Microcapsules of bioactive compounds from fruits and vegetables waste and their utilization: A review

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Abstract

Fruits and vegetables have crucial role in human nutrition. Due to presence of various bioactive compounds, they are consumed processed, minimally processed as well as raw. In fruit and vegetable processing industries, the generation of their wastes are becoming a serious nutritional and environmental issue. These wastes still contain many bioactive compounds and their recovery from industry wastes has received significant attention. These bioactive compounds are sensitive to heat, light and oxygen; therefore, entrapment of these bioactive compounds is necessary to provide them protection from degradation against the environmental conditions. Additionally, microencapsulation process has been used for improving bioavailability, stability, delivery and controlled release of bioactive compounds. The aim of this review is to compile the results of extraction of different bioactive compounds by different methodologies and then their incorporation into probiotics in order to develop a functional food product with improved health and nutritional characteristics.

Keywords: Microcapsules, encapsulation, bioactive compounds, waste

Introduction

Fruits and vegetables fall under the most exploited commodities amongst all horticultural crops (Sagar et al., 2018) [61]. They play a pivotal role in our diet as well as in our life as they provide a substantial number of fibers, vitamins, minerals and other health promoting factors in our daily diet (Rico et al., 2007) [49] and also presumed to reduce several degenerative diseases such as cancer and cardiovascular diseases. With the increase in the world population and transition in dietary practices, there is a remarkable increase in the demand for fruits and vegetables (FAO, 2017; Vilariño et al., 2017) [15, 72]. About 60% of food loss and waste is produced by fruits and vegetables among all horticultural commodities (Gustavsson et al., 2011) [22] because of their greater yield, high moisture content and deprivation of appropriate handling procedures (various preservation methods), have resulted in massive losses and wastes of fruits and vegetables (Sagar et al., 2018) [65]. Fruits and vegetables commodities produce the maximum wastes into the environment followed by domestic waste (Gowe, 2015) [22]. These huge amounts of waste cause unpleasant smell, disease and enormous environmental problems like pollution as they expel detrimental greenhouse gases when they decompose into landfills (Utama et al., 2020; Vilariño et al., 2017) [70, 72]. Therefore, there is a need to increase the shelf life of fruits and vegetables in order to limit the wastages, to reduce the cost of disposal and environmental issues regarding food wastes (Salehi, 2019a, 2019b) [55, 56].

Fruits and vegetables are excellent source of highly potential phytochemicals and have been studied for the extraction of polyphenols, various pigments, polysaccharides, proteins, dietary fibers, enzymes, flavor and aroma compounds and so on (Ran et al., 2019; Sharma et al., 2017; Kowalska et al., 2017) [47, 61, 30]. The wastes of fruits and vegetables have these valuable bioactive compounds comprise health beneficial attributes like anticancer, antimutagenic, antiviral, antioxidant, antitumor as well as reduces the risks of cardiometabolic diseases (Sharma et al., 2017) [61]. In accordance with recent studies on phytochemicals, polyphenols from plant sources (such as skins, seeds, pulp or pomace) considered as the main phytochemical (Kelly et al., 2019) [27] because of their high antioxidant properties (Garcia-Salas et al., 2010) [16], possesses both functional as well as health promoting factors and their little consumption could increases the possibilities of certain diseases to take place (Swallah et al., 2020) [65]. Polyphenols are secondary metabolites derived from plant sources (Gómez-Mejía et al., 2019; Rajauria, 2018) [20, 46] and constitute a large family of natural components with biological functions in humans (Ignat et al., 2011) [25].
Polyphenols are classified particularly in accordance with their chemical structure into five different major classes depending upon encompassing the number of phenol rings as well as the structural elements bind to these phenol rings (Rajauria, 2018) [46]. Namely, phenolic acids, flavonoids, stilbenes, tannins, anthocyanins, lignans and so on (Belščak-Cvitkovic et al., 2018; Larrauri et al., 2016) [4, 32]. The extraction of bioactive compounds from wastes is done through both conventional (classical) and non-conventional (novel) extraction techniques (Sharayei et al., 2019; Pimentel-Moral et al., 2018) [59, 43]. The conventional techniques include mainly solid-liquid extraction (such as Soxhlet), hydrodistillation and maceration (Giacometti et al., 2018) [18]. The non-conventional (novel) techniques which include ultrasound-assisted extraction (UAE) (Tiwari 2015) [60], microwave-assisted extraction (MAE) (Rodasamran and Sothernvit, 2019) [52], enzyme-assisted Extraction (EAE) (Puri et al., 2012) [44], pulsed electric field assisted extraction (PEFAE) (Redondo et al., 2018) [48], supercritical fluid extraction (SFE) (Pimentel-Moral et al., 2019) [43] and pressurized liquid extraction (PLE) (Pereira et al., 2019) [41]. Among these the most widely used techniques are microwave-assisted extraction (MAE), enzyme-assisted extraction (EAE) and ultrasound-assisted extraction (UAE). Some of these novel extraction techniques are also known as “Green” extraction techniques with less extraction time, energy efficient, decrease solvent consumption, low investment, increase environmental safety and provide special care for heat-sensitive compounds (Kate et al., 2016) [20]. Polyphenols are highly thermosensitive; therefore, selection of accurate extraction temperature should be taken into consideration otherwise beyond the particular extraction temperature causes denaturation of polyphenols or modifications in their structures (Mourtzinos et al., 2018) [39]. Encapsulation is a process of entrapment of one substance within another substance and thereby production of particles (having diameters ranging from a few nm to a few mm). The food industries anticipate more and more complex properties such as thermal barriers, delayed/controlled release, good stability and appropriate sensory attributes from food constituents which very often would not be accomplished without microencapsulation. The bioactive compounds have appeared as a health-promoting factor which enhance the designs of newly developed supplements as well as functional foods (Sharma and Singh, 2010) [60]. The key parameter for the successful incorporation of bioactive compounds into different food systems, is the stability of health-promoting bioactive compounds during processing and storage. These compounds are extremely sensitive to heat, light, water and oxygen, thereby, reduce the shelf life as well as bioavailability in food systems (Champagne and Fustier 2007) [6]. Furthermore, bioactive compounds form off-colors, off-flavors, carcinogenic compounds or other degradation products which also reduce the shelf life of the fortified food products. The food products incorporated with certain bioactive compounds when orally consumed, it shows first-pass effect (greatly reduced before it reaches the target or systemic circulation) which causes the transition in chemical structures as well as in biological activities of the biological components. Therefore, it is mandatory to ensure the stability, controlled release at an appropriate target, high uptake and bioavailability of these components. Besides these requirements, it is important to ensure the negligible impact on the organoleptic and qualitative attributes of fortified food systems. Aforementioned requirements can be ensured by using a powerful tool, encapsulation which embeds the wide variety of functional food ingredients (bioactive compounds) into a protective matrix and provide protection to those compounds (Thies, 2005) [68]. The different methods for microencapsulation of polyphenols are, namely, spray drying, coacervation, freeze drying, co-crystallization, molecular encapsulation, extrusion, and electrostatic extrusion (Bakryet al., 2016; Fang and Bhandari 2010) [2, 14].

Fruits and Vegetables Waste

It is found that maximum of fruit wastes and wastes emerge from industries generally resulted either after pressing the juice or after producing valuable food products. There is more concentration of bioactive components in the non-edible portions of fruits i.e., peels, twigs, seeds etc. in contrast to edible portions. It is evident that seeds of mango, avocado and jackfruit as well as peels of grapes, citrus fruits, and apples contain about 15% or more polyphenols in comparison to pulp (Gorinstein et al., 2001; Soong and Barlow 2004) [52, 64]. During the production of beverage, an enormous amount of fruit wastes is generated in the form of pulp, seeds, stem and skin (collectively called as pomace). Since pomace is highly perishable in nature thereby, prone to microbial spoilage causing serious environmental problems. Mostly pomace contains more bioactive components than its fruit juice (Majerska, 2019) [34]. During processing of fruits like lemon, orange and pomegranate, huge number of peels generally wasted. Many studies have been conducted to valorize these fruit processing wastes into valuable products (Choubey et al., 2020) [7]. Fruits are the potential source of phytochemicals especially carotenoids and polyphenols that produce antioxidant compounds. The consumption of fruits in a consistent way prevents or reduce the risk of CVDs (Cardio Vascular Diseases), cancer, diabetes etc. The consumption remains very minute even there is recommended amount of daily intake of fruits. So, there is a need to provide these phytochemicals by developing a novel functional food fortified with plant based bioactive compounds (Choubey et al., 2020; Saikia et al., 2015) [7, 54] or into pharmaceutical preparations as a nutraceutical (Dillard & Bruce German, 2010) [10] or can be used in biotechnology sector for the production of certain essential bioactive compounds, for instance, enzymes (Coman et al., 2019) [9]. Many studies have been conducted to valorize these fruit processing wastes into valuable products (Choubey et al., 2020; Saikia et al., 2015) [7, 54] and can be considered as a perfect rectifier in controlling huge waste and loss from fruits and vegetables (Salehi, 2017) [57].

Encapsulation of bioactive compounds from fruits and vegetable wastes

It is reported that polyphenols required to be protected or masked before their incorporation into food products because of their astrignency (unpleasant taste) and instability (Manach et al., 2004) [35]. There are some characteristics of an effectual system for the encapsulation and delivery of bioactive compounds incorporated into food materials: formulation using solvent free preparation methods with materials which are non-toxic and safe for consumption; incorporation of bioactive compounds into food matrices with limited impact on the organoleptic attributes and high physicochemical stability of food products; protection of encapsulated bioactive compounds from degradation (when exposed to
Anthocyanins have health beneficial effects due to their anti-inflammatory, anti-cancer, anti-diabetic and antioxidant properties (Rodriguez-Amaya, 2016) (51) and show stability under acidic conditions (Muhamad et al., 2018) (39) and stored under refrigeration conditions. Barretto et al., (2020) (3) studied the stability of spray dried encapsulated anthocyanin extracted from bark of Solanum melongena L. (Eggplant) as a natural dye when incorporated into yogurts. Thereby, anthocyanins used as an additive for yogurts and can add a nutraceutical value to eggplant as well as yogurt. Using 70% cereal alcohol acidified with citric acid (pH 2.0) without stirring for 48 hours at 5°C in the absence of light, anthocyanins were extracted and then the extracted anthocyanins were stored in the dark bottles (-18°C) for further application in yogurts. Initial monomeric anthocyanin content (using differential pH method), total phenolic content (by spectrophotometry using FC reagent and gallic acid as a reference standard), antioxidant activity (using DPPH radical scavenging activity), pH and acidity were analyzed in the extract. Total monomeric anthocyanin content in the extract was found to be 67.21 mg/100g of bark. Acidity in natural yogurt at time zero was 1.04%. Non-encapsulated and encapsulated anthocyanin were added to yogurt at concentration levels of 1.0, 1.5 and 2.0 g extract/100 mL of yogurt. Acidity in yogurt incorporated with non-encapsulated extract and encapsulated extract was found to be 1.4%, 1.7%, 2.05% and 1.18%, 1.29%, 1.47% respectively for 1.0, 1.5 and 2.0 g of sample extracts. Using non-encapsulated extract, anthocyanin content decreased but remain constant in encapsulated extract when added to yogurt. Phenolic content ranged from 225.17-291.98 mg of GAE per 100 g of yogurt in encapsulated extract and from 106.01-239.90 mg of GAE per 100 g of yogurt in non-encapsulated extract incorporated into yogurt. The results obtained for encapsulated extract formulation exhibited superior antioxidant property whereas non-encapsulated extract formulations during storage period exhibited significant anthocyanin degradation index and also showed loss of antioxidant potential.

The waste generation such stems, peels, seeds and leaves after processing of red pepper (Capsicum annuum) can be valorized as they are rich in carotenoids and phenolic compounds which provide intensive color to fruits, taste, flavor, aroma, and have health promoting effects (Khan et al., 2014) (39). Seregelj et al., (2019) (58) studied the formulation of functional yogurt incorporated with encapsulated phenolic compounds and carotenoids extracted from red pepper waste. The physicochemical and functional attributes of the red pepper waste encapsulate were affected by different encapsulation techniques. Freeze drying was found to be efficient for producing the best quality encapsulates in terms of moisture content, water activity, flowing, solubility, and color attributes. Yogurt incorporated with freeze dried encapsulates showed stability during the storage period of 21 days. So, high pigment retention (71.43%) and high polyphenol content (123.73%) were observed during the storage period. Polyphenol content was increased which may be due to degradation of protein aggregates and increasing in extracting power of polyphenols. Whereas spray drying found to be ineffectual in providing the high color intensity of encapsulates. Yogurt incorporated with freeze dried encapsulates was appeared to be more acceptable by sensory panelist because of its better color, appearance, flavor, and general acceptability scores in comparison to yogurt incorporated with spray dried encapsulates.

Apples (Malus pumila) contain many bioactive components such as vitamins, minerals and so on. Apple peels and seeds are found to be major wastes which contain high phenolic content, antioxidants, flavonoids (Vieira et al., 2009; Ajila et al., 2007) (71, 1) and have potential health benefits (Buono et al., 2012) (5). There is about 80% of phenolic content in the peel and 5-6-fold antioxidant potential in the peel than in the flesh of the apple (Leccese et al., 2009) (13). El-Messery et al., (2019a) (12) studied the microencapsulation of polyphenols extracted from apple peels and then application in yogurt to increase its nutritive value. Using 80% ethanol polyphenolic compounds were extracted from apple peel. The extract powder of polyphenolic compounds was encapsulated by spray and freeze-drying using maltodextrin and whey protein concentrate (8:2) and gum Arabic mixture (6:4) as coating materials and then homogenized (using ultrasonication and ultraturrax). The spray and freeze-dried microcapsules were stored at -18°C and ground to a fine powder at the time of use. Then encapsulated polyphenol compound extract powder (PCEP) were incorporated into yogurt. It was found that polyphenolic compounds were more stable in the encapsulated samples until the end of storage and there was no significant change in physicochemical (pH, viscosity, titratable acidity) and texture attributes of the samples. Thereby, this study manifested that PCEP microcapsules can be efficiently used in yogurt as a functional food ingredient to improve functionality of yogurt.

Olive mill wastes and leaves are some examples of waste which are considered as functional ingredients applied in food products (Mohammadi et al., 2016a; Mohammadi et al., 2016b; Rahmanian et al., 2014) (17, 38, 43). Petroetros et al., (2012) (42) studied the polyphenols recovered successfully from olive mill wastewater via membrane technology along with macro-porous resin chromatography (adsorptive-desorptive method) in order to purify and concentrate olive mill wastewater to recover polyphenols in 50/50% proportion.
of ethanol and water solution. Then polyphenols in a powder form was produced from the concentrate by using freeze drying. Now this freeze-dried polyphenol powder was encapsulated in heat resistant modified starch and also the integrity of encapsulation was analyzed through differential scanning calorimeter (DSC). It was found that 20% w/w polyphenolic content was present in the encapsulated product. The polyphenolic powder encapsulated product was incorporated into two types of yogurts: one was Greek yogurt which was not tightly sealed and the second one was European yogurt which was filled in tightly sealed plastic containers. In both cases it was observed that the pH of yogurt declined faster in the initial incubation phase as compared to when both yogurts were incorporated with polyphenols in concentration about 500-1000 ppm. The incorporated polyphenols provided protection against undesirable decline in pH (post acidification) during storage of yogurt. Thus, yogurt enriched with antioxidants from olive fruit exhibits a positive influence on consumer’s health. In recent studies it is reported that polyphenols from olive fruit may promote in vitro growth of many lactic acid bacteria (Giavasis et al., 2012) [19] and speed up the decline in pH during yogurt fermentation (Petrotos et al., 2012) [42].

The main polyphenolic compound present in olive leaves is oleuropein which have antibacterial, anti-inflammatory, antiviral and antioxidant properties (Taghvaei et al., 2015) [66]. Nanoliposomes are highly efficient which can entrap, preserve, and induce a controlled release of the preserved bioactive compounds. Also, nanoliposomes can improve the solubility of the hydrophilic materials in lipid networks. Generally, when extracts of olive leaves and waste encapsulated in nanoliposomes and incorporated into food, an undesirable taste (bitterness) and low stability of extract in food often occurred. These nanoliposomes were formed through ethanol injection process and using reagents such as phosphatidyl choline and cholesterol for wall material. The formulated nanoliposomes were analyzed in regard to their stability, particle size, zeta potential and encapsulation efficiency. It was studied that the extract from olive leaves can be entrapped by nanoliposomes with a great encapsulation efficiency, that is, 70.7-88.2%. It was observed that encapsulation of polyphenols from olive leaf extracts in nanoliposomes mask the bitterness and enhances the stability of extract in the yogurt. The antioxidant potential, acidity, pH, syneresis, and sensory attributes of aforementioned nanoliposome incorporated yogurt samples were analyzed.

The range of mean particle size of the nanoliposomes was found to be 25-158 nm. Encapsulated olive leaves extract exhibited no significant change in color and sensory properties of yogurt in contrast to non-encapsulated olive leaves extract. Also, rate of syneresis was limited in former. Hence, a desirable and functional food is formulated in order to increase their nutritional value as well as consumer acceptance (Tavakoli et al., 2018) [67].

In another study, polyphenols from olive fruit were encapsulated in maltodextrin. The procedure for encapsulation was same as performed by Petrotos et al., (2012) [42]. This encapsulation prevented discoloration of yogurt which often occurred due to the dark brown color of polyphenolic extract of olives, improved solubility of olive polyphenols, assisted the homogenization of polyphenols into yogurt matrix and their gradual release into the yogurt, probably the GIT. The effect of polyphenol enriched yogurt on physiological, hematological as well as metabolic condition was studied on 19 people (8 were males and 11 were females) with non-declared pathology volunteered to participate. The role of olive polyphenols in the microbiome of yogurt during fermentation, storage in refrigerator and consumption period was also studied. It was observed that polyphenol enriched yogurt (concentration of polyphenols was 50mg per day) may reduce LDL cholesterol, body weight, systolic blood pressure and lipid peroxidation in participants with non-declared pathology. Also, it was found that olive polyphenols enhance the growth of lactic acid bacteria in yogurt. Intake of yogurt having 50 mg of olive polyphenols over an extended period of time may stimulate favorable transitions in metabolic parameters and blood redox status in healthy as well as unhealthy participants. This study also showed some promising results for prevention of cardiovascular disorders and more research is required in this area (Georgakouli et al., 2016) [17].

Martins et al., (2014) [36], reported that Rubus ulmifolius belongs to Rosaceae family, commonly known as wild blackberry have high antioxidant property. Through decoction and hydroalcoholic extraction process, 24 phenolic acids were extracted from the flower buds of wild black berry. The hydroalcoholic extract exhibits maximum antioxidant property in contrast to decoction form. Microencapsulation technique was effectively applied to form microspheres comprising lyophilized hydroalcoholic extract of R. ulmifolius by atomization or coagulation and then incorporate into the yogurt. Microspheres were formed by sodium alginate used as a matrix and aqueous solution of calcium chloride used as a coagulation agent. In comparison of microspheres incorporated yogurt and a natural yogurt (without incorporation of microspheres and considered as a control), the former preserves the antioxidant property of the extract for a long time. Thereby, this study manifested the high antioxidant property of hydroalcoholic extract of R. ulmifolius and also the potency of the used microencapsulation technique for the preservation of natural phenolic extracts.

All parts of the pomegranate (Panicagranatum) plant such as stem, bark, arils, leaves and whole fruit juice have exhibited antioxidant potential. Polyphenols from pomegranate have demonstrated prevention of cancer, cardiovascular diseases as well as neurological disorders in humans (Lansky & Newman, 2007; Heber et al., 2006) [31, 39]. It was studied that by using spray drying pomegranate juice (PJ) and ethanolic extract (PE) were encapsulated with soybean protein isolates (SPI) and maltodextrin (MD) as coating materials and formed 4 systems, PJ-SPI, PJ-MD, PE-SPI and PE-MD. For each system the stability of microcapsules containing polyphenols and anthocyanins obtained under optimal conditions were studied during storage at 60°C for 56 days in oven. It was found that for better protective effects (lowest bioactive degradation rate constant) for anthocyanins and polyphenols had been observed in case of maltodextrin microencapsulation than SPI during storage. And when encapsulated bioactive compounds (PJ-MD) incorporated into yogurt, there is no distinct change as compared to those without encapsulation (PJ), barring PE-MD (Robert et al., 2010) [50].

The main waste of onion (Allium cepa) comprises the outer dry layers of onion bulb which is a source of polyphenols (anthocyana and flavonoids) (Slimestad et al., 2007) [63]. Polyphenols possess both antimutagenic and antioxidant properties (Dini et al., 2008; Singh et al., 2009) [11, 62]. Beyond these properties, anthocyanins can also be used a natural food.
colorant (Chung et al., 2016) [30]. In a study onion solid wastes are valorized by a green extraction procedure. Polyphenols present in onion solid wastes were extracted by using eco-friendly solvents like glycerol and water. Encapsulation of polyphenols in cyclodextrin involves the incorporation of polyphenols into cyclodextrin cavity. This enhances their antioxidant property which further enhance their solubility (Fang & Bhandari, 2010) [14]. Aqueous solutions of cyclodextrins considered as green solvents as there is a complex formation between hydrophobic cyclodextrin cavities and non-polar components which leads to reduce the free energy of system (Kyrkiakidou et al., 2016) [30]. LC-MS was done to identify the different polyphenols and colorants present in the extract. The polyphenols and colorants found in the extract were: quercetin and their derivatives and cyanidin 3-O-glucoside respectively. The extracted food colorant was found to be a stable when incorporated into yogurt. It produced red colored stable yogurt (Mourzinos et al., 2018) [39].

Orange (Citrus sinensis) peels contain high number of polyphenolic compounds. It was studied that different concentrations of orange peel were extracted in order to achieve high number of polyphenolic compounds then they were encapsulated via coacervation method and further they were incorporated into yogurt. The wall materials used in the encapsulation were whey protein concentrate (WPC) and gum Arabic (GA), in different ratios 3:1, 3:2 and 3:3 was studied. The ratios between orange peel extract (OPE) and wall materials to form microcapsules were 1:10 and 1:20. The encapsulation efficiency of capsules was found to be 95.4% when WPC:GA (3:1) and OPE:WPC/GA (1:10). When OPE was incorporated in yogurt no significant change in physicochemical, phenolic content and texture attributes of yogurt samples occurred. Besides nutritional value, sensory attributes of supplemented yogurt was also enhanced and gained consumer acceptance (El-Messery et al., 2019b) [38].

Conclusion
Phytochemicals extracted from the fruit and vegetable wastes are promising factor for enhancing the nutritional and health promoting properties. These phytochemicals are very sensitive as their direct use into food products impair their sensory and functional attributes. These bioactive compounds are prone to degradation during storage and processing of food which leads to loss of antioxidant activity. So, it’s a great challenge to the food industry to incorporate these sensitive bioactive ingredients into the food in order to develop a functional food with improved properties. Encapsulation technology is an excellent method to protect these sensitive bioactive ingredients having superior antioxidant property and controlled release as compared to non-encapsulated bioactive ingredients. The present study intends to valorize the fruits and vegetable waste by extracting the polyphenols from them. Microcapsules formed from various encapsulation technologies incorporated into probiotics in order to develop a functional food product with improved health and nutritional characteristics.

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