin, 1994, 2000). Gaseous composition of the atmosphere surrounding the fresh-cut produce has a profound effect on the microbial quality and shelf life. Low oxygen-modified atmospheres may inhibit the growth of spoilage micro-organisms and increase the shelf life of packaged produce (Al-Ati & Hotchkiss, 2003). Zhang, Samapundo, Pothakos,

Sürengil, and Devlieghere (2013) studied the effect of high carbondioxide packaging on the microbial spoilage of fresh-cut honeydew melon and suggested that 50% O₂ + 50% CO₂ is a potential gaseous composition in a modified atmosphere for extending the shelf life of fresh-cut honeydew melon. Montero-Calderón, Rojas-Graü, and Martín-Belloso (2008) investigated the effect of packaging conditions on microbial growth and other parameters influencing quality and shelf life of fresh-cut pineapple.

Respiration rate of the tissue and internal package atmosphere are of capital importance for efficient designing of appropriate packaging for a particular commodity (Conte, Scrocco, Brescia, Mastromatteo, & DelNobile, 2011).

4. Occurrence of micro-organisms on fresh-cut surfaces

Micro-organisms are ubiquitous in nature. Most of the literature available on the occurrence of micro-organisms in fresh-cut products emphasizes mainly on total bacterial count, yeast and mould counts and certain other specific microbial groups, such as coliforms and pectinolytic species. Food-borne pathogens including *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes* are often present on the surface of fresh produce and may cause public health problems. These organisms have long been recognized as dangerous pathogens causing various food-borne diseases. However, some non-pathogenic bacterial species, unfortunately, will also give positive results on *coliform* detection (Heard, 2002). Therefore, it is highly encouraged that contamination of raw and freshly processed vegetables with faecal pathogens should not be determined by the *coliform* test (Beuchat, Nail, Adler, & Clavero, 1998; Nguyenthe & Carlin, 2000). Nonetheless, such tests being simple to perform make them popular in the fresh-cut industry. Table 1 presents the data of the microbial counts of different fresh-cut fruits and vegetables reported in various studies. Microbial counts are reported to vary with type of fruit and vegetable. Gram-negative rods mostly *pseudomonads* have been reported to be the predominant group of micro-organisms on fresh produce. Some antagonistic strains of *pseudomonads* have also been identified and isolated from fresh-cut produce which restrict the growth of pathogenic species of *E. coli*. The strain L-59-66 of *Pseudomonas syringae* has been reported to prevent the growth of *E. coli* on apple wounds (Janisiewicz & Conway, 1999). A strain of *Pseudomonas graminis*, isolated from whole apple surface, was investigated for its effectiveness to reduce *E. coli* O157:H7, *Salmonella* and *Listeria innocua* on fresh-cut apples and peaches (Alegre et al., 2013a). Leverentz et al. (2006) have also reported the reduction in growth of *L. monocytogenes* and *Salmonella* on fresh-cut apple due to various antagonistic microbial spices. This may be called as biological control and the micro-organisms as bioprotective micro-organisms. Trias, Bañeras, Badosa, and Montesinos (2008) also isolated bioprotective *Leuconostoc* strains from fresh fruits and vegetables and investigated them for their antagonistic capacity against *L. monocytogenes*. Fluorescent *pseudomonads* are the dominant group of micro-organisms isolated from endive, spinach, cauliflower (Garg, Churey, & Splittstoesser, 1990; Jacques & Morris, 1995), chicory and mung bean (Bennik, Vorstman, Smid, & Gorris, 1998). *Enterobacteriaceae* may also be present on minimally processed products (Bennik et al., 1998). Another important group of microflora reported to be present on fresh-cut fruits and vegetables constitutes yeast and moulds. For instance, yeast populations of 10³–10⁴ have been reported for processed fruits (Nguyenthe & Carlin, 1994), and total bacterial populations of 10⁶ cfu/g have been reported for cantaloupe (Sapers & Simmons, 1998). Similarly, a mould population ranging from 10² cfu/g on cut lettuce to 10⁸ cfu/g in shredded carrots has been reported. Detailed studies are still required to be conducted to explore the knowledge regarding the occurrence of micro-organisms in minimally processed fruits and vegetables. Large number of human pathogens can contaminate fresh-cut produce and past years have witnessed an increase in the number of food-borne outbreaks associated with fresh and fresh-cut produce. For instance, outbreak in 2006 due to spinach-associated *E. coli* O157:H7 caused more than 200 illnesses and even some deaths. Fresh-cut cilantro and parsley have also been reported to be involved in food-borne illnesses (Campbell et al., 2001). Many cases of salmonellosis have been reported due to consumption of fresh-cut fruits (Sim, Hong, Yoon, & Yuk, 2013). In certain instances, pathogen levels may decline on the outer surface and rate of decline is influenced by produce type, humidity, temperature, atmosphere and type of packaging. Pathogenic growth on intact surfaces of the

Table 1. Total mesophilic and yeast and mould populations present in/on some fresh-cut fruits and vegetables

Fresh produce	Microbial population (Log cfu g ⁻¹)		References
	Total mesophilic count	Yeast and mould	
Broccoli florets	4.7	3.25	Jacques and Morris (1995)
Cabbage (coleslaw)	4.07–7.08	2.2	Garg et al. (1990)
Carrot sticks	4.99–5.77	4.25	Garg et al. (1990)
Chicory endive (shredded)	5.2	3.0	Jacxsens, Devlieghere, Falcató, and Debever (1999)
Cut chicory endive	4.00	–	Bennik et al. (1998)
Chopped lettuce	4.85	–	Odumeru et al. (1997)
Chopped bell peppers	3.5	–	Izumi (1999)
Fresh-cut brassica	4.91	–	Conte et al. (2011)
Fresh-cut mushrooms	8.3	–	Sapers and Simmons (1998)
Fresh fruit salad	3.0	–	Costa, Conte, Buonocore, and DelNobile (2011)
Japanese radish shreds	3.9	–	Izumi (1999)
Lettuce	6.39–7.69	4.14–5.29	Gras, Druet-Michaud, and Cerf (1994)
Minimally processed kiwi fruit	3.15	2.46	Benitez, Achaerandio, Pujola, and Sepulcre (2015)
Minimally processed broadleaf endive	3.83–4.82	–	Carlin, Nguyen-the, Da Silva, and Cochet (1996)
Minimally processed cantaloupe	1.05	–	Lamikanra, Chen, Banks, and Hunter (2000)
Processed lettuce	2.5–6.2	–	Francis and O'Beirne (1998)
Packaged salad (iceberg lettuce, carrot and red cabbage)	5.3–8.9	0.9–3.8 yeasts	Hagenmeier and Baker (1998)
		0.3–2.2 molds	
Fresh-cut pineapple	–	3–4	Montero-Calderón et al. (2008)
Pre-packaged ready-to-serve salad	5.5–8.3	<3–6.75	Lack, Becker, and Holzapfel (1996)
Potato strips	2.00	–	Gunes, Splittstoesser, and Lee (1997)
Raw vegetables	5.7	2.3	Kaneko et al. (1999)
Ready-to-use mixed salad	7.18	–	Vescovo, Orsi, Scolari, and Torriani (1995)
Salad mix	5.35	–	Odumeru et al. (1997)
Shredded lettuce	4.28	2.07	Delaquis, Stewart, Toivonen, and Moyls (1999)
Shredded carrots	2.9	1.1	Chervin and Boisseau (1994)
Trimmed spinach leaves	4.00	–	Izumi (1999)

produce is not common as they are not able produce the enzymes required to break down the protective outer coverings of the produce. The outer covering acts as a barrier and restricts the availability of nutrients and moisture.

5. Survival of pathogenic micro-organisms on cut surfaces and role of non-pathogenic micro-organisms

Damage to the outer protective covering of fruits or vegetables leads to the comfortable survival of food-borne pathogens and their multiplication, especially under non-refrigerated temperatures. Temperature control plays a vital role in restricting the growth and multiplication of microbes on fresh-cut produce. Though refrigeration or low temperature may result in reduced potential of multiplication, often micro-organisms may survive at such conditions. Injured tissues of fresh-cut products and the fluids released thereby act as a complete nourishing environment for micro-organisms.

Usually, the natural microflora of raw fruits and vegetables is non-pathogenic and may be present at the time of consumption (Ahvenainen, 1996). A large population of non-pathogenic bacteria on fresh-cut products may serve to reduce the risk of food-borne illness by acting as indicators of temperature abuse and age of the produce and may cause tangible changes on spoilage. Pathogenic micro-organisms do not necessarily cause spoilage of produce and in absence of any signs of spoilage, the produce may be consumed leading to the development of food-borne illness.

6. Effect of contaminated wash water

Most of the fresh produce reaching the packinghouse or industry retains populations of 10^4 – 10^6 cfu/g micro-organisms (Beuchat, 1996). In the fresh-cut produce production chain, washing of produce with sanitizing solutions is considered the only step to achieve a reduction in spoilage micro-organisms and potential pathogens (Alegre et al., 2013a; Allende, Aguayo, & Artés, 2004; Allende, Selma, López-Gálvez, Villaescusa, & Gil, 2008). Proper disinfection or decontamination procedures are considered critical in ensuring the safety of fresh and minimally processed fruits and vegetables. Washing and sanitization steps before cutting and during processing lower the microbial contamination on fruits and vegetables.

However, contamination due to wash water may result in increase in microbial load on the surface of produce and hence in the final fresh-cut products. Infiltration of wash water is also a concern as it may infiltrate the intercellular spaces under some favourable conditions, paying a way to pathogens to enter into the fruit tissue. Such infiltration or transfer of pathogens from wash water to fruit becomes an important safety concern and needs to be avoided under all circumstances.

Among all the factors to be considered when addressing safety issues for fresh-cut produce, the potential for pathogen contamination and food-borne outbreaks is the one receiving most attention. The psychrotrophic pathogen *L. monocytogenes* and thermophilic pathogens *Salmonella* and *E. coli* O157:H7 are among the most important pathogens involved in human illness associated with consumption of fresh produce (Rangel, Sparling, Crowe, Griffin, & Swerdlow, 2005). Indeed, outbreaks of salmonellosis and listeriosis due to consumption of vegetables have been reported (Nguyenthe & Carlin, 1994). Also, enterohaemorrhagic *E. coli* O157:H7 and *Salmonella* lead to outbreaks associated with the consumption of pre-prepared leafy green salad vegetables and other produce.

7. Preventive/novel approaches to reduce microbial risks

Development and application of antimicrobial edible coatings has emerged as an effective means to increase the shelf life of fresh-cut fruits and vegetables. Antimicrobial components from different natural sources have been successfully extracted and incorporated into the coating materials intended to protect the fresh-cut fruits and vegetables. Several categories of such antimicrobials incorporated into edible films and coatings include various organic acids, fatty acid esters, polypeptides, plant essential oils, nitrites and sulphites (Franssen & Krochta, 2003). Some coating materials having inherent antimicrobial properties are more suitable and cost effective and have already been investigated for coating of various food products including fresh-cut fruits and vegetables. (Franssen & Krochta, 2003; Gutierrez, Barry-Ryan, & Bourke, 2008; Mantilla, Castell-Perez, Gomes, & Moreira, 2013; Vargas, Chiralt, Albors, & González-Martínez, 2009). Popular among such materials is chitosan which can inhibit the growth of many pathogenic micro-organisms (Romanazzi, Nigro, Ippolito, DiVenere, & Salerno, 2002). Sipahi, Castell-Perez, Moreira, Gomes, and Castillo (2013) used

multilayered antimicrobial alginate-based edible coating for shelf life extension of fresh-cut watermelon (*Citrullus lanatus*). Trans-cinnamaldehyde and beta-cyclodextrin were used as antimicrobial components in these multilayered coatings. Microbiological analyses showed the effectiveness of the coatings as a carrier of natural antimicrobial compounds and effectiveness of the compound against microbial growth, which was particularly significant against psychrotrophics, coliforms, and yeast and moulds. Martinon, Moreira, Castell-Perez, and Gomes (2014) also developed a multilayered edible coating with antimicrobial (trans-cinnamaldehyde) agent to extend the shelf life of fresh-cut cantaloupe.

Muthuswamy, Rupasinghe, and Stratton (2008) investigated the potential of natural antimicrobial compounds extracted from cinnamon and their use as food additives to extend the shelf life of fresh-cut apples. It was found that the ethanolic extract of cinnamon bark significantly reduced the aerobic growth of bacteria inoculated on fresh-cut apples while storage at 6°C up to 12 days.

Essential oils from various spices and other plant sources have been studied as potential source of antimicrobial agents against both bacteria and mould. Essential oils serve as an alternative to chemical preservatives and have been introduced as the novel approach to improve microbiological quality of fresh-cut products (Oms-Oliu et al., 2010). However, the impact on organoleptic properties of foods intended to be protected, especially due to intense flavour of essential oils, has been the major limiting factor for their incorporation into various coating materials (Rojas-Grau, Soliva-Fortuny, & Martín-Belloso, 2009; Soliva-Fortuny & Martín-Belloso, 2003). Nonetheless, various studies on use of different types of oils such as rosehip oil (Paladines et al., 2014), lemon grass essential oil (Azarakhsh, Osman, Ghazali, Tan, & Mohd Adzahan, 2014), phytoncide essential oil derived from pine leaves (Kim, Kim, Chung, & Moon, 2014), sunflower oil (Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2008; Rojas-Grau, Tapia, Rodríguez, Carmona, & Martín-Belloso, 2007; Tapia et al., 2008), mint and basil essential oils (Karagözlü, Ergönül, & Özcan, 2011) and many others on fresh-cut produce have been reported in the literature.

Green tea extract was investigated to study its effectiveness as an antimicrobial on fresh-cut dragon fruit (Matan, Puangjinda, Phothisuwan, & Nisoa, 2015). Green tea is a good source of polyphenols, mostly including catechins and catechin derivatives such as epigallocatechin-3-gallate (EGCG), which is reported to add antimicrobial activity (Almajano, Carbó, Jiménez, & Gordon, 2008).

Biopreservation has been used to reduce microbial growth on various fresh-cut fruits and vegetables. Native microflora of fresh-cut fruits has been reported to reduce the growth of many pathogens (Abadias et al., 2014; Alegre et al., 2013b; Leverentz et al., 2006; Trias et al., 2008; Ukuku, Fett, & Sapers, 2004).

8. Conclusion

Commercialization of fresh-cut produce is at rise owing to its tremendous scope for expansion of this industry. Though consumption of fresh-cut products has gone up, an important concern to consumers is the safety of such sensitive products. Fresh-cut fruits and vegetables offer an array of advantages but are highly perishable. Nevertheless, with the growing interest in these kinds of products, further research is required to offer adequate technologies to processors in order to ensure safety while maintaining the nutritional and sensory properties. Unhygienic and ignorant practices in the preparation of fresh-cut produce, instead of providing convenience and health benefits to consumers, may pose risks to public health by causing food-borne illnesses. Therefore, utmost care has to be taken to improve and maintain microbiological quality of fresh-cut produce throughout its preparation and until its final consumption.

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