
A Review on Microencapsulation in Improving Probiotic Stability for Beverages Application

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ABSTRACT

Nowadays, probiotic bacteria are extensively used in beverages application to deliver beneficial health effect to the consumer upon ingestion. Different entrapment techniques can be used to maintain the viability of probiotic bacteria during processing as well as during storage of beverage products. Development of artificial microcapsules from entrapment techniques are to support the growth and to provide protection on probiotic cells from unfavorable external conditions that may affect the viability of probiotics in beverages. Techniques that usually applied for probiotic entrapment in beverages are microencapsulation, emulsification, spray drying and extrusion. Biomaterials such as alginate, carrageenan, whey protein, gelatin, chitosan and starch are the most commonly used matrix in entrapment of lactic acid bacteria. Entrapment of probiotic is applied on beverages products such as fruit juice, yoghurt and ice cream.

Keywords: *Entrapment; Beverages; Matrix materials; Probiotics*

INTRODUCTION

Nowadays, the demand for functional food by consumers is increasing due to awareness of obtaining wellness through diet [1]. Global market analysts forecast worldwide probiotics market size to grow greater than 3 billion US dollar in 2024. In 2017, Lactobacilli strains had market size worth 1.2 billion US dollar. By 2024, the forecast market size for *Bifidobacterium* strains will grow about 6% while for *Bacillus* strains may exceed 180 million USD [2]. The functional foods can be described as food incorporated with beneficial ingredients such as living probiotics that capable to develop health advantages upon consumption [3]. According to FAO/WHO (2001), probiotics are defined as “live organisms which, when administered in adequate amounts, confer a health benefit on the consumer” [4]. Even though health benefits from probiotics are well documented, the survival of living probiotics during processing, storage and gastrointestinal transit become huge issues since the consumers must ingest minimum amount 10⁶ CFU/mL of live probiotics in order to obtain benefits from it [3,5].

Based on analysis of probiotic products in many different countries, it is confirmed that probiotic strains showed poor survival in traditional fermented dairy products. The survival of probiotics in food products is influenced by large factors including pH, post-acidification in fermented products during storage, production of hydrogen peroxide, temperatures during storage, stability in dried or frozen form, limited growth in milk, insufficient proteases to break down milk protein to simpler nitrogenous substances and compatibility with traditional starter culture during fermentation [6]. Thus, the protection of living probiotics cells became serious issue. Probiotics are protected gently in an effective encapsulation system to prevent the cells from damage, to improve shelf storage life and to provide good protection from acidic environment [7]. Encapsulation is one of the promising methods in controlling and protecting the cells during storage and gastrointestinal transition. Encapsulation can be defined as “a process to entrap one substance (active agent) within another substance (wall material)” and it is a great way to deliver living cells and bioactive materials into foods [8]. Several encapsulation methods were commonly used in beverages such as microencapsulation, extrusion, spray drying, emulsion, and others.

Encapsulation approaches

Microencapsulation

In the pharmaceutical, nutritional, food science and biological fields, microencapsulation technology has been used for over 50 years [8]. Microencapsulation process involve encapsulation of functional ingredient within protective coating material and creating particles with a few nanometers to a few millimeters in diameters [8]. Active agent or core ingredient consists of substance that is encapsulated while coating or shell material consists of substances that provide the protection toward probiotics [9]. The microencapsulation in food system normally has numerous aims. However, the prevalent objective is to provide protection on encapsulated substance from environmental condition that may harm the cells. The reason for microencapsulation of living probiotics is to reduce the unavoidable decline of living cell numbers from the first incorporation of the probiotics concentrate to the food, until they reach their final target in the human intestine [3].

Extrusion

Extrusion technique is used to produce hydrocolloid capsules using a cheap and simple procedure that minimize injuries on probiotic cells while maintaining comparatively higher cell survivals [10]. In extrusion process, hydrocolloid solution that mixed with bacteria cells are pass through nozzles such as syringes and fall into hardening solution containing calcium chloride to produce beads (Figure 1). The cells are directly entrapped by the polymers leading to three-dimensional matrixes that cross links with calcium ions [11]. The diameter of the extruder, type of polymer used, viscosity of the sodium alginate solution and the distance of syringe and hardening solution influence the size and shape of the bead produced. The extrusion method produces beads size ranges from 2 to 3mm in diameter [12]. The size of the beads decreases when the concentration and viscosity of sodium alginate increase [13]. Mild process condition of extrusion technique ensures high retention of cell survival [14]. However, it is not suitable for large-scale production due to slow formation of beads [11].

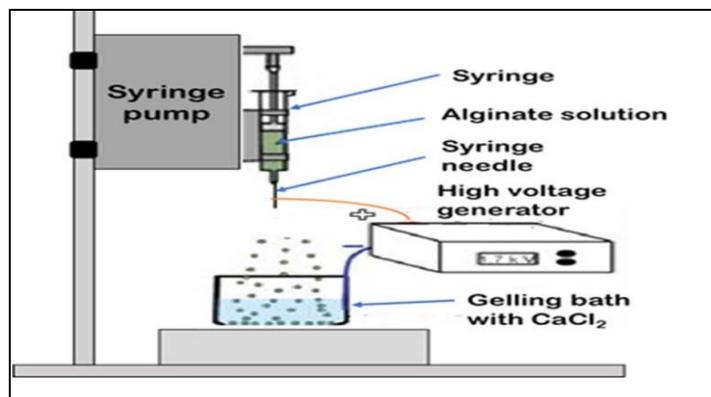


Figure 1: Schematic representation of extrusion technique. Adopted from [13]

Spray Drying

Spray drying technique normally used in food industry involves atomization of probiotics in an aqueous or oily suspension and carrier material into a drying gas, producing rapid water evaporation. Water evaporation is described as the difference between air outlet temperature and air inlet temperature [15]. Spray drying yield powders with particle sizes in the micrometre scale that contribute to a smoother mouth feel compared to micro-beads (Figure 2). Probiotics in powdery texture allows for addition in broad range of food products [16]. The mostly used biomaterials for spray drying are polysaccharides, lipids and proteins. The arabic gum, starches, corn syrups, and maltodextrins are classified under polysaccharides while monoglycerides, diglycerides and stearic acid are grouped under lipids. Meanwhile, the soy, milk serum, wheat, gelatin, and casein are classified under proteins [13]. Spray drying technique has low cost of production and high availability of equipment in the food industry [17]. Nevertheless, high temperature used during processing may not suitable for encapsulation of probiotics since it is lethal to the bacteria viability [14]. A proper choice of material can help to improve thermostability of the protective matrix, such as B-cyclodextrin and cellulose derivatives [18].

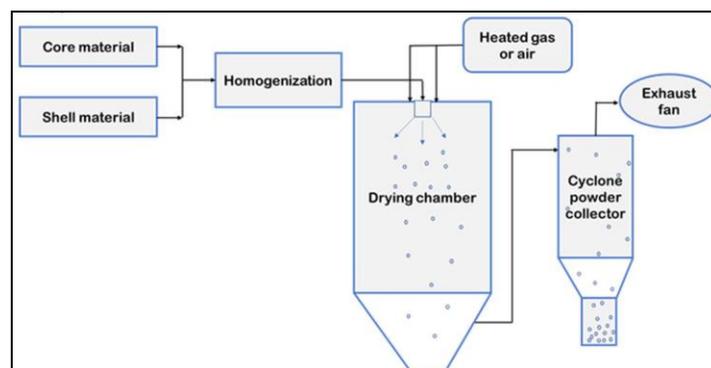


Figure 2: Schematic representation of spray drying technique. Adopted from [13]

Emulsification

Emulsification technique is suitable for probiotics encapsulation due to its flexible adjustment and control of the resulting capsule size. Emulsion involves a dispersed phase and a continuous phase in which the cell or polymer suspension is dispersed into an oil to form a water-in-oil emulsion (Figure 3). This technique requires homogenized mixing of the mixture with the aid of a surfactant and stirring to produce emulsion products [19]. After preparation, a water-in-oil emulsion has been formed, small beads within the oil are created by insolubilizing the dispersed hydrocolloid-cell mixture. The microcapsules are hardened by slowly adding a calcium chloride solution to the emulsion with continuous stirring after alginate capsules are produced. Fast gelling occurs when the calcium solution reacts with the dispersed alginate phase [3]. The emulsion method produces smaller diameter beads and is suitable for mass production. However, due to oil residual in the capsule, it may be unsuitable for development of low-fat food product applications [6]. Emulsification brings advantage because this technique is easy to scale up for mass production. However, it requires high operating cost due to the use of vegetable oil as raw material in the emulsification system [11].

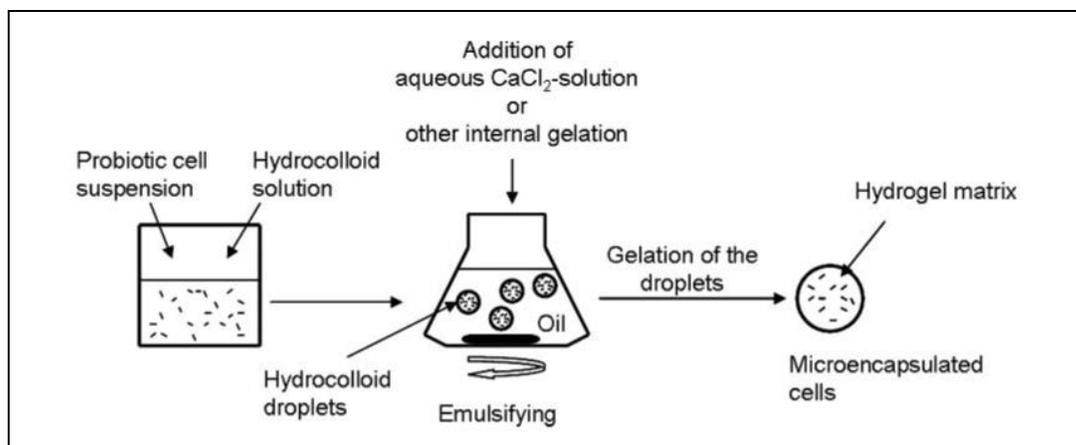


Figure 3: Schematic representation of emulsification technique to form emulsion. Adopted from [3]

Biomaterials used in encapsulation of probiotics in beverages application

Alginate

Alginates are unbranched anionic polysaccharides obtained from Phaeophyta known as brown algae. It comprises approximately 20-40% of the entire dry weight of these seaweeds [20]. Typical alginates that are available commercially are extracted from brown seaweed; for instance, they include *Laminaria digitata*, *Laminaria hyperborea*, *Laminaria japonica*, *Macrocystis pyrifera*, and *Ascophyllum nodosum* [21]. Alginate is also produced by bacteria such as *Pseudomonas* and *Azotobacter*. Alginate is largely applied in entrapment due to its ability to form gels in simple conditions, its great mechanical properties, high porosity, and biodegradability, easy manipulation, as well as good biocompatibility exhibited *in vivo* [20, 21]. In addition, alginate beads possess disadvantages such as sensitivity to acidic conditions in which the microparticle shows less resistance in the stomach conditions [10].

Other disadvantages include difficult for mass production in industry perspective because of high material costs and low capability for scaling up [22]. Moreover, the formation porous surface of alginate beads become problem when the aim is to protect the cells from harsh environment [10]. The defects can be counter balance by addition of other polymer compound in which it coats the alginate capsules or by using different additives to apply structural modification of the alginate. For instance, combination of alginate with starch helps in an enhancement of probiotics encapsulation efficiency [11]. Alginate and starch tend to be synergic in gelling. Therefore, it may help in providing extra protection to the entrapped probiotic cells [23].

Carrageenan

Carrageenan is used in entrapment of probiotic due to its ability to form gel that can entrap the cells. Carrageenan is a water-soluble anionic polysaccharide originated from the Rhodophyceae red algae through alkali extraction. Carrageenan contains repeated sequences of β -D-galactose and α -D-galactose with varying proportions of sulfate groups. Carrageenan that offered in commercial can be separated into three families depend on the number and arrangement of sulfate groups that carrageenan carrying. For instance, κ -(kappa) carrageenan, ι -(iota) carrageenan and λ -(lambda) carrageenan contain 1, 2 and 3 sulfate groups, respectively [24]. κ -Carrageenan and ι -carrageenan have an oxygen bridge between carbons 3 and 6 of the D-galactose. This oxygen bridge is responsible for conformational transitions as well as for the gelation of κ -carrageenan and ι -carrageenan. The absence of this bridge in the λ -carrageenan cause it unable to gel [15].

In a suitable condition, κ -Carrageenan and ι -carrageenan in aqueous solutions undergo thermo reversible sol-gel transition while no gelation takes place on λ -carrageenan that containing more electrolyte groups. Carrageenan is largely used in food industry as viscous or gel agents [25]. Carrageenan is widely used in food application to provide viscosity, primarily for gelling and textural functionality. It also has synergic behavior with other food hydrocolloids. In food application, κ -carrageenan is primarily used for its highest gelling ability of all type of carrageenan as it able to form strong, self-supporting, brittle gels and the gelling is mediated by presence of K^+ or Ca^{2+} ions, while ι -carrageenan is mainly used for production of softer gels and elastic gels besides offering good freeze stability. λ -carrageenan is primarily used as a thickener to provide creamy texture and mouthfeel in desserts [26].

Whey Protein

Whey proteins are a combination of globular protein obtained from whey, the liquid material produced as a byproduct from production of cheese [14]. Whey proteins are typically used because of their amphoteric properties [27]. Whey protein have ability to form cold-induced gel matrices with the presence of divalent ions such as Ca^{2+} to a preheated protein suspension [28]. Whey contains numerous nutritional compounds for example soluble milk proteins, mineral and milk sugar, and provide suitable food medium for viability and growth of probiotic cells. Whey protein can be used directly or with addition of dairy based powder like buttermilk powder and skim-milk powder [29]. Milk proteins are natural medium for probiotics and due to their physicochemical and structural properties, it can be used as probiotic delivery system. For instance, these proteins have great gelation properties and become advantageous to entrap probiotic cells [27].

Gelatin

Gelatin is combination of protein and peptide produced by partial hydrolysis of collagen obtained from the bones, skin, and connective tissues of animals. During hydrolysis process, natural molecular bond between single strands of collagen was broken down into a simple form [9]. Gelatin easily dissolves in hot water, and able to form gel after cooling. It also soluble in most polar solvents [9]. Gelatin solution is commonly used as a thermo reversible medium because it has viscoelastic flow properties and can be used alone or in combination with other compounds. Gelatin is suitable for cooperation with anionic polysaccharides because of its amphoteric characteristic [11]. Gelatin can be modified chemically for encapsulation of cells in order to prevent it from liquefy when located at physiological temperature. For instance, photo-cross-linkable gelatin–methacrylamide can be produced by addition of methacrylate groups to the side chains of gelatin [24].

Chitosan

Chitosan is a polysaccharide originated from chitin that naturally synthesized by numerous natural species as a structural part of their exoskeleton. Crabs and shrimp shells are the typical sources for commercially viable chitin. Chitin comprises of N-acetyl- β -d-glucosamine chains arranged in semi-crystalline structures [24]. Chitosan is water soluble below pH 6 [11]. In acidic conditions, amino groups undergo protonation that lead to an increase in solubility due to chitosan properties that relatively insoluble in water but can be dissolved by dilute acids [10]. Meanwhile, the alkalization of chitosan acidic solution up to a pH suitable with the presence of cells at around 7 pH leads to the precipitation of gel. A three-dimensional network is forms when the protonated amine that induces electrostatic repulsion among the chains is neutralized. This occur because of the hydrogen bonding and hydrophobic interactions. Chitosan becomes soluble at physiological pH when supplemented with phosphate salts in solution and becomes thermo responsive, forming a gel when heat up to physiological temperature [24]. The encapsulation of probiotic bacteria using chitosan with combination of alginate as coating matrixes create protection in simulated gastrointestinal environment [30]. Thus, it provides safe way to deliver viable probiotic cells to the intestine [30].

Application of encapsulation techniques and biomaterials on probiotics in beverages

The encapsulation techniques and biomaterials applied on probiotics for development of probiotics beverage products were summarized in Table 1.

Fruit juice as probiotic carrier

Fruit juice can be suitable medium for fortified with probiotics since it is rich in nutrient and free from starter cultures that may compete with probiotics for nutrients. In addition, fruit juices usually contain oxygen scavenging ingredient such as ascorbic acid that stimulate anaerobic condition. Fruit juice can support probiotics growth because it contains high sugar level which can be easily monitor by using refractometer [31]. Fruit juices and vegetable juices including calcium and vitamin fortified juices were already established in market sector as a functional drink. Several studies have been conducted to study the suitability of different fruit juices and vegetable juices such as tomato, orange, grape, carrot, pomegranate, beet and cabbage for production of probiotics beverages.

Table 1: Application of encapsulation techniques and biomaterials on probiotics in beverages

Strains used	Entrapment techniques	Biomaterials	Type of beverages	Storage time of product	References
<i>Lactobacillus rhamnosus</i> , <i>Bifidobacterium longum</i> , <i>Lactobacillus salivarius</i> , <i>Lactobacillus plantarum</i> , <i>Lactobacillus acidophilus</i> , <i>Lactobacillus paracasei</i> , <i>Bifidobacterium lactis</i> type Bi-04, <i>Bifidobacterium lactis</i> type Bi-07	Emulsion	Sodium alginate	Orange juice, apple juice	30 days	[31]
<i>Bifidobacterium animalis subsp. lactis</i> (BB-12)	Emulsion	Milk protein matrix	Pineapple juice, strawberry-apple juice	28 days	[32]
<i>Lactobacillus acidophilus</i> , <i>Bifidobacterium lactis</i>	Emulsion	Calcium - induced alginate - starch	Yoghurt	49 days	[33]
<i>Lactobacillus sp.</i> 21C2-10	Emulsion	Gelatine and maldoxtrine	Ice cream	180 days	[34]
<i>Lactobacillus paracasei subsp. paracasei</i> DC 412, <i>Lactobacillus bulgaricus</i> , <i>Streptococcus thermophilus</i>	Emulsion	Milk matrix	Yoghurt	28 days	[35]
<i>Lactobacillus rhamnosus</i>	Saturated monoglyceride structured emulsions (MSEs)	Skim milk, sunflower oil or anhydrous milk fats, saturated monoglycerides	Ice cream	14 days	[36]
<i>Lactobacillus delbrueckii ssp. bulgaricus</i> , <i>Streptococcus salivarius ssp. thermophilus</i> <i>Lactobacillus acidophilus</i> (NCDC- 005), <i>Lactobacillus helveticus</i> (NCDC- 232), <i>Bifidobacterium longum</i> (BB-46), <i>Bifidobacterium lactis</i> (BB- 12)	Emulsion and encapsulation	Sodium alginate, starch, gelatin	Yoghurt	21 days	[37]
<i>Bifidobacterium breve</i> R070 (BB R070), <i>Bifidobacterium longum</i> R023 (BL R023)	Emulsion and/or spray-drying,	Milk fat and/or denatured whey proteins	Yoghurt	28 days	[38]
<i>Lactococcus lactis</i> Gh1	Spray drying	Gum Arabic and <i>Synsepalum dulcificum</i> (miracle fruit)	Yoghurt	21 days	[39]

<i>Bifidobacterium animalis subsp. lactis</i> (BB-12)	Spray drying	Maltodextrin and/or inulin	Powdered probiotic passion fruit juice	30 days	[40]
<i>Lactobacillus casei</i> ATCC 393	Spray drying	Skim milk	Fermented milk	28 days	[41]
<i>Lactobacillus casei</i>	Encapsulation	Sodium alginate, chitosan	Mango juice	28 days	[42]
<i>Lactobacillus rhamnosus</i> , <i>Streptococcus thermophilus</i> , <i>Lactobacillus delbrueckii subsp. bulgaricus</i>	Encapsulation	Alginate, carrageenan	Yoghurt	15 days	[43]
<i>Lactobacillus acidophilus</i> , <i>Bifidobacterium bifidum</i>	Encapsulation	Sodium alginate, Calcium chloride	Grape juice	60 days	[44]
<i>Lactobacillus acidophilus</i> 5, <i>Lactobacillus casei</i> 01	Encapsulation	Alginate with chitosan	Commercial yoghurt, orange juice	28 days	[45]
<i>Lactobacillus rhamnosus</i> ASCC 290 <i>Lactobacillus casei</i> ATCC 334	Encapsulation	Calcium alginate-chitosan	Yellow mombin ice cream	150 days	[46]
<i>Lactobacillus acidophilus</i> ATTC-4356	Encapsulation	Sodium alginate, carrageenan	Ice cream	120 days	[47]
<i>Lactobacillus casei</i>	Encapsulation	Sodium alginate	Orange juice	28 days	[48]
<i>Lactobacillus casei</i> ATCC 393	Encapsulation	Alginates	Fermented milk	28 days	[49]
<i>Lactobacillus casei</i>	Encapsulation	Sodium alginate, amidated low-methoxyl pectin, and blends of sodium alginate and amidated low-methoxyl pectin	Yoghurt	20 days	[50]

Different lactic acid bacteria strains such as *Lactobacillus plantarum*, *L. delbrueckii*, *L. acidophilus*, *L. casei* and *L. paracasei* were used as probiotic cultures. It has been reported that *L. plantarum*, *L. acidophilus* and *L. delbrueckii* were showed resistance to high acidic environment during storage periods for 28 days at 4 °C [51]. As shown in Table 1, probiotics were incorporated into fruit juices using several approaches such as emulsion, spray drying and encapsulation techniques. Based on previous researches, encapsulated probiotics cells showed higher survivability compared to free cells in juice samples after storage period [42,44]. In addition, fruit juices containing encapsulated probiotics showed high stability in comparison to those containing free probiotics [31].

Ice cream as probiotic carrier

Ice cream can be an alternative food medium to deliver probiotics to consumers [52]. Ice cream with neutral pH ensures the viability of probiotics during storage period. Ice cream is consumed by the general population and the presence of beneficial ingredients such as dairy raw materials, vitamins and minerals make it suitable as a food carrier to incorporate with probiotics. Ice cream is a great probiotics carrier since it supports considerably higher viability of probiotic strains when compared to fermented milks during production and storage. However, probiotics in ice cream losses their viability unavoidably during product formulation, processing, storage and melting since they are exposed to several stresses related to pH, acidity, redox potential, freezing, oxygen, sugar concentration and osmotic effects, hydrogen during these different stages [53].

Encapsulation of probiotics help to provide protection to cells from unfavorable condition and damages during processing, storage as well as passage through the digestive tract [31]. For instance, entrapment of *Lactobacillus* sp. 21C2-10 using emulsion technique with use of gelatin and maltodextrin as wall materials protect probiotics in ice cream during 180 days of frozen storage [34]. Previous study had proved that encapsulation of probiotics provides significance effects on probiotics viability in ice cream product and in vitro condition. The use of carrageenan and sodium alginate as biopolymer materials for probiotics encapsulation provide protection to probiotics in food medium and improve the probiotics survival in simulated gastrointestinal environment [47].

Yoghurt as probiotic carrier

Yoghurt is extensively consumed as functional food because it has great taste, rich in nutritional values and brings beneficial health effects on human. Modern yoghurt fully utilizes ingredients such as milk, milk powder, sugar, flavor, fruit, coloring, stabilizers, emulsifiers, and specific lactic acid bacteria as pure starter culture to carry out fermentation process in yoghurt [54]. Conventionally, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus* were used as starter cultures in yoghurt manufacturing. These probiotics are claims to offer several health advantages, but they are not indigenous intestinal flora. Thus, for yoghurt to be considered as a functional food, these probiotics are incorporated as dietary adjuncts [55].

Processing and storage procedure, as well as gastric juice and bile salts action on functional foods containing probiotics contribute to reduction in number of viable probiotic cells after consumption. Probiotics have poor survivability in yoghurt because of less resistance to high acidic environment and aerated medium [50]. Previous researches showed that encapsulation of probiotics contributes to high viability of live probiotics in yoghurt when compared to free probiotics cultures during storage period at 4 °C [38,39,43]. Encapsulation techniques are applied on probiotics to increase cell resistance to acidic condition during storage and/or passage through digestive track [50].

CONCLUSIONS

The application of encapsulation techniques on probiotics help in incorporation of probiotic cells in beverages. However, the challenges are to choose the suitable encapsulation techniques and biomaterials as wall matrix to protect the probiotics. Microencapsulation has recognized as one of alternative methods

to maintain high probiotics survival and stability, as it protects cells both during food processing, storage and even in gastrointestinal environment. The application of microencapsulation in beverages industries are grow extensively from day by day following new methods, and it is applied on varying conditions of beverage products.

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