A Brief Overview of Beverage Emulsions for Fortification

Asieh Habibi, Sebahattin Serhat Turgut, Erkan Karacabey, and Erdogan Kucukoner
Department of Food Engineering, Faculty of Engineering, Suleyman Demirel University, Isparta, Turkey
Email: habibi_asieh@yahoo.com, {serhatturgut, erkankaracabey, erdogankucukoner}@sdu.edu.tr

***Abstract*—There has been increasing trend in beverage consumption especially soft drinks and functional beverages. The direct use of hydrophobic compounds in aqueous medium like beverages is in limited extent due to solubility problems. On the other hand, addition of hydrophilic compounds directly to an aqueous media leads to loss and changing structure of compounds during processing or storage. Thus, encapsulating bioactive compounds through microemulsion, nanoemulsion or emulsion compounds is an appropriate approach to combination and preservation of bioactive ingredients in beverages. This brief overview provides information about the ingredients used in beverage emulsions and researches have been carried out with high potential usage for fortification in beverage industry.**

***Index Terms*—nutraceuticals, emulsifier agents, encapsulation, aqueous based product.**

I. Introduction

Fortification of beverages with nutraceuticals leads to enhance the nutritional value of drink which results in improvement in its contribution to the diet. Addition of hydrophobic ingredients into beverages provides some technological challenges for producers [1]-[3]. Also, other bioactive compounds added to beverages undergo some structural changes during processing and storage. Thus, encapsulating hydrophobic and other bioactive compounds as small particles suspended within an aqueous medium, microemulsion, nanoemulsion, or emulsion, facilitate usage of these ingredients in aqueous-based products and protect them during processing and storage [2], [4].

The emulsion application in beverages is classified into two categories as flavor and cloud emulsions. The primary group contains lipophilic compounds and that is used to provide aroma and taste to beverages (such as lemon, lime, or orange oils). However, the second group increases the turbidity of beverage products and cloudiness. Cloudy appearance of emulsion leads to hidden sedimentation and ringing and it incorporates to beverages that contain the low proportion of juice [2], [3], [5].

Microemulsion and nanoemulsion in food industry provide some advantages such as good physical stability, high optical clarity and increasing bioavailability. Self-assembled mixture of water, oil, surfactant and sometimes co-surfactant and co-solvent creates microemulsions. Microemulsion is thermodynamically stable and transparent when it contains lipid type particles (5nm<r <100 nm). On the other hand, nanoemulsions, composed of oil, surfactant, and water, are thermodynamically unstable. Small particle sizes (r<100 nm) in oil in water nanoemulsions have a tendency to produce transparent or slightly turbid emulsion [4]. On the other hand, the phenomenon such as gravitational separation, flocculation, coalescence and Ostwald ripening contribute to phase separation in emulsion [2], [3], [5]. Some advantages such as the stability of nanoemulsion to gravitational separation and droplet aggregation accompanied by increasing shelf life product, and enhanced oral bioavailability of lipophilic compounds lead to preference of nanemulsion usage in food or beverage products [6], [7].

High and low energy methods are two substantial strategies to prepare nanoemulsions. Mechanical devices, which produce intense shear force in order to split oil droplets into smaller ones and to mix it with water phase, are used in high energy method. The mentioned devices may be classified as high-speed mixers, microfluidizers, homogenizers, and ultrasonicators. Low- energy technique is defined as the spontaneous formation of oil droplets in the surfactant-oil-water mixture under specific conditions (temperature, composition, stirring). Low energy method has some privileges compared to high-energy method. These benefits are attributed to the low cost of operation and equipment, high energy efficiency and simple implementation. Low energy technique includes some preparation methods such as phase inversion temperature (PTT), Spontaneous Emulsification (SE), Phase Inversion Composition (PIC), and Emulsion Phase Inversion (EPI) [6].

This review presents how and which different functional ingredients are commonly preferred for emulsion preparation considering up to date studies regarding the subject especially about potential usage these compounds in the beverage industry.

II. Emulsifier and Stabilizer Agents in Emulsions

A. Polysaccharide-based Emulsifiers

Beverage emulsions, as a unique group of emulsions, are consumed in a highly diluted form compared to their original concentrated form. Dilution of emulsion

Manuscript received April 28, 2019; revised August 10, 2019.

doi: 10.18178/ijfe.5.3.175-182

concentrates in sugar solution may decrease the emulsion stability. Thus, it can be said that application of hydrocolloids as emulsifiers and stabilizers such as gum Arabic in beverage emulsions has some detrimental effects on stabilization of the emulsion. Here, electrostatic (repulsion), van der Waals (attractive) and polymeric steric (repulsing) interactions are known to have a key role on the stability of diluted form of beverage emulsions [5], [8].

Acacia Senegal produces around 80% of gum acacia, whereas acacia seyal supplies approximately 10-15% of gum acacia. The sensitivity of two gum producing species (Senegal and Seyal) in different treatments was significantly various. Despite high molecular mass (~ 400,000 Da), it exhibits great emulsifying properties and low viscosity. The surface activity properties of gum Arabic is attributed to arabinogalactan segments attached to a polypeptide backbone [2], [8]. The stability of aqueous phase is mainly increased by steric repulsion and some contribution of electrostatic repulsion against aggregation [9].

There are some factors which influence emulsifying and rheological properties of gum acacia in beverage emulsions. Minerals decrease the stability of emulsion because of electrostatic screening effect. Both, pasteurization and demineralization relatively increases stability due to probable alteration of protein unfolding and removing the screening effect. Higher emulsion stability was obtained between pH 4.5-5.5 compared to pH 2.5. Gum demineralization led to increasing the viscosity of emulsion while the opposite was observed as a result of pasteurization treatment [8].The application of gum Arabic as an emulsifier is limited in beverage owing to a relatively high concentration of gum Arabic requirement to prepare the stable emulsion, and the reliable source to obtain high-quality gum Arabic [2], [10], [11].

Starches have a poor surface activity inherently because of existing hydrophilic glucose fraction. However, chemical modification is a method to overcome these limitations in which non-polar chains attach along their backbones to produce effective emulsifiers. These kinds of modified starches are extensively used in the beverages. The most prevalence usage of modified starches is as an octenyl succinate isolated from waxy maize. The emulsions stabilized by modified starch exhibit resistance to pH, temperature, and ionic strength changes being in between 3 to 9, 30 ℃ to 90 ℃ and 0- 100 mM NaCl, 0-25 mM CaCl2, respectively [2], [9]-[11]. The use of gum Arabic and modified starch emulsifier have been suggested to stabilize emulsions in orange terpene-based beverages at temperature of 25 ℃ and 35 ℃ [12].

The numerous surface–active polysaccharides used in the beverages as emulsifiers in emulsion (o/w). Beet pectin, soy soluble polysaccharide (SSPs) derived from Okara[2], [13], zein as insoluble biopolymer in water [14] and corn fiber gum which derived from milling process can increase the stability of oil-in-water emulsions [15]. Also, modified cellulose utilization in beverage emulsion showed a good stability between pH range of 2-11, for freeze-thaw cycle, and salt existence [2].

B. Protein-based Emulsifiers

There are various protein sources including meat, fish, milk, plants which have emulsifying properties. The major mechanism to inhibit droplet flocculation in protein–stabilized emulsions are electrostatic repulsion rather than steric repulsion derived from a thin interfacial coating that electrically charged. In addition, flocculation occurs at pH value close to their isoelectric points and excessive ionic strength at a certain level [16], [17].

The lower quantity of protein requirement compared to amphiphilic polysaccharides to stabilize o/w emulsion is one of the advantages of protein originated emulsifiers [18]. Since emulsions stabilized only by proteins are much more sensitive to pH, ionic strength and thermal processes than polysaccharides, formation interfacial complex between proteins and polysaccharides would be alternative to solve stability problems by increasing electrostatic and steric repulsion between droplets [2].

C. Globular Proteins

Whey protein, soy, pea and other plants are some sources of globular proteins having surface activity. A number of commercial whey proteins including whey protein concentration (WPC), whey protein isolate (WPI) and highly purified protein fraction such as β-lactoglobulin and α-lactalbumin are widely used in food and beverage industry. Whey proteins consisting of a combination of globular proteins: ~ 55% β-lactoglobulin; ~ 24% α-lactalbumin; ~ 15% immunoglobulin; and ~ 5% serum albumin [2]. The major whey proteins have isoelectric point around pH 5. Hence, the pH range 4-6 and also in isoelectric point (pH 5) increase the tendency of droplets to be aggregated. In addition, the denaturized whey proteins are formed at 70-80 ℃, so exposing to the temperature above the denaturation temperature accelerates droplet aggregation especially in the presence of salts that hides electrostatic interaction [2], [16], [19].

β-lacto globulin (BLG) has a molecular weight of 18.3 kDa and 162 amino acids with two disulfide bridges and a free thiol group at Cys121 that is not available to the solvent at or below neutral pH. This results in stability of BLG at low pH and its high resistance to proteolytic degradation in the stomach. Heat treatment of BLG stimulates aggregation phenomenon by the interchange of a chain-reaction of sulfhydryl-disulfide [20], [21]. α -lactalbumin, is the second most predominant whey proteins in cow milk that exhibits a smaller globular metalloprotein with four disulfide bridges. The privilege of using BLG compare to α –lactalbumin for nanoparticles preparation is ascribed to small molecular weight, highly unfolding and less hydrophobic proteins properties [20].

Lactoferrin is a globular glycoprotein derived from transferrin family existing in numerous mammalian secretion fluids. The molecular weight of glycoprotein in lactoferrin is 80 kDa. Lactoferrin illustrates the isoelectric point at pH 8, which leads to positively charged at both acidic and neutral pH. The primary emulsion containing lactoferrin (LF) coated corn oil droplet and produced gels when exposed to the temperatures over 70 ℃. The apo (iron free) form of lactoferrin is more heat sensitive than the halo (iron saturated) state, which prefers to produce large insoluble aggregates as exposing to its thermal denaturation temperature [22].

D. Flexible Proteins

The main sources of flexible proteins are gelatin from fish and animals, and caseins from bovine milk [5]. Gelatin derived from an animal collagen consists of a relatively high molecular weight protein prepared by heat treatment in the presence of either acid (Type A, PI ~ 7-9) or alkaline (Type B, PI~ 5). Because of a high isoelectric point of type (A) gelatin, it would be suitable for preparation of oil-in-water emulsions with positively charged droplets. Also, it shows high oxidative stability by warding off iron ions from the surface of oil droplet during varied pH range [23]. Gelatin typically produces relatively large droplet sizes, so it may be used with other ingredients to enhance its efficiency for preparing stabilized emulsion [2].

The most different casein based emulsifier utilized commercially is sodium caseinate, calcium caseinate and purified protein fractions. Droplet aggregation occurs between pH 3.5-5.3 when casein is used as coating droplets. Nevertheless, stabilized emulsion with caseinate would be more stable to heating than that stabilized by whey protein due to the relatively flexible casein molecules structure which does not change as much as globular protein do when exposing to heat treatment [2].

E. Small-molecule Surfactant

In general, small-molecule surfactants consist of a polar head-group and a non-polar tail group. The head group could be anionic, cationic or non-ionic, whereas the tail group may be different in the number, length, and degree of unsaturation of the chains. Surfactant usage has some merits compared to amphiphilic biopolymers. These advantages include fast adsorption of surfactant to the oil- water interfaces, producing smaller oil droplets, require lower concentration to stabilize emulsion, and their applicability to both low and high energy method [2], [24].

Tweens (polysorbates) are synthetic non-ionic surfactants that contain non-polar fatty acid group esterified to a polar polyoxyethylene sorbitan group. The functional properties of tweens depend on the nature of the fatty acid and polyoxyethylene. Water soluble molecules found in Tween 20 (monolaurate), Tween40 (monopalmitate), Tween 60 (monostearate) and Tween 80 (monooleate) can stabilize oil-in-water emulsion with relatively high Hydrophilic-Lipophilic Balance (HLB) index (14.9 and 16). Although they can generate stable droplets against aggregation during a wide range of pH and ionic strength values, they are likely to become unstable to coalescence at high temperature near to phase inversion temperature [2]. Smaller dimensions of surfactant (Tween 20) micelles hydrophobic core compared to Tween 60 or Tween 80 led to less solubilizing lemon oil in emulsion [25].

Binding different numbers and types of fatty acids to the sucrose segment generate various sucrose esters with a varied HLB values. The advantages of sugar esters utilization in emulsions are ascribed to low toxicity, high biodegradability as they are derived from natural sources (sucrose and vegetable oil) and desirable sensory attributes [26].

A natural food-grade surfactant derived from the bark of Quillaja Saponaria Molina tree contains saponins as major components. Saponins have high molecular weight glycosides consisting of sugar segment attached to a triterpene or a steroid aglycone. Saponins are surface active components due to having both hydrophilic regions and hydrophobic segments on the same molecule [27]. Quillaja saponin, as a replacement of gum acacia, was released under the trade name Q-Naturale from Natural Starch Company in 2008. Quillaja saponin can be effective at a low level, high oil load and good stability in some forms of alcoholic beverages, contrast to most forms of modified starch and gum Arabic. Moreover, it is used as a foam stabilizing agent in beverages [2].

III. Entrapment of Bioactive Compounds Through Carriervehicles to Prepare Emulsions

There are various lipophilic or hydrophilic components that probably present in or combined to beverage formulations for fortification. These substances undergo some physicochemical degradation during process and storage. The following parts present some experiences as for entrapment of nutraceutical components in order to preserve them in beverages.

A. Pigments

Carotenoids are the natural group of pigments derived from fruit, vegetables constituting 40 carbon molecules and multiple conjugated double bonds. Carotenoids are classified into two groups: the first group is carotenes containing carbon and hydrogen, α-carotene, β-carotene, lycopene and second group including lutein, zeaxanthin and xanthophylls consisting carbon, hydrogen, and oxygen. However, the use of carotenoids is restricted due to a strong color, its susceptibility to chemical degradation during processing, poor water solubility, and low bioavailability [7], [28].

β-carotene stabilization during processing is an important goal in food fortification. Reassemble nanomicelles of casein was formed to encapsulate β-carotene, with the particle size distribution of 80 nm and - 34mV zeta potentials, showed the protective effect against deterioration during sterilization, pasteurization, high hydrostatic pressure treatment and baking [28]. Moreover, globular protein like β-lactoglobulin is more effective to prevent degradation of β-carotene compared to those stabilized by Tween 20 [7].

Protein-polysaccharide conjugates are produced by Maillard reaction base on Amadori rearrangement. β-carotene emulsions stabilized by conjugated whey protein isolate-beet pectin (WPI-beet pectin) exhibited much smaller droplet sizes, more homogenous droplet size distribution, enhanced freeze-thaw stability, less alteration in centrifugal transmission profile and lower β-carotene loss in emulsion over storage compared to emulsions stabilized by WPI alone and WPI-beet pectin mixtures without heat treatment [13].

Lycopene as natural carotenoids was emulsified in orange oil-in-water beverage emulsions by utilizing pure whey isolate powder as emulsifier agent. Physical stability of beverage during chilled storage was improved by preventing Ostwald ripening when corn oil was added as carrier oil [29]. In another study, lutein fortified emulsions prepared by Quillaja saponin as an emulsifier accompanied by ascorbic acid showed higher overall stability compared to Tween 80, whey protein and casein during storage for ten days at 45 ℃ [30]. Low molecular weight chitosan (LMWC) as a new carrier can improve lutein bioavailability in food and pharmaceutical application [31].

Anthocyanins belong to the flavonoid group of phytochemical used as a natural ingredient due to their high potential health benefits and desirable color. The interaction between glycoprotein fraction of gum Arabic and anthocyanins from purple carrot create hydrogen bonds resulting in enhanced anthocyanin stability [32]. Procyanidins are known as oligomers and polymers of flavan-3-ols. The extracted cranberry procyanidins (CPS) was encapsulated within zein protein as a carrier. Raising CPS–zein concentration ratio, led to increasing nanoparticle size from 392 nm to 447nm [14].

Betalains are natural pigments derived from cactus pear (Opuntia spp.) as Native American fruits. The encapsulated betanins using gelatin-maltodextrin (G:M) mixture with ratio of 2.5:7.5 demonstrated high antioxidant activity, a spherical shape of microparticle (6.14 μm) with a range of melting points from 205 ℃ to 235 ℃ [33].

B. *Lemon Oil*

Sucrose monoesters are widely used in beverage emulsions. Lemon oil nanoemulsion with small mean particle diameters (d~81 nm), stabilized by sucrose monoesters, illustrated an appropriate stability during storage for 1 month at room temperature. Sucrose monoesters are highly unstable to acidic conditions. Increasing temperature, the addition of co-solvent (glycerol and propylene glycol) and decreasing Iysolecithin concentration as a co-surfactant lead to droplet growth resulted from phase inversion temperature [34]. Entrapping orange peel oil (limonene), which is flavoring ingredient, with whey protein-pectin nanocomplexes had shown a high encapsulation efficiency (~88%) at pH 3 [35].

The type of oil used in emulsions has a pronounced effect on emulsion stability. For instance, lemon oil was formed transparent and stable microemulsion at a high ratio of surfactant to oil, while vitamin D and vitamin E did not have capabilities to form microemulsions due to relatively large molecular dimensions of vitamin oil than the hydrophobic core of micelles. On contrary, lemon oil emulsions exhibited lower stability to droplet growth which ascribed to Ostwald ripening effects [25].

Maximum solubilization capacity (Csat) is a substantial criterion to determine condition for micro and nanoemulsion formation. Sucrose monopalmitate (SMP) exhibited a higher Csat value of lemon oil compared to Tween 80 due to different molecular geometries. The acid stability of colloidal dispersions would be enhanced by the combination of SMP with Tween 80. Transparent microemulsion consisting of swollen micelles will be formed if total lemon oil concentration is lower than Csat [4]. In another research, an acidic beverage emulsion was stabilized by sucrose monopalmitate (SMP) and anionic Iyso- lecithin was suggested thanks to its high stability and physicochemical performance [26].

C. *Lipophilic Vitamins*

Flavored beverages are often acidified and subjected to heat processing to assure shelf stability. The effect of the thermal process on vitamin E encapsulation stabilized by whey protein in orange oil-in-water emulsion showed that heat treatment has a substantial beneficial effect on emulsion stability. The high-temperature treatment for a short period of time (90 ℃ for 45 s) exhibited the highest stability after 28 days at 4 ℃. The loss of vitamin E for all heated beverage was considerably low [36].

Furthermore, low-energy method (emulsion phase inversion, EPI) is another emulsion preparation technique in which water-in-oil emulsion is formed when water is titrated into the organic phase (containing a mixture of oil and hydrophilic surfactant). Subsequently, a multiple emulsion and the oil-in-water emulsion are prepared under suitable conditions. Low-energy method (EPI), was used to incorporate vitamin E into aqueous-based foods and beverage products. This technique helps to provide stable oil-in-water nanoemulsions [6].

Lecithin and Q-Naturale, as a natural small molecule surfactant, were used to prepare nanoemulsion containing vitamin E in the oil phase. Both nanoemulsions stabilized by Q-Naturale and lecithin showed good thermal stability (30-90 ℃ for 30 min). Nanoemulsions stabilized by Q-Naturale were stable over a various range of pH values (pH 3-8) and salt concentration (0-300 mM NaCl) [37]. Incorporation of medium chain triglyceride (MCT, ≥ 20 %) prior to homogenization in order to prepare vitamin E emulsions led to small droplet formation. Q-Naturale emulsifier showed the ability of forming small particles in emulsion (d<400nm) from oil phase consisting high level of vitamin E (60-80%) [27]. Beverages such as water or apple juice were fortified with vitamin E (15%) using high–pressure homogenization and a food-approval starch sodium octenyl succinate did not form any ringing and creaming during 6 month storage at room temperature. The average nanoparticle emulsion was found to be around 100 nm [38].

In terms of beverage fortification with vitamin D, it is should be mentioned that vitamin D should be solved in a suitable oil type carrier before incorporation into nanoemulsion because of their crystalline form at room temperature. The factors such as surfactant type, surfactant quantity and stirring conditions contribute to initial size of the droplets in nanoemulsions. Nanoemulsions prepared by spontaneous emulsification (SE) method formed small droplet diameters (d<200 nm) using tween 80 (non-ionic surfactant) at a surfactant-to-oil ratio (SOR) $\geq$ 1:1 at high stirring speed (800 rpm). These systems exhibited stabilization of droplet growth at room temperatures (<10% in diameter after one month storage) though they were unstable to heat treatment (T>80 ℃). Addition of co-surfactant (sodium dodecyl sulfate SDS) would be increased thermal stability of nanoemulsion [39].

D. Fatty Acids

The sensitivity of omega-3 fatty acids to oxidation has an unacceptable effect on functionality, consumer acceptability and safety of the enriched food products [40]. Whey protein addition to fish oil emulsion increased the electric repulsion at pH 6.8 leading to alteration in the emulsion stability, so it would be appropriate for omega-3 fatty acids to be incorporated into milks and milk containing drinks. On contrary, stabilization of fish oil emulsion at pH 3.4 was achieved by the addition of fish gelatin into emulsion. Whey protein-Fish oil gelatin WPI- FG emulsions exhibited higher physicochemical stability compared to emulsions stabilized by individual proteins [23]. Enrichment of fruit juice was carried out by addition of nano complex fish oil containing 40, 50 and 60 mg EPA/DHA. The optimum condition to prepare stable complex was determined as 0.1g/100ml sodium caseinate and 0.2g/100ml gum Arabic at pH 4. The entrapped oil and particle size in sodium caseinate/gum Arabic complex were obtained as 78.88% and 232.3 nm, respectively [41]. In another study, fortification of pomegranate juice using fish oil (gelatin-gum Arabic) coacervates did not show any significant increase in mean particle diameter of enriched juice after 42 days storage at 4 ℃ [42].

The use of nanoparticles is another method preferred to stabilize emulsion identified as Pickering emulsions. The main advantage of particle used in products is irreversible adsorption of nanoparticles at the oil-water interface comparing to high dynamic interfacial properties of the biopolymer emulsifiers. Synthetic particles including organic and inorganic ones and also biological particles can be utilized in emulsion stabilization. For examples, silica nanoparticles stabilized emulsion illustrated a higher stability rather starch and tween 20 for beverage application purpose [24].

The stabilization of corn oil- in-water was carried out by using combination of lactoferrin (LF) (pI~8) and BLG (pI~5). The usage of this combination showed an appropriate electrostatic attraction at pH Mixed interfacial coatings provide an appropriate pH stability, thermal processing, and salt stability [22]. Moreover, the corn oil-in-water emulsion stabilized by lactoferrin did not show droplet aggregation at low pH values (pH$\leq$ 6). Emulsions at pH 3 were found to be stable to the high level of salt (200 mM NaCl or $CaCl_2$). The pH of the solution and counter-ion type was reported as two influential factors in terms of emulsion stability to salt addition [43]. Combination of lactoferrin and caseinate exhibited better physical stability in omega-3 rich oil emulsion against pH changes and salt addition (pH 3–7, 0–50 mM CaCl2 at pH 7) than those containing only caseinate (pH 5–7, 0–2 mM CaCl2 at pH 7) [44].

Biopolymer type and pH have pronounced effect on the electrical charge on the biopolymer-coated lipid droplets. Rice bran oil emulsion stabilized by modified starch (MS) and WPI had higher stability during storage at temperature of 37 ℃ up to 20 days against lipid oxidation than those stabilized by gum Arabic (GA) at both pH 3 and 7 [45].

E. Phenolic Compounds

Epigallocatechin-3-gallate (EGCG) is one of the major polyphenolic components found in green tea [46]. Green tea products accompanied by high level of EGCG and other catechins are a preventative measure for cardiovascular disease, cancer, obesity, neurodegenerative disease [21]. Also, they exhibit antimicrobial and anti-inflammatory properties [47]. The most important factors should be controlled in order to prevent water-soluble components of EGCG from decomposition/degradation are adjustment of alkaline and neutral condition, oxygen concentration, and temperature. Also, the loss of green tea catechins after one month of storage was obtained at least 50% in commercial soft drinks. EGCG has produced a colorless aqueous solution. When it dissolved freshly, the oxidation and dimer formation lead to its degradation to a yellowish-brown solution over the time. Thus, it is essential to design a proper formulation to prevent undesirable browning in green-tea beverages in order to improve their stability. Addition of EGCG to the preheated β-lactoglobulin solution (78-85 ℃ for 20 min) was an effective approach for nano-entrapment of EGCG. Application of this kind of nano complex in clear beverage due to excellent transparency and particle size smaller than 50 nm was recommended. Nano encapsulation of EGCG showed 3.2 fold slower degradation during 8 days compared to free EGCG [21].

Gum Arabic – Maltodextrin complex prepared by using homogenization and spray drying method was effective ways for encapsulation of EGCGs. Loading efficiency, spray dried particle and mean average diameters of EGCG in aqueous suspension were reported as 96%, 20 µm, 40 nm and 400 nm, respectively. The antioxidant properties of EGCG remained through carbohydrate complex [47].

Formation of protein-polysaccharide conjugates such as casein (CN) – maltodextrin (MD) accompanied by Maillard reaction was used appropriately for nanoencapsulation of vitamin D as a hydrophobic and EGCG (hydrophilic nutraceuticals), respectively. Protection of these bioactive compounds was improved against oxidation and low pH by conjugation. At vitamin D: casein ratio of 3:1, the 100% of the protein-polysaccharide conjugates were smaller than 100 nm [48].

Plant curcumin Longa.C.longa extracts is the substantial component of turmeric spices used in Indian cooking [46], [49]. Nanoemulsion encapsulation of curcumin is an important implication in formation and design of encapsulated bioactive compounds, especially polyphenols. Whey protein concentrates 70% and tween 80 were used as emulsifiers to encapsulate curcumin dissolved in medium chain triglyceride oil droplets by using ultra sonication. The average particle size and zeta potential in prepared nanoemulsion were found as 141.6 nm and 6.9 mV, respectively. The prepared curcumin nanocapsules exhibited good stability during pasteurization, a wide range of pH values from 3.0 to 7.0 and various ionic strengths (0.1-1 µm) [50].

IV. Application of Some Techniques to Determine Emulsion Stabilization

Beverage manufactures define some standardized criterias to accept a special bache of product that has appropriate physicochemical properties. Particle size distribution and zeta potential measurement are two of the most predominant parameters that have a key role in emulsion stabilization [2]-[5], On the other hand, microstructure assessment of emulsions can provide valuable information to figure out the changes occurred in the system. Some apparatus used for this purpose are Optical Microscopy (OM), confocal laser scanning microscopy (CLSM), Confocal Raman Microscopy (CRM), scanning and transmission electron microscopy (SEM and TEM), respectively or atomic force microscopy (AFM). Selecting the suitable technique depends on the physical state and the information need to know. The CLSM method is the most popular method to consider the structure of food emulsion systems. It provides cross-sectional localization of exclusively labeled components (protein, lipids) or overall 3-dimensional structure of oil particles in the emulsion. Also, information about morphology of emulsion, overall 3-dimensional structure of oil droplets, and localization of individual components like clustering of minerals can be achieved by using other microscopic methods (CRM, SEM, OM, AFM, and TEM [1].

A. Particle Size Distribution Measurement

The optimum particle size distribution is an important parameter to produce stable emulsions against gravitational separation, flocculation, coalescence, and Ostwald ripening. The particle size distribution (PSD) of an emulsion is measured as the concentration of droplets within different size classes using a commercial instrument. Reporting data and its interpreting on a beverage emulsion is a predominant factor to formulate emulsion. The concentration of particles within a particular size class is reported as either volume or number percent, while the size of the particles in a special size category is reported as either the mid-point particle radius or diameter. The most three values to report mean particle sizes are the volume-weighted mean diameter (dV or d43 = Σnidi4/ Σnidi3), surface-weighted mean diameter (dS or d32= Σnidi3/ Σnidi2), and the number weighted mean diameter (dN or d10 = Σnidi/ Σni). The volume- weighted mean diameter is more susceptible to the existence of large particles than the number-weighted mean diameter. If the measured d43 is small, then it means that the emulsion would remain stable. The differences between mentioned three values can represent the broad or multimodal particle size distribution. Polydisperse systems of beverage emulsions are "monomodal", "bimodal" or "multimodal" depends on the number of peaks (one, two, or more peaks) in the particle size distribution. Monmodal distribution provides narrow range particle size with the best long-term stability [2], [3], [5].

B. Droplet Charge

Adsorption of ionic species to droplet surfaces leads to electrical charge of droplets in beverage emulsion. Some factors such as ionic composition, physical properties of the surrounding aqueous phase, type, organization and concentration of the ionized species have detrimental effect on electrical properties of a droplet surface. Measuring electrical charge on the oil droplet in beverages has some benefits: assessing droplet stability to aggregation, determining electrostatic interaction, and the droplets manner interaction with electrical charges surfaces (such as bottles, mouth, storage vessels). Zeta-potential is a suitable approach to measure electrical characteristics of an oil droplet due to accounting the adsorption of any counter ions or ionic species to the droplet surface. Generally, zeta-potential is measured versus pH under suitable defined measurement condition [2], [3]. Zeta-potential is mainly affected by chemical nature of the polymer stabilizing agent and the pH value of medium [51].

V. Conclusion

Because of increasing obesity, cancer or other diseases consumers are changing their criteria/habits while choosing foods. Over the recent years, there has been increasing demands to consume the functional foods including beverages. Thus, the beverage manufacturers have been presenting new products to market in order to meet the consumer needs. Fortification of beverages with nutraceuticals, substitution natural ingredients to artificial components and using functional materials with highly beneficial health effects properties can increase marketing in the beverage industry. Optimization of formulation and condition are obligatory to produce stable emulsions. Ingredients and processing conditions are chosen depends on the expected physicochemical characteristics at finished products. Fortification along with suitable optical properties especially for opaque beverages is important to obtain a high quality product. Obtaining beverage emulsions with small particle size to produce clear fortified beverage can be achieved by selecting suitable coating materials and defined the optimum condition to prepare a stable emulsion. However, the combination of hydrophobic compounds to beverages has still remained the main challenge to fortification beverage. Thus in the present study, the potential use of some ingredients and factors for fortified beverages was considered. It can provide some ideas to manufacturers and researchers to produce new products with high beneficial health effects.

REFERENCES

[1] K. P. Drapala, D. M. Mulvihill, and J. A. O'Mahony, "A review of the analytical approaches used for studying the structure, interactions and stability of emulsions in nutritional beverage systems," *Food Structure*, vol. 16, pp. 27-42, 2018.

[2] D. T. Piorkowski, and D. J. McClements, "Beverage emulsions: Recent developments in formulation, production, and applications," *Food Hydrocolloids*, vol. 42, pp. 5-41, 2014.

[3] S. Friberg, K. Larsson, and J. Sjoblom, *Food Emulsions*, 4 ed, New York: Marcel Dekker, 2004, ch. 12, pp. 493-532.

[4] J. Rao and D. J. McClements, "Lemon oil solubilization in mixed surfactant solutions: Rationalizing microemulsion and nanoemulsion formation," *Food Hydrocolloids*, vol. 26, pp. 268-276, 2012.

[5] D. J. McClements, *Food Emulsions: Principles, Practice, and Techniques*, 2 nd ed, Boca Raton: CRC Press, 2005, ch. 1, pp. 8-19.

[6] S. Mayer, J. Weiss, and D. J. McClements, "Behavior of vitamin E acetate delivery systems under simulated gastrointestinal conditions: Lipid digestion and bioaccessibility of low-energy nanoemulsions," *Journal of Colloid and Interface Science*, vol. 404, pp. 215-22, 2013.

[7] C. Qian, E. A. Decker, H. Xiao, and D. J. McClements, "Physical and chemical stability of ß- carotene-enriched nanoemulsion: Influence of pH, ionic strength, temperature, and emulsifier type," *Food Chemistry*, vol. 132, pp. 1221-1229, 2012.

[8] R. A. Buffo, G. A. Reineccius, and G. W. Oehlert, "Factors affecting the emulsifying and rheological properties of gum acacia in beverage emulsions," *Food Hydrocolloids*, vol. 15, pp. 53-66, 2001.

[9] R. Chanamai and D. J. McClements, "Comparison of gum arabic, modified starch, and whey protein isolate as emulsifiers: Influence of pH, CaCl2 and temperature," *Journal of Food Science*, vol. 67, no. 1, pp. 120-125, 2002.

[10] R. Charoen, *et al*., "Influence of biopolymer emulsifier type on formation and stability of rice bran oil-in-water emulsions: Whey protein, gum arabic, and modified starch," *Journal of Food Science*, vol. 76, no. 1, pp. 165-172, 2011.

[11] C. Qian, E. A. Decker, H. Xiao, and D. J. McClements, "Comparison of biopolymer emulsifier performance in formation and stabilization of orange oil-in-water emulsions," *Journal of the American Oil Chemists Society*, vol. 88, no. 1, pp. 47-55, 2011.

[12] S. J. Reiner, G. A. Reineccius, and T. L. Peppard, "A comparison of the stability of beverage cloud emulsions formulated with different Gum Acacia- and Starch – based emulsifiers," *Journal of Food Science*, vol. 75, pp. 236-246, 2010.

[13] D. Xu, X. Wang, J. Jiang, F. Yuan, and Y. Gao, "Impact of whey protein-beet pectin conjugation on the physicochemical stability of ß-carotene emulsions," *Food Hydrocolloids*, vol. 28, pp. 258-266, 2012.

[14] T. Zou, Z. Li, S. S. Percival, S. Bonard, and L. Gu, "Fabrication, characterization, and cytotoxity evaluation of cranberry procyanidins-zein nanoparticles," Food Hydrocolloids, vol. 27, pp. 293-300, 2012.

[15] M. P. Yadav, D. B. Johnston, A. T. Hotchkiss, and K. B. Hicks, "Corn fiber gum: A potential gum arabic replacer for beverage flavor emulsification," *Food Hydrocolloids*, vol. 21, no. 7, pp. 1022-1030, 2007.

[16] E. Dickinson, "Flocculation of protein-stabilized oil-in-water emulsions," *Colloidals and Surfaces B: Biointerfaces*, vol. 81, no. 1, pp. 130-140, 2010.

[17] D. J. McClements, "Protein-stabilized emulsions," *Current Opinion in Colloid & Interface Science*, vol. 9, no. 5, 305-313, 2004.

[18] R. Chanamai and D. J. McClements, "Comparison of gum arabic, modified starch, and whey protein isolate as emulsifiers: Influence of pH, CaCl2 and temperature," *Journal of Food Science*, vol. 67, no. 1, pp. 120-125, 2002.

[19] H. J. Kim, E. A. Decker, and D. J. McClements, "Role of postadsorption conformation changes of beta-lactoglobulin on its ability to stabilize oil droplets against flocculation during heating at neutral pH," *Langmuir*, vol. 18, no.20, pp. 7577-7583, 2002.

[20] A. O. Elzoghby, W. M. Samy, and N. A. Elgindy, "Protein-based nanocarriers as promising drug and gene delivery systems," *Journal of Controlled Release*, vol. 161, pp. 38-49, 2012.

[21] A. Shpigelman, G. Israeli, and D. Livney, "Thermally –induced protein-polyphenol co-assemblies: Beta lactoglobulin-based nanocomplexes as protective nanovehicles for EGCG," *Food Hydrocolloids*, vol. 24, pp. 735-743, 2010.

[22] T. Schmelz, U. Lesmes, J. Weiss, and D. J. McClements, "Modulation of physicochemical properties of lipid droplets using ß-lactoglobulin and or lactoferrin interfacial coatings," *Food Hydrocolloids*, vol. 25, pp. 1181-1189, 2011.

[23] A. R. Taherian, M. Britten, H. Sabik, and P. Fustier, "Ability of whey protein isolate and /or fish gelatin to inhibit physical separation and lipid oxidation in fish oil-in-water beverage emulsion," *Food Hydrocolloids*, vol. 25,pp. 868-878, 2011.

[24] A. Chaudhari, Y. Pan, and N. Nitin, "Beverage emulsions: Comparison among nanoparticle stabilized emulsion with starch and surfactant stabilized emulsions," *Food Research International*, vol. 69, pp. 156-163, 2015.

[25] K. Ziani, Y. Fang, and D. J. McClements, "Encapsulation of functional lipophilic components in surfactant-based colloidal delivery systems: Vitamin E, vitamin D, and lemon oil," *Food Chemistry*, vol. 134, no. 2, pp. 1106-1112, 2012.

[26] S. J. Choi, *et al*., "Formulation and properties of model beverage emulsions stabilized by sucrose monopalmitate: Influence of PH and Lyso-Lecithin addition," *Food Research International*, vol. 44, pp. 3006-3012, 2011.

[27] Y. Yang and D. J. McClements, "Encapsulation of vitamin E in edible emulsions fabricated using a natural surfactant," *Food Hydrocolloids*, vol. 30, pp. 712-720, 2013.

[28] M. J. S. Abajo, C. González-Ferrero, A. Moreno-Ruiz, A. Romo-Hualde, and C. J. González-Navarro, "Thermal protection of b-carotene in re-assembled casein micelles during different processing technologies applied in food industry," *Food Chemistry*, vol. 138, pp. 1581-1587, 2013.

[29] E. Meroni and V. Raikos, "Formulating orange oil-in-water beverage emulsions for effective delivery of bioactives: Improvements in chemical stability, antioxidant activity and gastrointestional fate of lycopene using carrier oils," *Food Research International*, vol. 106, pp. 439-445, 2018.

[30] F. Weigel, J. Weiss, E. A. Decker, and D. J. McClements, "Lutein- enriched emulsion-based delivery systems: Influence of emulsifiers and antioxidants on physical and chemical stability," *Food Chemistry*, vol. 242, pp. 395-403, 2017.

[31] R. Arunkumar, K. V. H. Prashanth, and V. Baskaran, "Promising interaction between nanoencapsulated lutein with low molecular weight chitosan: Characterization and bioavailability of lutein in vitro and in vivo," *Food Chemistry*, vol. 141, pp. 327-337, 2013.

[32] C. Chung, T. Rojanasasithara, W. Mutilangi, and D. J. McClements, "Enhancement of colour stability of anthocyanins in model beverages by gum Arabic addition," *Food Chemistry*, vol. 15, no. 201, pp. 14-22, 2016.

[33] R. C. Munoz, B. E. Barragan-Huerta, and J. Y. Fernandez, "Use of gelatin-maltodextrin composite as an encapsulation support for clarified juice from purple cactus pear (Opuntia stricta)," *Food Science and Technolgy*, vol. 62, pp. 242-248, 2015.

[34] J. Rao and D. J. McClements, "Optimization of lipid nanoparticle formation for beverage applications: Influence of oil type, cosolvents, and cosurfactants on nanoemulsion properties," *Journal of Food Engineering*, vol. 118, pp. 198-204, 2013.

[35] S. Ghasemi, S. M. Jafari, E. Assadpour, and M. Khomeiri, "Production of pectin-whey protein nano-complexes as carriers of orange peel oil," *Carbohydrate Polymer*, vol. 117, pp. 369-377, 2017.

[36] V. Raikos, "Encapsulation of vitamin E in edible orange oil-in-water emulsion beverages: Influence of heating temperature on physicochemical stability during chilled storage," *Food Hydrocolloids*, vol. 72, pp. 155-162, 2017.

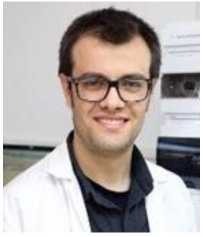
[37] B. Ozturk, S. Argin, M. Ozilgen, and D. J. McClements, "Formation and stabilization of nanoemulsion-based vitamin E delivery systems using natural surfactants: Quillaja saponin and lecithin," *Journal of Food Engineering*, vol. 142, pp. 57-63, 2014.

[38] C. Chen and G. Wagner, "Vitamin E nanoparticle for beverage applications," *Chemical Engineering Research and Design*, vol. 82, pp. 1432-1437, 2004.

[39] M. Guttoff, A. H. Saberi, and D. J. McClements, "Formation of vitamin D nanoemulsion-based delivery systems by spontaneous emulsification: Factors affecting particle size and stability," *Food Chemistry*, vol. 171, pp. 117-122, 2015.

[40] E. Arab-Tehrany, *et al*., “Beneficial effects and oxidative stability of omega-3 long-chain polyunsaturated fatty acids,” *Trends in Food Science and Technology*, vol. 25, no. 24-33, 2012.
[41] H. Ilyasoglu and E. S. Nehir, “Nanoencapsulation of EPA/DHA with sodium caseinate gum Arabic complex and its usage in the enrichment of fruit juice,” *Food Science and Technology*, vol. 56, pp. 461-468, 2014.
[42] A. Habibi, J. Keramat, M. Hojjatoleslamy, and F. Tamjidi, “Preparation of fish oil microcapsules by complex coacervation of Gelatin-Gum Arabic and their utilization for fortification of pomegranate juice,” *Journal of Food Process Engineering*, vol. 40, no. 2, pp. 1-11, 2016.
[43] T. Tokle and D. J. McClements, “Physicochemical properties of lactoferrin stabilized oil-in-water emulsions: Effects of pH, salt and heating,” *Food Hydrocolloids*, vol. 25, pp. 976-982, 2011.
[44] U. Lesmes, S. Sandra, E. A. Decker, and D. J. McClements, “Impact of surface deposition of lactoferrin on physical and chemical stability of omega-3 rich lipid droplets stabilized by caseinate,” *Food Chemistry*, vol. 123, pp. 99-106, 2010.
[45] R. Charoen, *et al*., “Influence of interfacial composition on oxidative stability of oil-in-water emulsions stabilized by biopolymer emulsifiers,” *Food Chemistry*, vol. 131, pp. 1340-1346, 2012.
[46] I. S. Santos, B. M. Ponte, P. Boonme, A. M. Silva, and E. B. Souto, “Nanoencapsulation of polyphenols for protective effect against colon-rectal cancer,” *Biotechnology Advances*, vol. 31, pp. 514-523, 2013.
[47] I. Peres, *et al*., “Preservation of catechin antioxidant properties loaded in carbohydrate nanoparticles,” *Carbohydrate Polymer*, vol. 86, pp. 147-153, 2011.
[48] G. Markman and D. Livney, “Maillard-reaction based nano-capsules for protection of water-insoluble nutraceuticals in clear drinks,” in *Proc. 11th International Congress on Engineering and Food*, Greece, 2011.
[49] J. Gomez-Estaca, M. P. Balaguer, R. Gavara, and P. Hernandez-Munoz, “Formation of zein nanoparticles by electrohydrodynamic atomization: Effect of the main processing variables and suitabilityfor encapsulating the food coloring and active ingredient curcumin,” *Food Hydrocolloids*, vol. 28, pp. 82-91, 2012.
[50] T. P. Sari, *et al*., “Preparation and characterization of nanoemulsion encapsulating curcumin,” *Food Hydrocolloids*, vol. 43, pp. 540-546, 2015.
[51] C. E. Mora-Huertas, H. Fessi, and A. Elaissari, “Polymer-based nanocapsules for drug delivery,” *International Journal of Pharmaceutices*, vol. 385, pp. 113-142, 2010.

Asieh Habibi was born in Esfahan, Iran on 19th September, 1985. She received her B.S. and M.S. at Food Science & Technology from Islamic Azad University in Iran. Her research interest is about Food Fortification, Nutraceutical Encapsulation, and Food Hydrocolloid.



Sebahattin S. Turgut was born in Ankara, Turkey on April 03, 1989. He received his B.S. degree in food engineering from Ankara University, Turkey in 2012 and M.S. degree in Food Engineering Department of Suleyman Demirel University, Turkey in 2016. He is now a Ph. D. candidate at the same institution. The research interests are about food biochemistry, unit operations, and novel technologies.

Erkan Karacabey was born in Ankara, Turkey on August 24, 1979. He completed Doctorate in Food Engineering Department of Middle East Technical University in 2009. He has been in Canada for one year as a Visitor Scientist in PARC (Pacific Agricultural Research Center of AAFC. He has publications and projects about Unit Operations in Food Engineering. He has been working as an Associated Professor in Food Engineering Department of Suleyman Demirel University since 2012.

Erdogan Kucukoner was born in Gumushane, Turkey on December 05, 1965. He completed Doctorate in Food Science and Technology Department of Missisipi State University in 1996. He has publications and projects about functional foods, food chemistry/biochemistry, dairy techologies, food additives and unit operations in food engineering. He has been working as an Professor in Food Engineering Department of Suleyman Demirel University since 2007.