（様式５）(Style5)

学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

氏名：　Asmaliza Binti Abd Ghani
Name

学位論文題目：　Encapsulation of Functional Oils by Spray Drying and their Stability in Spray-Dried Powder
Title of Dissertation　(噴霧乾燥による機能性油の粉末化と粉末内機能性油の安定性)

学位論文要約：
Dissertation Summary

Microencapsulation technology of valuable food ingredients has undergone remarkable development in the past five decades, and widely entered our daily lives. It is widely used for encapsulation of flavors and functional oils. This is a key technology to obtain stable functional oil such as fish oil by encapsulated functional oil in the powder. Microencapsulation processes are the process by which solid, droplets of liquid and dispersion can be enclosed in microscopic particle by the formation of a coating of wall material around the functional compound (core material).

ω-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are beneficial in preventing diseases such as cardiovascular disease (Siscovick et al., 2017), inflammatory (Yang et al., 2017), cancer (Correia-da-Silva et al., 2017) and hypertension (Minihane et al., 2016). PUFAs play a vital role in maintaining human health and are found abundantly present in marine fish oils. A recent trend in food industry shows the increase of food products containing ω-3 PUFAs (Götz et al., 2013). However, PUFAs contain two or more unsaturated double bonds carbon structure that is naturally oxidized when PUFAs are exposed to oxygen, light, and heat during storage. In addition, the oxidation of functional oil such as fish oil will produce an unfavorable smell. This is a major problem in the application of PUFAs as functional oil in foods products. In the food industry, spray drying is the most common encapsulation method of functional oil. This is due to its efficiency, cost effectiveness and readily available of the technology and equipment (Dobry et al., 2009).

This study focuses on the emulsification and spray drying processes to produce a stable encapsulated oil powder. By using two steps of homogenization with a high shear mixer and high-pressure homogenizer, spray-dried powders with different oil-droplet diameters were formed and evaluated in the surface-oil content, stability of functional oil in spray-dried powder and morphology of spray-dried powder. Numerous studies have investigated the effect of oil-droplet diameter in encapsulated functional oil and flavor spray-dried powder on the stability of their oil in spray-dried powder (Soottitantawat et al., 2003, Soottitantawat et al., 2005, Nakazawa et al., 2008, Chen et al., 2016).

The selection of core material and emulsifier and composition of wall material are also considered important. Wall materials can influence the physicochemical properties of the spray-dried powder. Maltodextrin (MD) is a hydrolyzed starch commonly used as wall material in microencapsulation of food ingredients (Gharsallaoui et al., 2007). MD has some advantages as wall material such as its relatively low cost, neutral aroma, and taste, low viscosity at high solids concentrations and good protection against oxidation. Increasing dextrose equivalent (DE) of MD means the decrease of the molecular weight, which is an important factor of stability in the encapsulation of functional oil. MD with higher DE value are less permeable to oxygen and encapsulated the flavor better than lower DE value (Reineccius, 1991).

Sodium caseinate (NC) have high emulsifying, viscosity modifying, water-binding, fat binding and foaming properties (Ennis and Mulvihill, 2000). In addition, NC has been used as an emulsifier in the food industry due to its flexibility and amphipathic nature which enable them to absorb on the emulsion interface (Chen et al., 2006). NC is also altered by physical, chemical and enzymatic treatments to produce preferable functional properties (Haard, 2001). Meanwhile, transglutaminase (TrG) enzyme received an attention because of its ability to cross-link with protein (Yokoyama et al., 2004). Moreover, several researchers have claimed that by increasing the DE of MD blended with NC, it will also increase the encapsulation efficiency of oil in powders (Hogan et al., 2001, Danviriyakul et al., 2002).

In this study, squalene oil (SQ) was used as a model for PUFAs, relatively low cost and could be quantified using gas chromatography. Current research into the biochemical and biophysical properties of SQ shows that it has beneficial components; it is mainly used in the cosmetics industry and in food supplements and pharmaceuticals (Spanova and Daum, 2011).

The objective of this study was to investigate the effect of the oil-droplet diameter of emulsified SQ at homogenization pressures of 20 MPa and 100 MPa. The influence of NC and PNC as an emulsifier on the stability of spray-dried powder during storage was also evaluated.

SQ emulsions were spray dried in a pilot plant spray dryer (Ohkawara L-8; Ohkawara Kakohki Co. Ltd, Yokohama, Japan). SQ content in powder was analyzed using a GC-FID (Shimadzu 2010; Shimadzu Corporation, Kyoto, Japan). The cross-sectional structures of powders were observed using scanning electron microscopy (SEM) (JSM 6060; JEOL Ltd, Tokyo, Japan).

The particle diameter of the encapsulated SQ powders was 26–37 μm for homogenization at both 20 MPa and 100 MPa, indicating that particle diameters of the SQ powders were not influenced by the homogenization pressure. The moisture content of the SQ powders ranged from 1.3% to 2.0%. SQ retention in encapsulated powder is defined as the ratio of the amount of SQ in the powder to that in the dried solid of the feed emulsion. At 20 MPa homogenization, SQ retention was over 100% in 3 wt% NC and 97% in 5 wt% NC. Oil retention in powders decreased at 100 MPa homogenization to 97% in 3 wt% NC and 87% in 5 wt% NC. These results showed that PNC did not affect oil retention in SQ oil powders. In a study of microencapsulated essential oil of basil, Garcia et al. (2012) showed that oil retention was affected by homogenization, but was unaffected by the oil type used. The surface oil ratio of SQ powder is defined as the surface oil content to the total oil content in the powder. Surface oil ratios were about 1% for homogenization at 100 MPa and independent of NC content. Nonetheless, for homogenization at 20 MPa, the surface oil ratio decreased from 5.4% to 1.79% when the amount of NC was increased from 3 wt% to 5 wt%. Munoz-Ibanez et al. (2015) showed the importance of estimating the capillary number in controlling the oil-droplet breakup under right atomization conditions and/or emulsion formation in a microencapsulation application. A higher surface oil content in the powder was observed with the large droplet diameter than with the small droplet diameter.

The cross-sections of the spray-dried powder shown in Figure 1 indicate larger oil-droplet diameters (a′–d′) at 20 MPa high-pressure homogenization and smaller oil-droplet diameters (e′–h′) at 100 MPa high-pressure homogenization; all the cross-sectional images show vacuoles inside the powder. The cross-section shell images of spray-dried powder with 5 wt% NC (c′ and g′) and PNC (d′ and h′) may indicate a rigid and harder shell than with 3 wt% NC (a′ and e′) and PNC (b′ and f′). This difference is more clearly seen for 100 MPa high-pressure homogenization, as shown in images g′ and h′ in Figure 1.

3 wt% NC　　3 wt% PNC　　5 wt% NC　　5 wt% PNC

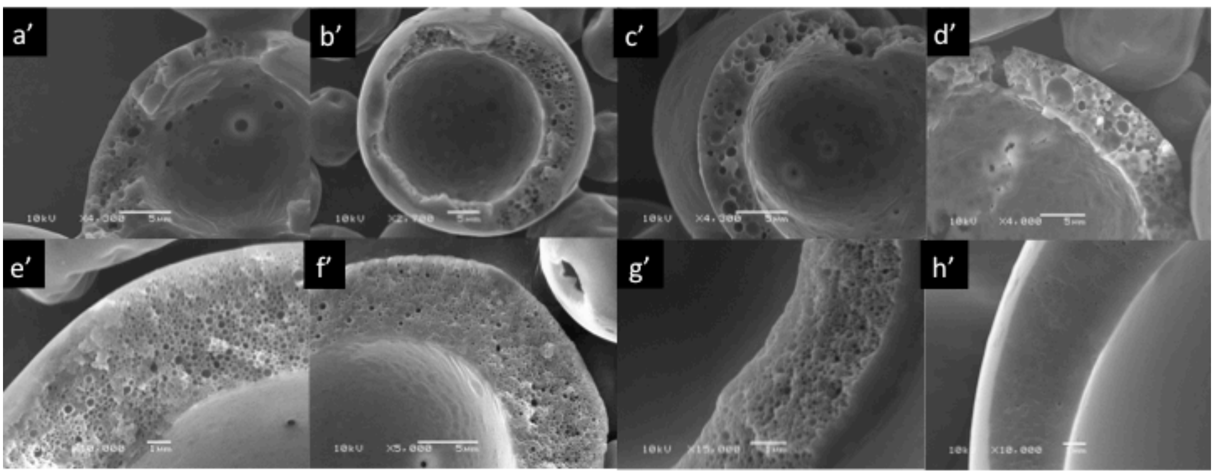


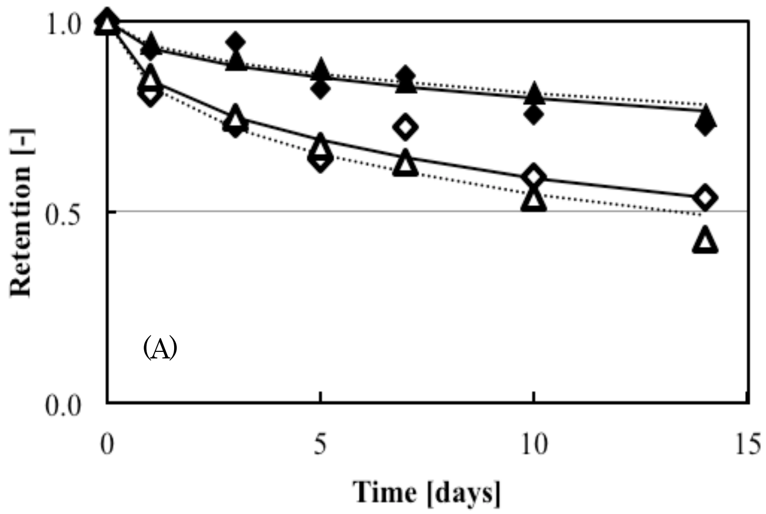
Figure 1 SEM images of cross-sectional structures. The spray-dried powders were formed at 20 MPa (a’–d’) and 100 MPa (e’–h’). (from Abd Ghani et al., 2016)

Figures 2 (A) and (B) show the stability of SQ in the spray-dried powders at 105 °C as a plot of SQ retention to storage time. SQ retention is defined as the ratio of the remaining amount of SQ oil to the initial amount in the powder. SQ retention in the powders decreased gradually during storage. The solid symbols indicate retention after 100 MPa homogenization and the open symbols indicate retention after 20 MPa. Both were correlated using the Avrami equation (Weibull distribution function), as shown in the following equation:

$$R = \exp\,[(-kt)^{n}] \tag{1}$$

where R [−] is SQ retention, t [days] is storage time, k [1/days] is the degradation rate constant, and n is a parameter for the degradation mechanism and was defined as 0.5.

The lines calculated using the Avrami equation correlated well to SQ retention in the powder with $n = 0.5$. As reported by Hancock and Sharp, (1972) the Avrami equation can correlate several mechanisms of degradation in solid-state reactions by varying the mechanism number from 0.5 to 2. The degradation kinetic might be diffusion controlled in the sphere powder for a mechanism number of 0.5. SQ degradation may be affected by oxygen diffusion in the wall material of the spray-dried powder. However, the mechanism of SQ degradation is still under investigation. SQ retention in the powders was similar for 3 wt% and 5 wt% for NC at the same homogenization pressure (Figure 2 A). On the other hand, at the same homogenization pressure, the degradation rate of SQ retention in the powder increased in 3 wt% PNC and decreased in 5 wt% PNC (Figure 2B). This expected behavior could be related to the low level of casein in PNC in the SQ powders (Mora-Gutierrez et al., 2014, Sørensen et al., 2007). The stability of SQ retention in powders depended considerably on the oil-droplet diameter, and less on PNC. The surface oil in the spray-dried powder was also measured during storage at 105 ºC. The good correlation lines for SQ retention during storage with a mechanism number of 0.5 suggested that the oxidation of SQ could depend on oxygen diffusion in the powder. The degradation rate constant was obtained using these correlations.



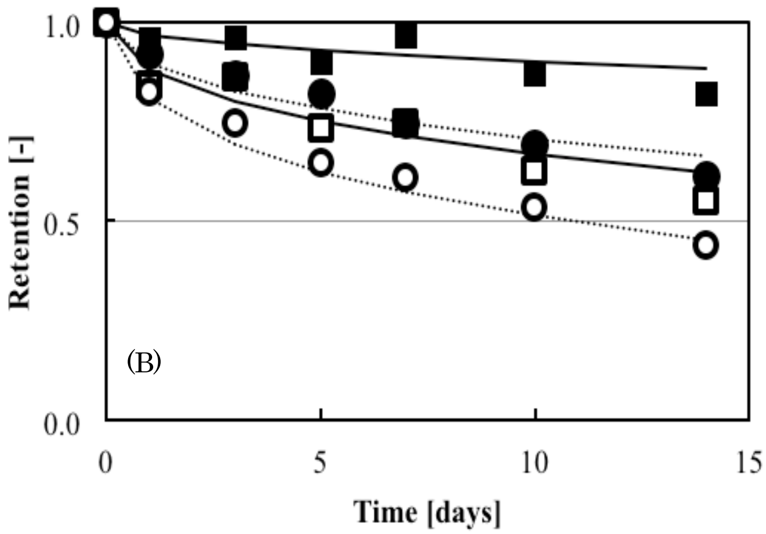


Figure 2 Stability of SQ in the spray-dried powders at 105 °C: (A) NC powders; (B) PNC powders. Solid symbols represent 100 MPa and open symbols represent 20 MPa, with 3 wt% NC: ▲, △; 3 wt% PNC: ●,○; 5 wt% NC: ◆, ◇; and 5 wt% PNC: ■, □. (from Abd Ghani et al., 2016)

In the SQ powder system, an explanation for the effect of the oil-droplet diameter on oxidative stability could be proposed using the propagative transfer rate of radical oxidation between oil-droplet particles in the powder.

Effect of dextrose equivalents of MD on the fish oil in spray-dried powder stability was investigated by measuring the peroxide value (PV) of surface fish oil, encapsulated fish oil, and total fish oil in spray-dried powder stored at 60 ºC. The physical properties of the spray-dried powder were also investigated.

The feed solutions were homogenized using a high-pressure homogenizer at 25 MPa for 4 min before spray-dried. The oil content in spray-dried powder was quantified using an Iatroscan MK-5 TLC-FID (Iatron Laboratories Inc., Tokyo, Japan). Peroxide value (PV) was measured using a potentiometric titration system (Easy Ox Titrator; Mettler-Toledo International Inc. Switzerland).

The cross-section structures of the spray-dried powder were observed using SEM and confocal laser scanning microscope (CLSM). Fig. 3 shows SEM images of the cross-section structures for the spray-dried powder for three different DEs (11, 19, and 25) of MD. Vacuoles could be found in the cross-section images of those powders and the vacuole size for DE = 11 was larger than other MD of DE = 19 and 25.

Spray-dried powder particles with a vacuole could be observed by the green fluorescence ring, and the diameter of each particle and vacuole was measured. Fig. 4 shows CLSM images representing the internal structure of the spray-dried powder with MD and DE = 11, 19, and 25. In counting 400 particles, the vacuole percentages of the spray-dried powder were 73 ± 0.01% for MD with DE = 11, 40 ± 0.04% for DE = 19, and 38 ± 0.05% for DE = 25.

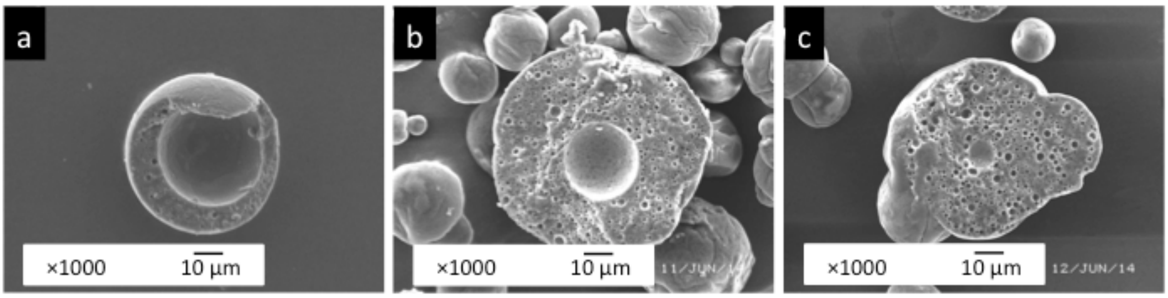


Figure 3 SEM images of cross-section structures (a, b, c) of spray-dried powder. MD (DE = 11): a; MD (DE = 19): b; and MD (DE = 25): c. (from Abd Ghani et al., 2017)

The vacuole diameter was also measured for 30 particles of the cutting images: 24 ± 12 μm for MD with DE = 11, 8.6 ± 5.6 μm for DE = 19, and 5.8 ± 3.6 μm for DE = 25. These vacuole percentages showed that powder prepared with MD and DE = 11 had not only a higher number of vacuoles but also larger vacuole diameters. MD with low DE produces high viscosity emulsions (Hogan et al., 2001, Di Mittia et al., 2015). In our studies, the viscosities of feed emulsion for MD with DE = 11 was 231 mPa·s, when the DE decreased, the viscosities decreased to 48 mPa·s for DE = 19 and 44 mPa·s for DE = 25. Paramita et al. (2010) reported on the effect of additives on the morphology of spray-dried powder, finding the vacuole percentage in spray-dried powder is not affected by viscosity but is influenced by outlet air temperature during spray drying and the wall material composition.

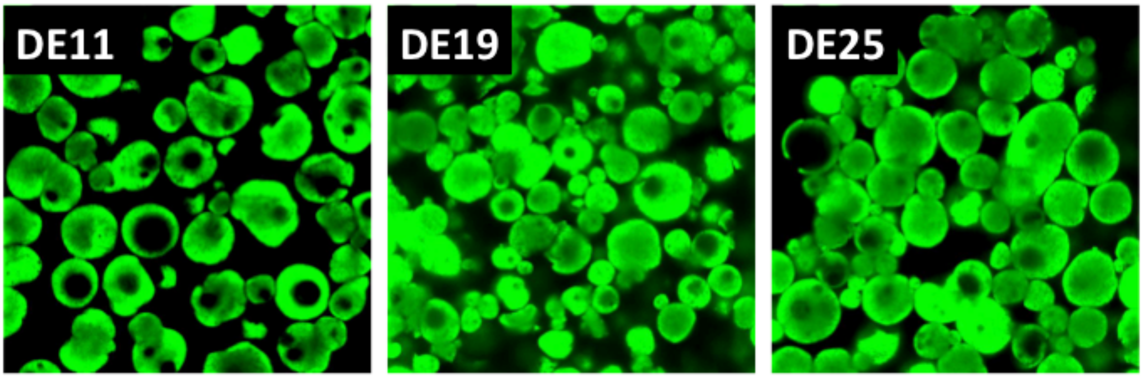


Figure 4 CLSM images of spray-dried powder with MD (DE = 11, 19, and 25). The wall materials were stained using sodium fluorescein. (from Abd Ghani et al., 2017)

Fig. 5 shows the PV changes in the spray-dried powders based on the weight of the powder for surface oil (a), encapsulated oil (b), and total oil (c) stored at 60 °C. DE of MD affected the PVs of the encapsulated oils. Encapsulated oil for MD of DE = 11 had higher oxidation than those for MD of DE = 19 and 25. This PV behavior could be seen in the plot of PV changes based on powder weight as shown in Fig. 5 b and c. Wang and Zhou (2012) in their study of encapsulated soy sauce demonstrated that higher DE values of MD had higher stability in terms of caking strength. These data indicated that the surface oil ratio and selection of wall material is important in forming stable fish-oil powder by spray drying, as well as for other encapsulated oil powders.

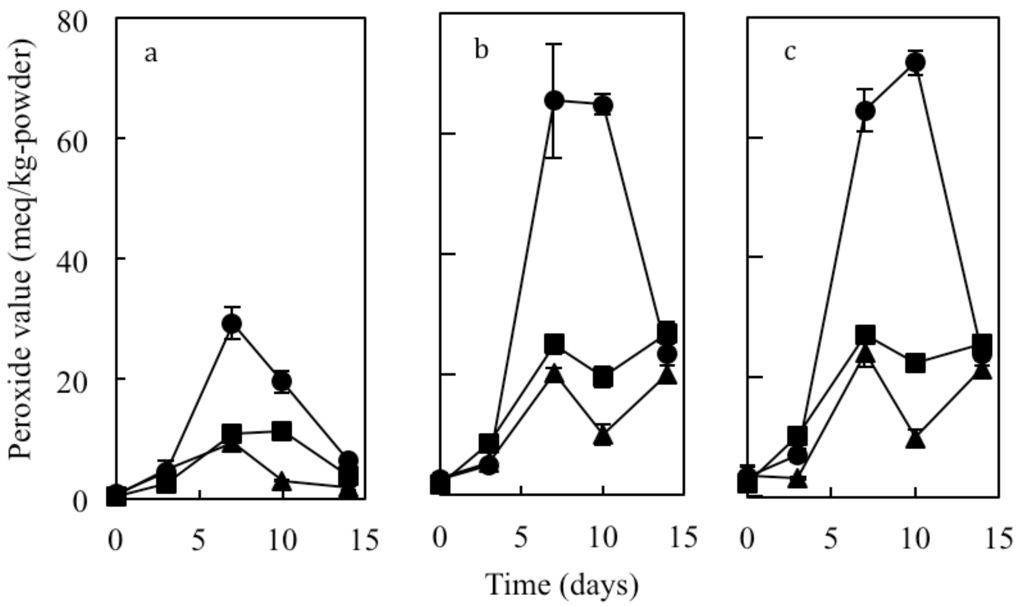


Figure 5 Peroxide values of spray dried powders stored at 60 °C for based on the weight of powder. a for surface oil, b for encapsulated oil, and C and c for total oil. MD (DE = 11): ●; MD (DE = 19): ■; and MD (DE = 25): ▲. (from Abd Ghani et al., 2017)

Several researchers investigated the stability of functional lipids and flavors in spray-dried powder (Drusch et al., 2007, Shen et al., 2010, Domian et al., 2014, Cano-Higuita et al., 2015). However, the focused of these studies were not on the effects of wall material such as in different DE value of MD and oil-droplet size in powders on the oxidation stability. The stability of pure functional compounds obviously the factor affecting stability of matrix powder. Encapsulation of stable oil might consider the mass transfer of oxygen in the matrix of spray-dried powder (Abd Ghani et al., 2016). In our experiment, powder prepared with almost same diameter of oil-droplet and particle of powders. These parameter is crucial to control since the surface oil content will affected the initial oxidation stability.

References:

Abd Ghani, A., Matsumura, K., Yamauchi, A., Shiga, H., Adachi, S., Izumi, H., Yoshii, H. **2016**. Effects of oil-droplet diameter on the stability of squalene oil in spray-dried powder. *Drying Technology,* 34, 1726−1734.

Abd Ghani, A., Adachi, S., Shiga, H., Neoh, T.L., Adachi, S., Yoshii, H. **2017**. Effect of different dextrose equivalents of maltodextrin on oxidative stability in encapsulated fish oil by spray drying. *Bioscience, Biotechnology and Biochemistry,* 81, 705–711.

Cano-Higuita, D.M., Malacrida, C.R., Telis, V.R.N. **2015**. Stability of curcumin microencapsulated by spray and freeze drying in binary and ternary matrices of maltodextrin, gum Arabic and modified starch. *Journal of Food Processing and Preservation*, 39, 2049–2060.

Chen, L.Y., Remondetto, G.E., Subirade, M. **2006**. Food based materials as nutraceutical delivery systems. *Trends in Food Science and Technolgy*, 17, 272–282.

Correia-da-Silva, M., Sousa, E., Pinto, M.M.M, Kijjoa, A. **2017**. Anticancer and cancer preventive compounds from edible marine organisms. *Seminars in Cancer Biology*, In press.

Danviriyakul, S., McClements, D.J., Decker, E., Nawar, W.W., Chinachoti, P. **2002**. Physical stability of spray-dried milk fat emulsion as affected by emulsifiers and processing conditions. *Journal of Food Science*, 67, 2183–2189.

Di Mattia, C., Paradiso, V.M., Andrich, L., Giarnetti, M., Caponio, F., Pittia, P. **2015**. Effect of olive oil phenolic compounds and maltodextrins on the physical properties and oxidative stability of olive oil o/w emulsions. *Food Biophysics*, 9, 396–405.

Dobry, D.E., Settell, D.M., Baumann, J.M., Ray, R.J., Graham, L.J., Beyerinck, R.A. **2009**. A model-based methodology for spray-drying process development. *Journal of Pharmaceutical Innovation*, 133–142.

Domian, E., Sulek, A., Cenkier, J., Kerschke, A. **2014**. Influence of agglomeration on physical characteristics and oxidative stability of spray-dried oil powder with milk protein and trehalose wall material. *Journal of Food Engineering*,125, 34–43.

Drusch, S., Serfert, Y., Scampicchio, M., Schmidt-Hansberg, B., Schwarz, K. **2007**. Impact of physicochemical characteristics on the oxidative stability of fish oil microencapsulated by spray-drying. *Journal of Agricultural and Food Chemistry*, 55,11044–11051.

Ennis, M.P., Mulvihill, D.M., **2000**. Milk protein. In G.O Phillips and P.A. Williams (Eds), Handbook of hydrocolloids, Cambridge, England: CRC Press (pp. 189–217).

Garcia, L.C., Tonon, R.V., Hubinger, M.D. **2012**. Effect of homogenization pressure and oil load on the emulsion properties and the oil retention of microencapsulated basil essential oil (Ocimum basilicum L.). *Drying Technology*, 30, 1413–1421.

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A. and Saurel, R. **2007**. Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food Research International*, 40, 1107−1121.

Götz, N., Bulbarello, A., König-Grillo, S., Düsterloh, A. and Völker, M. **2013**. Long-chain polyunsaturated omega-3 fatty acids in food development. *Sight and life*, 27, 12-17.

Hancock, J.D., Sharp, J.H. **1972**. Method of comparing solid- state kinetic data and its application to the decomposition of kaolinite, brucite, and BaCO3. *Journal of American Ceramic Society*, 55, 74−77.

Hogan, S.A., McNamee, B.F., O’Riordan, E.D., O’Sullivan M. **2001**. Emulsification and microencapsulation properties of sodium caseinate/carbohydrate blends. *International Dairy Journal*, 11, 137–144.

Minihane, A.M. **2016**. Impact of genotype on EPA and DHA status and responsiveness to increased intakes. *Nutrients*, 8, 123.

Munoz-Ibanez, M., Azagoh, C., Dubey, B.N., Dumoulin, E., Turchiuli, C. **2015**. Changes in oil-in-water emulsion size distribution during the atomization step in spray-drying encapsulation. *Journal of Food Engineering*, 167, 122–132.

Nakazawa, R., Shima, M., Adachi, S. **2008**. Effect of oil-droplet size on the oxidation of microencapsulated methyl linoleate. *Journal of Oleo Science*, 57, 225–232.

Paramita V, Iida K, Yoshii H, Furuta, T. **2010**. Effect of additives on the morphology of spray-dried powder. *Drying Technology*, 28, 323–329.

Reineccius, G.A. **1991**. Role of carbohydrates in flavor encapsulation. *Journal of Dairy Science*, 45, 144-146.

Shen, Z., Augustin, M.A., Sanguansri, L., Cheng, L.J. **2010**. Oxidative stability of microencapsulated fish oil powders stabilized by blends of chitosan, modified starch, and glucose. Journal of Agricultural and Food Chemistry, 58, 4487−4493.

Siscovick, D.S., Barringer, T.A., Fretts, A.M., Wu, J.H.Y., Lichtenstein, A.H., Costello, R.B., Kris-Etherton, P.M., Jacobson, T.A., Engler, M.B., Alger, H.M., Appel, L.J., Mozaffarian, D. **2017**. Omega-3 polyunsaturated fatty acid (fish oil) supplementation and the prevention of clinical cardiovascular disease. *Circulation*, In press.

Soottitantawat, A., Yoshii, H., Furuta, T., Ohkawara, M., Linko, P. **2003**. Microencapsulation by spray drying: Influence of emulsion size on the retention of volatile compounds. *Journal of Food Science*, 68, 2256–2262.

Soottitantawat, A., Bigeard, F., Yoshii, H., Furuta, T., Ohkawara, M., Linko, P. **2005**. Influence of emulsion and powder size on the stability of encapsulated d-lemonene by spray drying. *Innovative Food Science & Emerging Technologies*, 6, 107–114.

Spanova, M., Daum, G. **2011**. Squalene – biochemistry, molecular biology process biotechnology and applications. *European Journal of Lipid Science and Technology*, 113, 1299–1320.

Wang, W., Zhou, W. **2012**. Characterization of spray-dried soy sauce powders using maltodextrins as carrier. *Journal of Food Engineering*, 109, 399–405.

Yang, W., Chen, X., Liu, Y., Chen, M., Jiang, X., Shen, T., Li, Q., Yang, Y. Ling, W. **2017**. N-3 polyunsaturated fatty acids increase hepatic fibroblast growth factor 21 sensitivity via a PPAR- γ-β -klotho pathway. Molecular Nutrition & Food Research, In press.

Yokoyama, K., Nio, N., Kikuchi, Y. **2004**. Properties and applications of microbial transglutaminase. *Applied Microbiology and Biotechnology*, 64, 447–454.