Recent developments and prospects in the composition, extraction, stability, delivery system, digestion and food applications of plant oil bodies

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Abstract

Oil bodies (OBs) are micron- or submicron-sized sub-organelles widely found in plants seeds and nuts. The structure OBs is composed of a core of triglycerides covered by a phospholipid-protein layer, which ensures the stability of the OBs under extreme environmental conditions and further protects core lipids as energy reserves. As naturally pre-emulsified oil-in-water emulsions, OBs have been gradually applied to replace synthetically engineered oil droplets. In this paper, the recent research on the composition, extraction, stability, delivery system, digestion, food applications and future perspectives of plant OBs are reviewed. Recent studies have focused on the OBs surface protein identification and function, large-scale extraction techniques such as enzyme assisted, high pressure, ultrasound, and extrusion and the reconstituted OBs. Electrostatic deposition of polysaccharides significantly improves the stability of OBs emulsions. OBs emulsions have promising applications to encapsulate bioactive compounds, deliver targeted drugs, and prepare gels and edible functional films. The digestive behavior of OBs emulsions is similar to that of protein-stabilized emulsions, which can increase the satiety, effectively help reduce calorie intake and improve the bioavailability of functional factors. It has also promoted the development of simulated dairy, spices and meat products.

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Oil bodies (OBs) are micron- or submicron-sized sub-organelles widely found in plants seeds and nuts. The structure OBs is composed of a core of triglycerides covered by a phospholipid-protein layer, which

ensures the stability of the OBs under extreme environmental conditions and further protects core lipids as energy reserves. As naturally pre-emulsified oil-in-water emulsions, OBs have been gradually applied to replace synthetically engineered oil droplets. In this paper, the recent research on the composition, extraction, stability, delivery system, digestion, food applications and future perspectives of plant OBs are reviewed. Recent studies have focused on the OBs surface protein identification and function, large-scale extraction techniques such as enzyme assisted, high pressure, ultrasound, and extrusion and the reconstituted OBs. Electrostatic deposition of polysaccharides significantly improves the stability of OBs emulsions. OBs emulsions have promising applications to encapsulate bioactive compounds, deliver targeted drugs, and prepare gels and edible functional films. The digestive behavior of OBs emulsions is similar to that of protein-stabilized emulsions, which can increase the satiety, effectively help reduce calorie intake and improve the bioavailability of functional factors. It has also promoted the development of simulated dairy, spices and meat products.

Keywords : Oil bodies; Composition; Extraction; Stability; Delivery system; Digestion; Food applications

Introduction

Organisms store lipids in sub-organelles as energy reserves. These micron- or submicron-sized sub-organelles can be found in plants seeds and nuts, as well as few in some animal cells, fungi and insects (Huang, 1996) called oil bodies (OBs). Since 1970, scientists have reported on the structure, extraction and application of OBs from various natural sources. OBs either intracellularly or in isolated preparations have exhibited unique physical and chemical stability due to the presence of a surface membrane composed of phospholipids (PLs) and hydrophobic proteins such as oleosins preventing their aggregation or coalescence and protecting core lipids from extreme environmental conditions (Nikiforidis, Matsakidou & Kiosseoglou, 2014). It has isolated more than twenty kinds of seed OBs in the range of 0.5˜2.0 μm, including peanut, sunflower and walnut with high oil content and soybean, corn, germ and flax with low oil content (Huang, 2018). The small size of OBs provides a large surface area per unit of triacylglycerols (TAGs) and facilitate binding to lipase or other subcellular structures for rapid TAGs mobilization during germination (Tzen, Cao, Laurent, Ratnayake & Hung, 1993). OBs is extracted from plant materials by using aqueous media and the difference of specific gravity and solubility between OBs and other components. The isolated OBs droplets can be dispersed in aqueous environments to form natural emulsions. As a naturally pre-emulsified oil-in-water emulsions, OBs can be widely applied in food production, which is beneficial to the low energy consumption and it has excellent stability and emulsifying performance (Nikiforidis, 2019).

This paper reviews the research advances of OBs derived from plants. It provides a general description of OBs composition and structure and the methods to maintain the stability of OBs emulsions. The technologies for large-scale extraction and delivery systems of OBs are highlighted. In addition, the digestive behavior and current applications of plant-based OBs mainly in food research are discussed. The review reports the future research direction and development potential of natural OBs.

2.Composition and structure of OBs

The composition of the OBs conforms to a formula describing a spherical particle surrounded by a shell of a monolayer of phospholipids embedded with proteins. The spherical particle is a neutral lipid core consisting of TAGs as a rich source of oil in storage form, a small amount of diglycerides, free fatty acids, and Vitamin E, etc (Abdullah, Weiss & Zhang, 2020). The neutral lipids of the OBs account for about 92%˜98%, the contents of PLs and proteins are about 0.6˜2.0% and 0.6˜3.0%, respectively (Ding et al., 2019; Tzen et al., 1993).

2.1 Neutral lipids

The lipid composition of OBs was examined by chromatography. It was found that the content of TAGs was the highest, and palmitic acid, stearic acid, oleic acid, linoleic acid were the main fatty acids (Zaaboul, Raza, Chen & Liu, 2018; Xu et al., 2021). TAGs can not only be as energy reserves for germination and post germinative growth of the seedlings, but also are important for cell division and expansion, membrane

lipid remodeling and organ formation (Yang & Benning, 2017). If the seeds have been stored for a long time, the hydrolysis of TAGs and PLs by internal or external lipase or nonspecific acyl hydrolases may lead to the increase of minor lipids, especially free fatty acids, causing rancidity and quality degradation (Huang, 1992).

2.2 PLs

The electron microscope results showed that the core of OBs was an electronically opaque TAGs matrix surrounded by one electron-dense layer. This single electron-dense layer contrasted with the two parallel electron dense layers presented in the unit membrane of the two PLs layers that composed of the cell membrane. It was determined that the surface of the OBs was surrounded by a "half-unit" membrane of one PLs layer, in which the hydrophobic tail faced inward to interact with the TAGs, and the hydrophilic head was exposed to the cytoplasm (Tzen & Huang., 1992). Typically, PLs accounted for more than 80% of the surface area of the OBs, and the thickness was 0.9 nm˜2.5 nm (Purkrtova, Jolivet, Miquel & Chardot, 2008; Yang, Su, Zhang, Jia & Phillips, 2020). It was determined by TLC that the major PLs in *Echium plantagineum* OBs was phosphatidylcholine (PC; 52.4%), followed by phosphatidylserine (PS; 32.6%), phosphatidylethanolamine (PE; 12.3%) and phosphatidylinositol (PI; 4.2%) (Payne, Lad, Foster, Khosla & Gray, 2014). ^{31}P NMR measured the highest content of PC (50.6%) in peanut OBs, followed by PI (13.6%), PE (12.7%), PS (10.8%) and phosphatidic acid (PA; 10.0%) (Niu, Chen, Liu & Duan, 2021). PC had a stronger hydrophobic interaction with the OBs membrane proteins, therefore, the OBs were much more stable. Tzen et al. (1993) analyzed the PLs in the OBs from seven kinds of oilseeds found that they contained substantial amounts of the uncommon negatively charged PS and PI. It has been proposed that these negatively charged PLs can interact with the basic amino acid residues of the oleosins on the surface of the PLs layer. The fatty acid composition of the PLs in the OBs was determined to be high in saturated fatty acids, which contributed to the anchorage of the oleosins and thus to the stability of the OBs (Furse et al., 2013; Katavic, Agrawal, Hajduch, Harris & Thelen, 2006; Payne et al., 2014).

2.3 Proteins

It was studied that the PLs on the surface of the natural OBs could not be approached by external phospholipase A2 or phospholipase C. The integrity of the OBs was due to the shielding effect of proteins, while the OBs termed to polymerized after trypsin treatment. Experiments also showed that the artificially prepared particles of TAGs surrounded by a layer of PLs would polymerize rapidly. However, when the particles contained proteins on their surfaces, like natural OBs, they became stable and did not aggregate or coalesce, even they were brought to squeeze against one another *in vivo* and *in vitro* . This confirmed the importance of proteins in maintaining OBs stability, since proteins provided not only amphiphilic surfaces but also steric hindrances (Tzen et al., 1992). At present, the research mainly focuses on the identification and function of OB interface proteins.

The dominant OBs-associated proteins are called oleosins. Oleosin has a low molecular mass of 15˜26 kD with three structural regions. Two amphiphilic N- and C-terminal regions moieties located at the surface of the OBs, with the positively charged residues interacts with the negative charge of the phosphate molecule towards the lumen of the organelle and the negatively charged residues facing the cytosol, which makes the OBs surface negatively charged and generates electrostatic repulsion to maintain the stability of the OBs, also can prevent external phospholipase from acting on the PLs membrane (Napier, Stobart & Shewry, 1996). A central hydrophobic region of about seventy residues formed by two approximately 11 nm long antiparallel strands connected by a "proline knot" is inserted in the acyl moieties of PLs and the TAGs matrix and forms a hairpin-like structure (Tzen, Lie & Hung, 1992).

Caleosin is another group of protein on the OBs surface, with a molecular mass of 25˜35 kDa. It has the same molecular structure as oleosin, including a hydrophilic N-terminal group, a hydrophilic C-terminal region, and a central hydrophobic region inserted into the core of TAGs (Næsted, Frandsen, Jauh, Hernandez-Pinzon & Mundy, 2000). Caleosin is characterized by a more hydrophilic N-terminal segment as it contains a Ca^{2+} binding region (Chen, Tsai & Tzen, 1999). It has been reported that caleosin may be involved in OBs fusion, membrane-fission and/or fusion and lipid intracellular trafficking and metabolism (Frandsen, Mundy & Tzen,

2001; Næsted et al., 2000).

Steroleosin is a comparatively bigger protein with molecular mass more than 35 kDa. It firstly identified as a minor protein in sesame OBs, comprised a hydrophobic anchoring segment at the same length as caleosin, followed by a sterol-binding dehydrogenase domain, which could anchor the soluble sterol binding dehydrogenase domained to the surface of the OBs through the N-terminal hydrophobic fragment (Lin, Tai, Peng & Tzen, 2002).

Tnani, López, Jouenne & Vicient (2011) identified new proteins in maize embryos OBs besides oleosin, caleosin and steroleosin, such as karyopherin-beta and a stress-induced membrane pore protein were involved in membrane transport. Jolivet et al. (2004) found that oleosins accounted for 79% of OBs proteins in *Arabidopsis thaliana ecotype* WS, and the 18.5 kDa oleosin was the most abundant. Meanwhile, they found a probable aquaporin and a glycosylphosphatidylinositol-anchored protein, which had never been found in plant OBs, without known functions. Plant nsLTP, a soluble protein with a molecular mass of less than 20 kDa, presented in rice bran OBs, which contributed to the transfer of fatty acids, PLs, glycolipids and steroids between membranes, and played a key role in the process of plant resistance to pathogens. Embryo-specific protein belonging to plant antimicrobial peptides family and storage proteins such as gi|31432342, gi|24899397 and gi|12039336 were also identified in rice bran OBs (Xu et al., 2021).

2.4Bioactivecomponents

OBs may contain a small amount of bioactive components that are contribute to their chemical stability, such as tocopherols, phytosterols, γ-oryzanol, essential amino acids and isoflavones depending on the source (Fisk & Gray, 2011; Gallier, Gordon & Singh, 2012; Nantiyakul, Furse, Fisk, Foster, Tucker & Gray, 2012; White, Fisk & Gray, 2006; Zaaboul et al., 2018). Bioactive components make natural OBs with high nutritional value and the development and utilization prospects are very broad.

3. OBsextraction

The microstructure of plant cells was observed, and abundant natural OBs of different sizes were clearly found (Fig. 3). Starch and proteins were distributed around the OBs, and each region was separated by the cell wall to maintain the integrity of the OBs. Therefore, the destruction of cell wall is the key to extracting natural OBs. Due to the hydrophilic nature of the OBs surface, it can be dispersed into aqueous phase to form a uniform emulsion (Nikiforidis, 2019). Based on this characteristic, the isolation process of OBs can be divided into four steps: (1) the structure of plant cell wall is destroyed by mechanical crushing, and the OB is separated from protein body and starch grain to release in aqueous medium; (2) the slurry is filtered to produce a " milk ", which is a suspension of OBs in an aqueous medium, with reduced seed particulate material; (3) OB is concentrated into cream by centrifugation of the milk to obtain crude OBs; (4) the crude OB is washed to remove the impurities on the surface and obtain pure OBs (Nikiforidis et al, 2014). Current extraction methods of OBs mainly include aqueous extraction and enzyme assisted extraction based on the different principles of disrupting the cell wall.

3.1Aqueous extraction

The aqueous extraction of OBs requires soaking seeds in an aqueous medium, then blending or pressing to disrupt cell walls and released intracellular materials. Urea, sucrose, deionized water, salt, alkali and buffer solution including Tris-HCl and PBS are often used as the grinding medium for aqueous extraction. In 1992, 0.6 M sucrose solution was first used to extract plant OBs including maize, rice, soybean, rapeseed, jojoba, wheat bran, flax, sesame, tripsacum, teosinte, yucca, mustard, jojoba, sunflower, peanut, palm, castor bean, Brazil nut and oat bran, the grinding medium also contained 1 mM EDTA, 10 mM KCI, 1 mM $MgC1_2$, 2 mM DTT, and 0.15 M Tricine buffer (Tzen et al, 1993; Tzen & J., 1992; Tzen et al, 1992). Since 1996, the extraction medium was simplified (Chuang, Chen, Chu & Tzen, 1996; Tzen, Peng, Cheng, Chen & Chiu, 1997). Lacey, Wellner, Beaudoin, Napier & Shewry (1998) found that the OBs extracted by urea (9 M) was not affected by exogenous proteins, and integral oleosin proteins could be completely preserved. Urea can disrupt non-covalent bonds of proteins allowing the removal of passively associated proteins from the

OBs surface (Millichip, Tatham, Jackson, Griffiths, Shewry & Stobart, 1996). However, urea is not a food grade material, thus limiting its use. At present, deionized water and buffer solution are often selected as extraction media (Nikiforidis & Kiosseoglou, 2009; Iwanaga, Gray, Fisk, Decker, Weiss & McClements, 2007; Lan et al., 2020), while, the OBs still contain some intracellular substances such as exogenous proteins and phytochemicals, and endogenous proteins are also vulnerable to destruction. Therefore, in order to simplify the subsequent washing steps, De Chirico, di Bari, Foster & Gray (2018) pointed out that the purity of oilseed rape seed OBs extracted with sodium bicarbonate solution (0.1 M) in the grinding and washing steps was the same as that produced by washing a crude preparation with 9 M urea, and the physical stability of the OB was improved. The results provided a new method for the aqueous extraction of intact and pure OBs.

3.2Enzyme assisted extraction

Plant cell walls are composed of cellulose, hemicelluloses, lignin and pectin. Thus, in addition to mechanical means, the use of carbohydrase such as cellulase, hemicellulose, pectinase, xylanase and β-glucanase may also destroy the cell wall. The high specificity of enzymes greatly limits the hydrolysis due to the diversity of cell wall components and raw materials. In this case, the complex enzyme has the activities of multiple enzymes can be considered. During the extraction process, the use of proteases is not considered. Although the recovery of OBs can be improved, the associated proteins may be destroyed into small peptides through hydrolysis. Kapchie, Wei, Hauck & Murphy (2008) compared the efficiency of mixing Multifect Pectinase FE (Pectinase, cellulase, and hemicellulase complex), Cellulase A (Cellulase, β-glucanase, hemicellulase, and xylanase complex) and Multifect CX 13L (β-glucanase complex) in equal proportions with aqueous extraction of soybean OBs found that the total soybean oil recovered from OBs extracted by enzymatic method was 36.42%˜63.61% and the yield of soybean oil in OBs could reach 84.65% after three successive extractions of the residue. However, the total soybean oil recovered from OBs was only 28.65%˜34.28% by conventional method. Xu et al. (2021) studied the structures, physical properties and chemical compositions of rice bran OBs extracted by plant exacted enzyme, xylanase and their mixture, and showed that the yield of OBs obtained by the mixture of xylanase and plant extracted enzyme was 76.95%. Extracting OBs from plant raw materials is a relatively new research field. It is necessary to invest in developing special equipment and consider the actual production conditions to achieve industrial production. Towa, Kapchie, Hauck, Wang & Murphy (2011) conducted a pilot scale equipment in which the yield of OBs isolated from soybean to 93.40% compared to a laboratory-scale process.

3.3 Comparison of different methods

The selection of OBs extraction method mainly depends on the extraction yield, complexity, production cost, environmental friendliness, and safety. Aqueous extraction is a traditional method widely applied for extraction of OBs has the advantages of no chemical pollution and low energy consumption, however, there are certain limitations that it requires large amount of solvents, and thus makes up-scaling difficult. Although the enzymatic extraction of OBs requires a long reaction time, and the enzyme has strict requirements on temperature and pH, the extraction efficiency of OBs is higher. There are major problems that must be solved to develop special enzyme, reduce the dosage of enzyme and ultimately reduce the cost of enzyme in the industrialization of enzymatic extraction of OBs technology. Furthermore, the extraction procedures require simple equipment that can be operated safely to facilitate industrial production and commercial application of OBs.

3.4 The main influencing factors ofextraction process

In order to further improve the OBs yield, reduce the cost and obtain high quality and purity OBs, the key parameters in the extraction process can be controlled. Generally, there are many factors affecting the extraction procedures including pretreatment, pH, purify, medium ratio and centrifugal force.

3.4.1 Pretreatment

The pretreatment of plant materials usually involves grinding and soaking. Grinding provides better exposure

of OBs to the water as a result of cells structure rupture, and soaking allows water molecules to penetrate into the network of cells for more efficient extraction. For seed raw materials, mechanical grinding is usually carried out in the extraction medium after soaking for 8˜72 h. For the processing by-products such as wheat bran and rice bran, after sieving and removing impurities can be soaked or not for a short time, followed by mixed with the extraction medium for homogenous extraction of OBs (Lan et al., 2020; Nantiyakul, Furse, Fisk, Tucker & Gray, 2013; Tzen et al., 1992; White et al., 2006).

At present, pretreatment methods such as mechanical crushing, extrusion, high pressure, and ultrasound have been successfully used for aqueous oil extraction, but it is worth noting that these pretreatment methods have the potential to improve the OBs extraction yield (Mat Yusoff, Gordon & Niranjan, 2015; Koubaa, Mhemdi, Barba, Roohinejad, Greiner & Vorobiev, 2016). The crushing degree of raw materials directly affects the extraction yield of OBs. The size of raw material cells should be considered during mechanical crushing. When the particle size of the material is smaller than the cell size, the cell wall can be destroyed and the contact area between the OBs cells and the extraction medium will be enlarged. While, excessive crushing may destroy the complete structure of the OBs. Nikiforidis et al. (2009) showed that the extraction yield of OBs was the highest at any pH and number of extraction steps applied when the corn particle size was less than 0.8 mm. However, there was a possibility that a single OB merge into a larger droplet.

The extrusion process increases the pressure generated by compression to exceed the maximum shear stress that the plant cell wall can withstand, causing the cell wall to rupture (Peng et al., 2021; Li, Zhou, Zhang, Wang & Cong, 2020). It is not conducive to the solvent percolation for the fine powder raw materials. Extrusion can also reshape the material into porous expanded particles, which increases percolation rate of the solvent and hence better efficiency of OBs extraction (Liu et al., 2020). Romero-Guzmán, Jung, Kyriakopoulou, Boom & Nikiforidis (2020) used a twin-screw press to extract OBs at pH 7.0, and showed that the yield can reach 90% and twin-screw press was a promising alternative to scale-up the OBs aqueous extraction and the water usage was significantly reduced.

It has been reported that the high pressure-processing transmitted isostatic pressure (100˜400 MPa) to plant materials could be useful for destroying cells and increasing the solubility of bioactive compounds (Bueno, Gallego, Chourio, Ibáñez, Herrero & Saldaña, 2020; Ninčević Grassino et al., 2020). However, the structure of the product changed at a pressure of around 600 MPa, resulting in a decrease in extractability (Butz, Edenharder, Garcıa, Fister, Merkel & Tauscher, 2002; Dobrinčić, Repajić, Garofulić, Tuđen, Dragović-Uzelac & Levaj, 2020). Kapchie et al. (2008) demonstrated that the highest OBs yield of 71.39% was obtained with the ultrahigh pressurizations of soybeans flours at 200 MPa for 5 min at 25 °C, while, the OBs yield was 21.82% with material pressurized at 500 MPa.

Ultrasound induced impacts which can be attributed to the cavitation phenomenon referring to bubble formation, growth and implosion during the propagation of the ultrasonic wave into medium. The bubble implosion will create a hot spot with a temperature of up to 5000 K and a pressure of 5000 atm (Khadhraoui, Ummat, Tiwari, Fabiano-Tixier & Chemat, 2021). This may result in slight or high effects on cell walls, enhancing the penetration of the solvent into the internal structure and facilitating the release of the target compound. In addition, the strong shear forces and turbulence generated by the propagation of the ultrasonic waves appear to further accelerate the exchange between the raw material and the surrounding extraction solvent. These mechanisms lead to "an increase in the depth and speed of solvent penetration into plant internal structures" (Soria & Villamiel, 2010; Chemat et al., 2017). Loman, Callow, Islam & Ju (2018) found that 1.5 W/mL pulsed ultrasound treatment for 5 min every 3 h could significantly improve the performance and separation of OBs from protein during the enzyme processing. It was studied that ultrasonic pretreatment would increase the OBs yield, but the yield showed a downward trend after a certain time, because the ultrasonic energy was not uniformly distributed in the pretreatment process (Albu, Joyce, Paniwnyk, Lorimer & Mason, 2004; Kapchie et al., 2008; Toma, Vinatoru, Paniwnyk & Mason, 2001).

3.4.2 pH

pH is an important parameter in the OBs extraction process, which affects the separation of exogenous

proteins and the integrity of OBs. The isoelectric point of the OBs is between 4.0˜6.0 due to the existence of proteins on the surface (Wang et al., 2019; Tzen et al., 1993). Therefore, it is not considered that the extraction pH is lower than 6.0. At this point near the isoelectric point of the proteins, the natural and independent OBs in the cells will aggregate after separation. The pH value of the medium when extracting OBs is usually between 6.5˜11.0. In this pH range, the surface of the OBs maintains a negative charge, and electrostatic repulsion and steric hindrance effect exist between the OBs. When the pH value is higher than 9.0, both proteins and OBs are soluble and the extraction yield is improved (De Chirico et al., 2018). Study have indicated that an extraction yield as high as 95% could be reached when a finely comminuted germ material was extracted 3 times at pH 9.0 (Nikiforidis et al., 2009).

It is known that the pH value of the extraction buffer not only has a direct influence on the extraction yield of OBs, but also has a great influence on the protein composition of the extracted OBs, which affects the OBs properties, and then affects the OBs utilization. Zhao, Chen, Chen, Kong & Hua (2016) studied jicama, sunflower, peanut, castor bean, rapeseed, and sesame to explore the effects of pH (6.5˜11.0) on protein compositions of OBs. The results showed that there were many extrinsic proteins (globulins, 2S albumins and enzymes) presented in pH 6.5-extracted OBs. Globulins was mostly removed at pH 8.0 and 2S albumins were removed at pH 11.0. At pH 11.0, highly purified OBs were obtained from jicama, sunflower, peanut, and sesame, whereas there were still enzymes remained in the castor bean and rapeseed OBs. Therefore, the extraction pH could be selected according to the properties of OBs product: for high protein-containing OBs products, neutral or even acidic pH should be selected; for highly purified OBs products, high alkaline pH should be selected. It was clear that as the extraction pH increases, the exogenous protein was gradually reduced, however, it is not clear whether oleosins could also be removed by alkaline pH (Chen & Ono, 2010). Cao, Zhao, Ying, Kong, Hua & Chen (2015) confirmed that alkaline pH not only removed contaminated proteins but also oleosins, and more and more oleosins were removed with increasing alkaline pH. The research showed that pH also affected the rheological properties of OBs, gels were formed from OB emulsions with solid content of 40% except pH 11.0-OBs, for liquid-type OBs products, pH 11.0 should be selected; for solid-type OBs products, pH 9.5 should be selected (Zhao, Chen, Yan, Kong & Hua, 2016). Zaaboul et al. (2018) first investigated the main compounds of peanut OBs and found that oleic acid and linoleic acid were the major fatty acids in OBs regardless of pH. Tocopherol content went from 270.76 to 278.2 mg/g when pH was increased. On the contrary, phytosterols content decreased when pH was increased, with 631.49 μg/g at pH 6.8 and 614.96 μg/g at pH 11.0.

3.4.3Purified OBs

It is necessary to determine whether pure OBs or a mixture of OBs and storage proteins are needed when extracting OBs. For some applications, especially foods, such as salad dressings, where proteins have been used to modulate the macro properties of the system, so mixtures containing OBs and storage proteins are beneficial (Nikiforidis, Biliaderis & Kiosseoglou, 2012; Karefyllakis, Octaviana, van der Goot & Nikiforidis, 2019). However, in other applications, pure OBs may be required. To obtain pure OBs, several cleaning steps are required including urea washing, sucrose washing, deionized water washing, salt washing, alkali washing and buffer solution washing including Tris-HCl washing and PBS washing. Generally, the washing medium is similar to the extraction medium, while the medium used in extracting crude OBs and obtaining pure OBs can be different. It was reported that OBs washed in (9 M) urea were significantly enriched in lipids and low in proteins compared with unwashed, water-washed, and salt-washed OBs and washing significantly reduced the total phenolic content of the oat OBs but significantly increased concentrations of vitamin E (White et al., 2006). For soybean OBs from different varieties, the contents of vitamin E and total phenolics were decreased by urea washing (Fisk et al., 2011). Murphy & Cummins (1989) explored the influence of washing times on OBs found that the composition of OBs was not affected by washing times, but multiple washing times would greatly reduce the final production of OBs. The OBs apolipoprotein was tightly bound to the surface of OBs and could not be removed by washing and pure OBs fraction could be obtained by two floatation steps at most.

3.4.4 Other influencing factors

The key factors affecting OBs extraction also include medium ratio, centrifugal force, incubation time, temperature, stirring speed and so on. The viscosity of the system is determined by the ratio of the solid to the disperse medium. When the viscosity is too low, it may result in less force borne by OBs during the recovery process and reduce the collision and damage of OBs particles. However, when the water content of the system is low, the viscosity is high and OB is not easy to free, resulting in low yield. It had been confirmed that the OB particle size distribution presented a wide distribution at a high solid-phase loading ratio, and droplet aggregation was observed in the optical image, while, in a more diluted seed grinding system, the OB was smaller and integrity (De Chirico et al., 2018). The centrifugal force can affect the size distribution of the OBs, If the centrifugal force is too low, small OBs will be emulsified or too high, OBs trend to coalesce. Current studies select centrifugal forces ranging from 5000 RCF to 20000 RCF (Zhang et al., 2017). Some researchers modified the traditional extraction method by giving the raw materials a certain incubation time and temperature under a stirring speed (Niu et al., 2021; Nantiyakul et al., 2012). These operations affected the denaturation of proteins, the dispersion and aggregation of OBs, and also affect the extraction yield of OBs. For enzymatic extraction, the amount of added enzyme is also an important factor. Generally, there are many factors affecting the yield of OBs during the extraction process, while systematic and statistical optimization of the significant factors has not been attempted to obtain the optimal extraction conditions and the ideal yields.

4.Stabilityof OBs emulsions

OB is mainly dispersed in an aqueous media to form a natural emulsion system, which can deliver stable preemulsified oil into appropriate food systems to obtain products. This will reduce the need to extract and purify the oil using organic solvents and then emulsify it using a homogenizer, thereby achieving more sustainable and environmentally friendly processing operations. The utilization of OBs in food products requires a thorough understanding of their functional properties under complex environmental conditions. During food processing, storage, transport and utilization, the pH value of the system may change. For taste, preservation and modification of physicochemical properties, salt is added to the food. Many emulsion-based food products may undergo various kinds of thermal treatments such as pasteurization, sterilization, temperature fluctuations, baking and cooking and freezing and thawing process. Studies have shown that the rheology and stability of OBs suspensions are susceptible to the effects of pH and salt concentration, resulting in reduced electrostatic repulsion between OBs and instability such as flocculation (White, Fisk, Mitchell, Wolf, Hill & Gray, 2008). Therefore, to utilize OBs commercially in food systems, the stability of OBs must be enhanced in order to adjust to environmental changes (in terms of pH, ionic strength and thermal treatment).

OBs and polysaccharides have similar types of charges which can cause the effect of electrostatic deposition and steric repulsion of polysaccharides between emulsion droplets (Iwanaga, Gray, Decker, Weiss & McClements, 2008). Many researchers have attempted to use a method that changed interfacial composition by coating OBs with a layer of polysaccharides to improve the stability of OBs to environmental stresses. The most commonly reported polysaccharides are pectin, carrageenan, xanthan gum and gum arabic. The formation of polysaccharide-OB complex on the surface layer depended on the pH values, polysaccharide concentration, and temperature. Laccase cross-linked beet pectin coated soybean OBs had better stability at pH change (3.0˜7.0), NaCl addition (0˜500 mM), and freeze-thaw cycle (-20 for 22 h; 40 for 2 h) (Chen, McClements, Gray & Decker, 2010). It had been shown that soybean OBs emulsions stabilized with ι-carrageenan were more stable to creaming due to depletion flocculation than the emulsions stabilized with κ or λ-carrageenan after 7 d storage and the soybean OBs emulsions by coating a ι-carrageenan layer at pH 3.0 and 7.0 had improved stability to environmental stresses (Wu et al., 2012; Wu, Yang, Teng, Yin, Zhu & Qi, 2011). Lan et al. (2020) screened xanthan gum from ten stabilizers to stabilize safflower OBs, and determined that when the addition amount was 0.3%, the OBs coated with xanthan gum had thermal stability, but was affected by ultrasonic strength (Sukhotu et al., 2016). Ding et al. (2019) successfully and effectively encapsulated soybean OBs by using maltodextrin (MD)–chitosan (CS)- Epigallocatechin- 3-gallate (EGCG) covalent conjugates (CSEG) as coating material and applying spray drying technology, and the emulsifying activity and thermal stability of OBs microparticles were improved and significantly reduced the amount of

oil released during the whole digestion process.

5.Delivery system of OBs

5.1Emulsions-based delivery system of OBs

Emulsions-based delivery system in food industry is widely recognized to be a promising method of encapsulating and delivering bioactive compounds in product manufacturing and storage processes for preventing chemical degradation and increasing bioavailability (McClements, 2015). Liu, Wang, He, Cheng & Ma (2020) reported that soybean OBs could be used as the novel carriers in delivering the curcumin to improve the stability of curcumin and its release rate during digestion. Chiang, Chen, Liou & Chao (2019) found that the self-assembly nanoscale OBs enabled targeted delivery curcumin, and curcumin-loaded nanoscale OBs displayed a strong anti-proliferative effect on tumor cells. Studies also have showed that OBs also were effective carriers for volatile flavor compounds. Fisk, Linforth, Taylor & Gray (2011) indicated that OBs offered the enhanced flavor delivery through elevated headspace flavor persistence. Water-washed OBs were spray dried further stabilized with capsules to embedment volatile lipophilic actives (D-limonene), with a retention rate of 55.59% (Fisk, Linforth, Trophardy & Gray, 2013).

5.2 Emulsion-gels

Emulsion-gel is a typical semi-solid food system consisting of gel matrix filled with oil/fat droplets (Dickinson, 2012). Proteins such as gelatin, soy protein, casein and polysaccharides such as starch, carrageenan, pectins, alginate and flax gum are the most recently used as the matrix (Bi, Chi, Wang, Alkhatib, Huang & Liu, 2021; Dickinson, 2012; Fontes-Candia, Ström, Lopez-Sanchez, López-Rubio & Martínez-Sanz, 2020; Hu, Karthik & Chen, 2021; Li, Gong, Hou, Yang & Guo, 2020; Saavedra Isusi, Madlindl, Karbstein & van der Schaaf, 2020). Emulsion-gels have broad application prospects in food industry such as fat reduction, probiotics release and flavor control due to their diversity in structure and composition (Lin, Kelly & Miao, 2020). OBs can replace the traditional oil/fat droplets in the gel due to their nutritive value and natural emulsification properties resulting in great application prospects in food industry (Nikiforidis et al, 2014). However, to the best of our knowledge, there are very few studies on the emulsion-gels filled with OBs. Kirimlidou, Matsakidou, Scholten, Nikiforidis & Kiosseoglou (2017) revealed that filling the gelatin matrix with OBs instead of oil/fat droplets, the OBs could be well dispersed in the gel network, and had no negative effect on the rheological properties of the composite gels. Mert & Vilgis (2021) adopted xanthan gum and pectin to stabilize natural OBs suspension based on electrostatic deposition, and converted it into soft solid oleogel structure. This discovery not only improved the stability of OBs suspension, but also provided a new idea for the design of gel structure composed of OBs. Yang et al. (2020) constructed an emulsion-gel with soybean OBs as filling oil/fat droplets and $\varkappa$-carrageenan as gel matrix, and found that OBs emulsion-gels exhibited better lubrication properties and an ultralow boundary friction coefficient (μ) was achieved, which was significant to study the oral processing of OB emulsion-gels when it was used in semi-solid food. Nikiforidis & Scholten (2015) prepared a high internal phase emulsion gel with volume fractions of 0.91 and elastic properties by using natural OBs, and its shear elastic modulus was between 10^2 and 10^5 Pa.

5.3 Edible films

Edible films based on biodegradable materials, such as proteins, polysaccharides, lipids have potential uses in food packaging and as carriers of active compounds like antioxidants and antimicrobials (Jeya Jeevahan et al., 2020). However, the water barrier property of water-soluble hydrocolloid edible films is poor. It has been reported that vegetable oil was added into the biopolymer film matrix to reduce the water vapor permeability (Jeevahan & Chandrasekaran, 2019). The biopolymer-oil mixture needs to be homogenized to obtain a uniform distribution with small-sized droplets, thus increasing the tortuosity factor and improving the water barrier performance of the film (Vargas, Perdones, Chiralt, Cháfer & González-Martínez, 2011). To avoid the homogenization, small sized natural OBs can be added to the initial biopolymer solution to replace vegetable oil. OB is a kind of natural composite film based on the special structure, but it has reported that the initial OB film was less elastic and easy to tear, and then used Tris-HCl plasticizer, Ca^{2+}/Mg^{2+}crosslinking agent and carboxymethyl cellulose could effectively modify the mechanical properties of OBs membranes, making

them have a wide range of tensile strength and elongation properties (Wang, 2004). The interaction between the surface of maize germ OBs and caseinate molecules resulted in the effective binding of the OBs to the protein matrix, and then the composite film with milky white appearance, strong hydrophobicity, relative viscosity and flexibility was prepared. The fact that mechanical and optical characteristics of the composite films marked alterations upon storage due to water uptake or OBs movement (Matsakidou, Tsimidou & Kiosseoglou, 2018; Matsakidou, Biliaderis & Kiosseoglou, 2013).

5.4 Reconstituted OBs

According to relative proportions of TAGs, PLs and proteins, these three basic components can be used to reconstruct the stable OBs by ultrasonic technology (Tzen et al., 1992). Recently, the reconstituted OBs expression/purification system has been developed for the purification of recombinant proteins or enzymes immobilization in one step by linking a desired protein or enzyme to oleosin on the surface of OBs (Bai, Yan, Zhang, Yu & Bai, 2014; Bettini, Santino, Giancane & Valli, 2014; Chiang, Chen, Chao & Tzen, 2005). This novel technique provides a promising alternative for purifying recombinant proteins regarding to the equivalent purification efficiency at a lower cost (Tseng, Huang, Huang, Tzen, Chou & Peng, 2011). The reconstituted OBs can be used like natural OBs to carry nutrients (such as curcumin), probiotics and medicines (Bettini et al., 2013; Santiago & Devanadera, 2016). By changing the amount of three components, it is possible to obtain reconstituted OBs of different sizes and the physicochemical stability and structure of the reconstituted OBs with different sizes are also different (Peng, Lin, Lin & Tzen, 2003). Typically, nanoscale reconstituted OBs are generated by self-assembly by changing the ratio of matrix oil to oleosins to target the delivery of hydrophobic drugs (Chiang, Lin, Lu & Wang, 2011; Chiang, Lin, Yang & Chao, 2016).

6.Digestion of OBs

OBs emulsions can be used as carrier to transport natural, minimally processed and pre-emulsified oil to appropriate food system, and also contain lipophilic bioactive components such as tocopherol, oryzanol and sterol. The digestive behavior of OBs emulsions is similar to that of protein-stabilized emulsions, with flocculation of the OBs occurring under gastric conditions. Under intestinal conditions, bile salt replaces interfacial peptides and phospholipids and destroys the flocculate and the hydrolysis of triglycerides leading to the spontaneous formation of a novel multiple emulsion (Gallier, Tate & Singh, 2013). In general, lipids digestion in OBs is slow and the digestive efficiency may affect by the following three aspects (Wang, Ye & Singh, 2020). Firstly, the OBs membrane has a negative impact on the digestion efficiency. Secondly, the appearance of new prunin isoforms in oleosins and the rearrangement of protein profile may limit the lipids bioaccessibility. Thirdly, long chain fatty acids, the main lipolytic products, accumulated at the surface of the OBs limited the activity of pancreatic lipase (Gallier & Singh, 2012; Trombetta et al., 2020). While, in the complex food matrix, a protective layer is formed around OBs under the influence of macromolecules such as protein and polysaccharide, which promote the flocculation of OBs droplets and inhibit the ability of pepsin and lipase, so as to reduce the absorption rate of lipids and related lipophilic compounds (Wu et al., 2012). The digestion rate of lipids in OBs-emulsion food products is slower than that in free OBs emulsion, which will affect gastrointestinal tract physiology and may result in increasing satiety, effectively helping to reduce calorie intake (White, Fisk, Makkhun & Gray, 2009). As carriers for the delivery of bioactive compounds and pharmaceutical drugs, in the process of digestion, OBs form mixed micelles to dissolve functional factors and improve their bioavailability (Zheng, Zhang, Peng & Julian McClements, 2019).

7. Food applications of OBs

OBs as natural emulsions have shown prospects in the preparation of liquid or semi-liquid food products such as milk, yogurt, mayonnaise and salad dressings (Nikiforidis et al., 2012).

Consumer demand for cow's milk alternatives has increased as a result of lactose intolerance of some people and the demand for vegetarianism and health (Aydar, Tutuncu & Ozcelik, 2020). Plant-based milk substitute (PBMS) is often perceived as healthy possibly due to they contain dietary fiber, vitamins, minerals, and antioxidants (Jeske, Zannini & Arendt, 2018). Generally, PBMS is emulsified systems composed of OBs, solid particles and starch granules all dispersed in an aqueous phase and the presence of water-soluble pro-

teins on the surface of the OBs (e.g. oleosins, caleosins, and steroleosins) improves the texture, stability and nutrition of PBMS (Huang, 2018; Zaaboul, Raza, Cao & Yuanfa, 2019). Ultrafiltration is commonly used to produce an OBs-based emulsion with a lipid content similar to cow milk and rich in polyunsaturated and monounsaturated fatty acids to obtain PBMS and the PBMS can maintain its aroma characteristics and bioactive constituents upon heating and exhibit appreciable microbial, physical and oxidative storage stability (Naziri, Koupantsis, Mantzouridou, Paraskevopoulou, Tsimidou & Kiosseoglou, 2017). Shakerardekani, Karim & Vaseli (2013) prepared pistachio milk by using pistachio OB as a natural pre-emulsified emulsion and adding 5.0% sugar, 0.02% vanilla and 0.0% salt.

Fat globules play an important role in the production of yogurt. The acidification of milk induces the formation of continuous gel networks of aggregated protein molecules where fat globules are incorporated (Horne, 1999). The strength of the gel depends on the size of the fat globules and the extent of their surface interactions with the gel network (Kirimlidou et al., 2017). The physical properties and microstructure of the gel are reflected in the texture and sensory characteristics of yogurt (Öztürk, Aydın, Sözeri, Demirci, Sert & Akın, 2018). Gallier et al. (2012) studied the differences in chemical, physical and structural properties between the almond OBs, and bovine milk fat globules. It was found that almond OBs contained mostly long-chain unsaturated fatty acids, phytosterols and nosphingomyelin. Bovine milk fat globules were rich in saturated fatty acids and cholesterol, larger in size, and richer in sphingomyelin and phosphatidylethanolamine. The monolayer membrane around almond OBs and the trilayer membrane around bovine fat globules might affect the stability of the lipid droplets in a food matrix and the way the lipids were digested. Mantzouridou, Naziri, Kyriakidou, Paraskevopoulou, Tsimidou & Kiosseoglou (2019) replaced the cow milk fat globules in yogurt with maize germ OBs, which had a good acidification kinetic pattern, forming a liquid structure acidic gel and the quality and stability of yogurt improved compared with that prepared with whole cow milk. Romero-Guzmán, Köllmann, Zhang, Boom & Nikiforidis (2020) obtained a plant-based mayonnaise by controlling the conditions of the aqueous extraction process of OBs and co-extracted proteins and soluble fibers.

8. Conclusion and future perspectives

At present, the basic structure of OBs has been determined. The unique conformation of the OBs membranes makes the OBs highly stable. This special structure can be used as reference for the development of synthetic oil droplets such as interface stabilizers for Pickering emulsions. It is necessary to study the interaction and structural dynamics between phospholipid and protein molecules, and investigate the mechanical properties of the OBs membranes to provide the OBs membranes with stability and stretchability. In order to promote large-scale extraction of OBs, it is necessary to design a process with low cost, easy to be sustained, high yield and complete structure of OBs. There has been a tendency to develop alternative extraction processes including enzyme assisted extraction and supercritical fluid extraction. Complete OBs extraction can be realized under the condition of ensuring that the OBs membrane is not destroyed by screening specific enzyme and adjusting operating parameters. At the same time, high pressure, ultrasonic, microwave, steam flash and other auxiliary processing technology can be developed to achieve efficient extraction. The application of OBs dispersion as a naturally pre-emulsified oil-in-water emulsion is a research hotspot. It can be considered as an alternative to animal fat in the development of healthier meat products to reduce fat percentage and improve fatty acid profile. However, its potential allergenic behavior and toxicity cannot be ignored if put into actual production and *in vitro* and *in vivo* studies are needed to take care of the associated side effects. It is also worth exploring the important functions of individual OBs components.

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