Journal of Food Measurement and Characterization (2021) 15:3026–3035
https://doi.org/10.1007/s11694-021-00897-8

ORIGINAL PAPER

FT-IR spectroscopy based investigation of stability in wheat germ oil body emulsions as affected by general processing treatments

Rabia Güneş[1] · İbrahim Gülseren[1,2]

Received: 5 December 2020 / Accepted: 18 March 2021 / Published online: 23 March 2021

Abstract

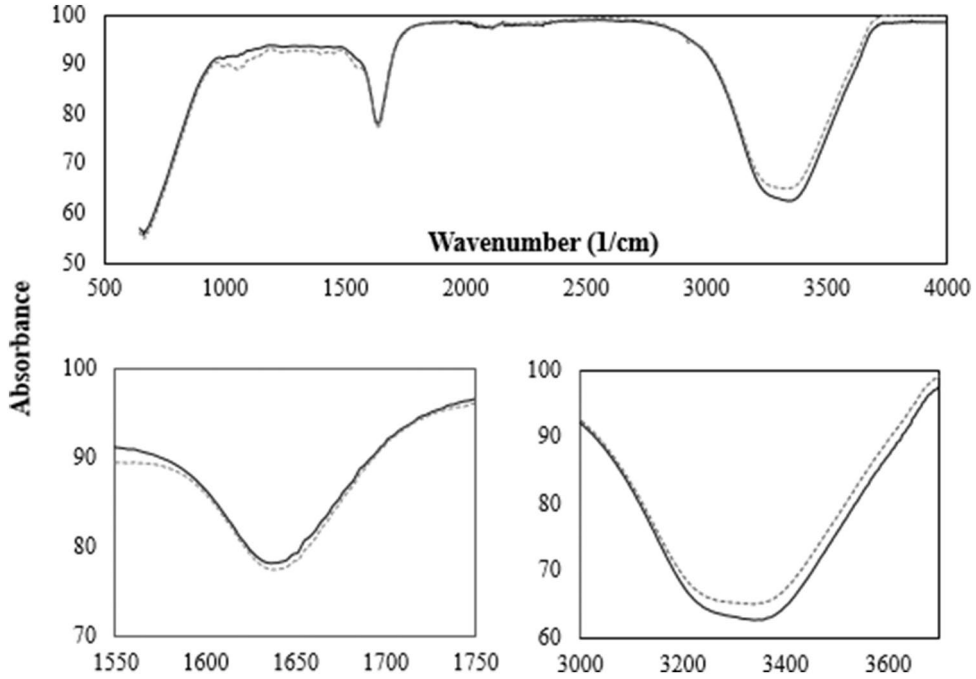
In many plants, unsaturated fatty acids are naturally stored in organelles that are named as oil bodies (OB). Wheat germ is a by-product of wheat flour manufacture that is generated in high tonnage globally. Here, an attempt was made to valorize wheat germ through the generation of natural OB emulsions and their extraction was based on an aqueous method. The natural stability limits for OB emulsions were investigated primarily using FT-IR spectroscopy as influenced by processing treatments such as lyophilization, heating and acidification. Differential scanning calorimetry (DSC) and particle size analysis were carried out to complement the FT-IR data. While freeze-drying seemed to have minimal influence on the structural attributes of OB systems, varying pH and extended heating induced destabilization at varying extents. The mean size values for the unprocessed samples were <8 μm in the first 15 days of refrigerated storage, which was characterized by significant increases in size ($d_{32} \geq 9$ μm) after 15 days. For the reconstituted samples, while the mean size values were <6 μm in the first 21 days of storage, $d_{32} \geq 8$ μm was observed after 21 days. The location and magnitude of DSC peaks were significantly different between unprocessed vs. lyophilized and reconstituted samples implying processing induced changes in protein structure. At neutral pH, thermal sensitivity of OB emulsions were more pronounced compared to their pH 3 or 9 counterparts. The findings were interpreted primarily in regards to oleosin denaturation and oleosin-oil interactions. Although utilization of hydrocolloids and/or emulsifiers and further homogenization might be necessary to enhance the stability of the current samples, the global capacity in wheat germ generation could justify utilization of wheat germ OB emulsions in food formulations.

✉ İbrahim Gülseren
ibrahim.gulseren@izu.edu.tr

[1] Department of Food Engineering, İstanbul Sabahattin Zaim University (İZÜ), Halkalı-Küçükçekmece, Istanbul, Turkey

[2] İZÜ Food and Agricultural Research Center (GTAUM), Küçükçekmece, Istanbul, Turkey

Graphic abstract



Keywords Valorization · Industrial by-products · Emulsion stability · Oleosomes · Particle size analysis · Differential scanning calorimetry (DSC)

Introduction

Encapsulation of polyunsaturated fatty acid bearing vegetable oils impart oxidative stability to food emulsions and emulsion-based delivery systems [1]. Natural protective mechanisms have been utilized as an alternative method to stabilize unsaturated fatty acids against oxidation [2]. In some plants, these fatty acids are naturally stored in small spherical organelles called "oil bodies" (OB), which are used as an energy source for seed germination and seedling growth in plants [3].

Oil bodies obtained from vegetable sources such as corn, soybean, rapeseed, sunflower, and wheat have attracted the attention of many researchers due to their interesting properties [4]. Oleosin proteins, which are hydrophobic and have relatively low molecular weights (15–20 kDa), have been thought to naturally stabilize OB [5, 6]. Their concentration could account for up to 2–8% of the total seed protein [7]. Apart from oleosins, two different types of proteins, namely caleocins (27 kDa) and steroleosins (39–40 kDa) with higher molecular weights surrounding OB have been identified [8–10].

According to the study by Huang [7], oleosins formed the surface of almost the entire OB. Thus, they played a fundamental role on the stability of the OB emulsions by providing electrostatic and steric repulsion between the oil droplets. In addition, Fisk et al. [11] have shown that OB were stable against oxidation and the extent of oxidation was significantly lower in OB emulsions compared to emulsions formulated with other surfactants.

Various aqueous methods of obtaining naturally emulsified OB from plant materials have been proposed as an alternative to oil extraction based on organic solvents (for example, see Nikiforidis et al. [12]). Solvents used in traditional oil extraction technologies could pose safety problems due to their highly flammable nature and cause environmental pollution. In addition, solvent extraction could induce detrimental effects on the functionality of the seed proteins rendering them solely instrumental in animal feed [13, 14].

However, solvent extraction methods are characterized by their high efficiency and low cost characteristics [15], while aqueous extraction potentially lowers efficiency [12]. In some studies, oil yield significantly increased when aqueous extraction methods were combined with enzymatic treatments [16]. Since aqueous extraction of OB generated a pre-emulsified formulation, thus generated samples were utilized in the preparation of emulsion-based products such as mayonnaise, cream liqueurs, salad dressings and creams [15, 17]. OB systems have enjoyed increasing extent of interest in both food applications and emulsion based delivery systems,

all of which have been thoroughly reviewed [18–20]. For example, oil bodies have been incorporated into various formulations including protein gel matrices [21]. While homogenization reduced the mean diameter of oil bodies in peanut milk, roasting of the peanuts stabilized the oil bodies during the sterilization treatments [22].

Due to its rich nutritional content, wheat germ is a highly nutritious food ingredient and serves as a good source of proteins, B vitamins (thiamine, riboflavin and niacin), vitamin E, unsaturated fatty acids and minerals. Wheat germ has an oil content of 8–14%, and approximately 55% of that is linoleic acid. Wheat germ oil is particularly rich in its α-tocopherol and β-tocopherol content [23]. Since wheat germ is a typical by-product of wheat flour production in large quantities worldwide, rational approaches to valorize its nutritional and economical potential are highly sought after.

As far as we know, there are no studies on wheat germ OB emulsions and their stability in the literature, while the fundamental data on wheat oleosins can be located in protein databases such as UniProt. In the seminal work of Tzen and Huang [24], wheat oil bodies were reconstituted and their structural attributes were investigated. Consequently, in this study, manufacture and stability of OB emulsions were investigated.

In this context, we made a first attempt to isolate OB from industrially generated wheat germ samples. OB emulsions were investigated for their stability limits in the absence of any further homogenization or added emulsifiers. In that respect, FT-IR spectroscopy was utilized along with the other complementary tools, since FT-IR spectroscopy is a commonly utilized tool (see for example, [25–27]) in the analysis of both organic and inorganic nanomaterials.

Materials and methods

Materials

Industrial wheat germ samples were donated by a local manufacturer (Sinangil Flour Company, Turkey). All chemicals were purchased from Sigma-Aldrich Corp (Schnelldorf, Germany) including NaOH (06203), HCl (07102) and sodium azide (71290).

Methods

Preparation of natural wheat germ OB emulsions

Preparation of wheat germ OB emulsions was carried out based on the methodology published by Nikiforidis et al. [12] with minimal modifications. No further homogenization have been administered and no further inclusion of emulsifiers or organic solvents have been carried out. First of all, the industrial wheat germ samples were ground using a laboratory mill (KINEMATICA POLYMIX PX-MFC 90D) and particle size was brought to the level of approx. 0.8 mm. Ground samples were mixed with water at a ratio of 1:5, and medium pH was immediately brought to pH 9 using 1 M NaOH. The mixture was kept stirred (1500 rpm) for 1 h, and the mixture was passed through three layers of cheesecloth, which yielded the raw OB emulsion. If necessary, insoluble solids were separated from the samples utilizing a brief centrifugation process (3500×*g*, 15 min). When necessary, OB emulsions were concentrated using a lab-scale ultrafiltration unit (Sartorius Stedim Vivaflow 200 Ultrafiltration Unit) using an ultrafiltration of a molecular weight cutoff (MWCO) value of 10 kDa (Sartorius VF20P0). In all cases, a small amount of sodium azide (0.02%) was added to samples and buffer solutions as a bacteriostatic.

Particle size distribution

Particle size distributions of the OB emulsions were determined using a SALD-2300 system (Shimadzu). All particle size distributions were reported on % volume frequency basis. The refractive indices of water and oil phases were taken as 1.33 and 1.45, respectively [28].

Lyophilization of OB emulsions

Freeze-drying of OB emulsions was carried out using a TRS 2/2V freeze-drier (Teknosem, İstanbul, Turkey). For analytical purposes, samples were stored at 4 °C for up to 4 weeks and periodic analyses were performed. When reconstitution was necessary, freeze-dried samples were brought back to their original volume using water.

Heating and acidification protocols

The heating of OB emulsions was carried out using a water-bath set to 80 °C. The sample tubes (15 ml) were placed in the water-bath and aliquots were withdrawn at pre-determined intervals (i.e., minute 0, 10, 20 and 30). Sample pH was adjusted to pH 3 or 7, using small quantities of 1 M HCl or 1 M NaOH as necessary.

Fourier transform infrared (FTIR) spectroscopy analysis

FT-IR spectroscopic analysis of the samples was carried out using an IRTracer-100 FT-IR spectrophotometer (Shimadzu, Japan) equipped with a DLATGS detector system, and MIRacle ATR module with a resolution of 2 cm^{-1} (Pike Technologies, USA). FT-IR absorption spectra were collected from 4000 to 650 cm^{-1} [29].

Differential scanning calorimetry (DSC)

Thermal behaviour of OB emulsions was analyzed using a differential scanning calorimeter system (DSC 60 Plus, Shimadzu Instruments, Japan). Approx. 20 mg of samples were placed into aluminum pans and heated from 30 to 125 °C at a heating rate of 10 °C min^{-1}.

Statistical analysis

In all cases, at least three replicates were analyzed for each sample. The sample mean and its corresponding standard deviation were reported. When necessary, a representative run was shown.

Results and discussion

The influence of freeze-drying and reconstitution

Firstly, natural oil body (OB) emulsions or their freeze-dried and reconstituted counterparts were studied using FT-IR spectroscopy. The whole wavenumber range (500–4000 cm^{-1}) was plotted on Fig. 1. While the spectra for both samples were quite similar (Fig. 1a), slight differences were observed, especially in the range of 1600–1700 and 3200–3400 cm^{-1}.

Between 1600–1700 cm^{-1}, the signals mostly correspond to Amide I band (Fig. 1b) [29]. Amide I band is represented by C = O stretching (approx. 80%) and N–H bending (< 20%) vibrations, which in turn represent the amide (i.e., peptide) bonds generated by the binding of amino acids in proteins. Based on these findings, native OB proteins were minimally affected by freeze-drying and reconstitution processes, which in turn could imply the potential stability of and ease of utilization for freeze-dried OB emulsions. The peak between 3200–3400 cm^{-1} is related to the O–H stretching vibrations, which are generated by the water molecules in the system (Fig. 1c) (see for example, Delmotte et al. [30]). Once again, peak intensities (for example at 3337.30 cm^{-1}) were reasonably comparable between the two samples, which indicated the interactions of water with the other components were minimally affected due to lyophilization. These findings indicated that freeze-drying could offer a simple stabilization and long-term storage method for natural OB emulsions generated from industrial wheat germ streams.

To test this hypothesis, particle size distribution of natural or freeze-dried and reconstituted OB emulsions were monitored for 4 weeks during 4 °C storage and Sauter mean diameter (d_{32}) was periodically analyzed (Fig. 2). Pronounced changes were observed in mean particle sizes, especially beyond 15 days of storage. For the unprocessed samples, the mean size values were < 8 μm in the first 15 days of storage, which was characterized by significant increases in size ($d_{32} \geq 9$ μm) after 15 days (Fig. 2a). This finding demonstrated that aggregation and growth of the emulsion droplets took place over extended storage. It is noteworthy that these samples were prepared on as is basis and no extraneous emulsifiers were added. Furthermore, no additional homogenization was administered. For the reconstituted samples, while the mean size values represented by d_{32} were < 6 μm

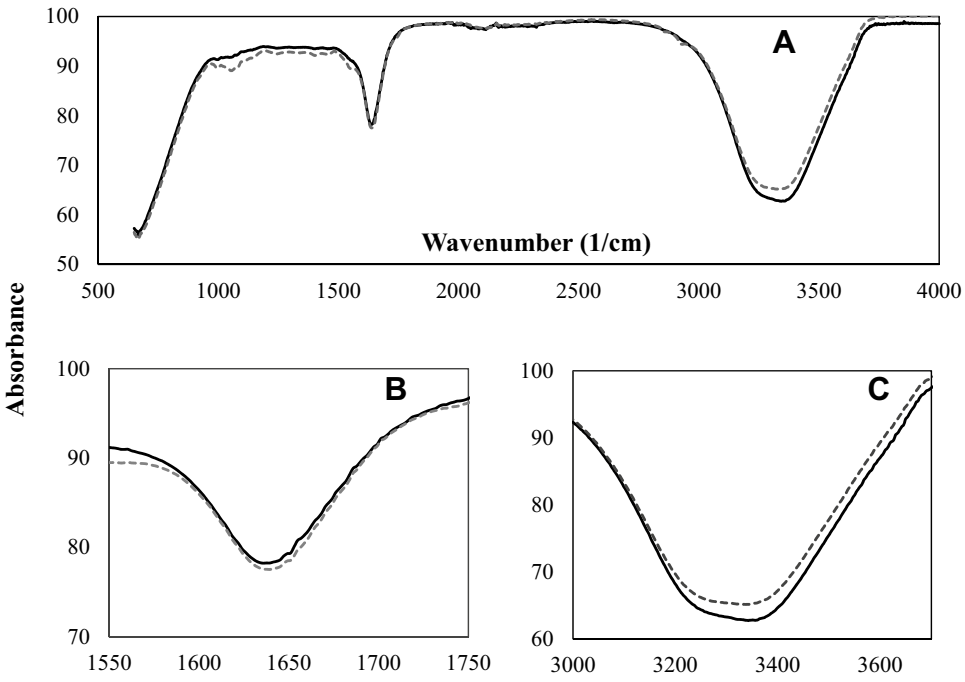


Fig. 1 FT-IR spectra for natural OB emulsions or freeze-dried and reconstituted OB emulsions. Wavenumber ranges of **a** 500–4500 cm^{-1}, **b** 1550–1750 cm^{-1}, and **c** 3000–3700 cm^{-1} were plotted. Solid line: natural OB emulsion; dashed line: freeze-dried and reconstituted OB emulsion

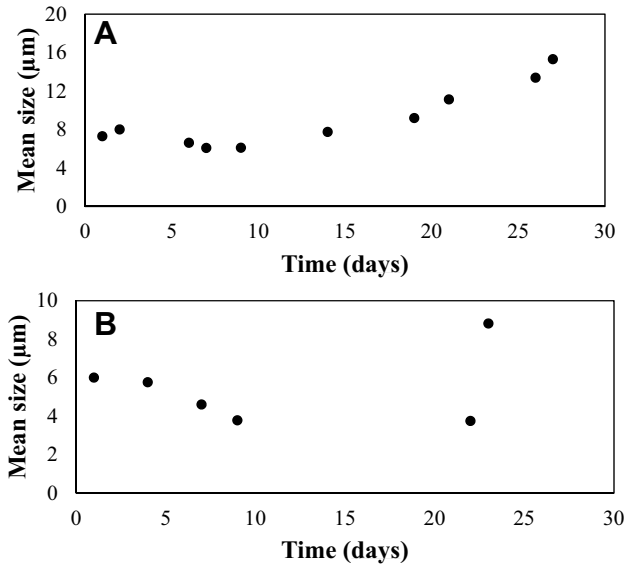


Fig. 2 Mean particle size (d_{32}) for **a** natural OB emulsions, or **b** freeze-dried and reconstituted OB emulsions. Samples were stored at 4 °C for 4 weeks immediately after preparation. Standard deviation values were < 5% of the sample mean in all cases ($n = 3$)

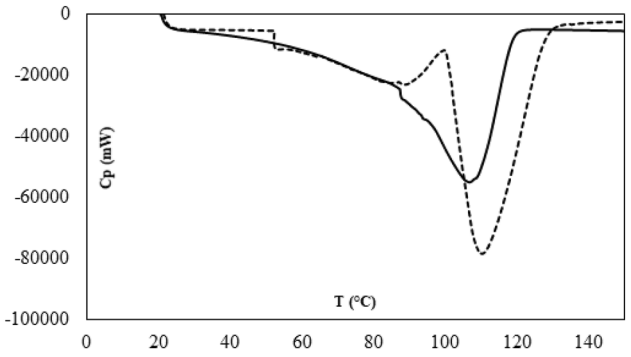


Fig. 3 DSC thermograms for natural OB emulsion (solid line) or freeze-dried and reconstituted OB emulsion (dashed line) samples. Representative runs

in the first 21 days of storage, $d_{32} \geq 8$ µm was observed after 21 days (Fig. 2b). Since oil bodies tend to range between 0.5 to 2.5 µm in diameter [24], both unprocessed and processed oil bodies could be hypothesized to undergo a certain extent of aggregation as shown by the particle size measurements.

Although some differences existed in IR spectra or particle size data, the storage stability of lyophilized samples were comparable to the unprocessed samples. Consequently, lyophilized OB emulsions can be stored under refrigerated conditions, and may reduce the storage space requirements.

Finally, thermal analysis of the samples was carried out using differential scanning calorimetry (DSC) (Fig. 3). The trends in the thermograms were comparable although the location and magnitude of DSC peaks were significantly different. Most importantly denaturation point of the proteins shifted by approx. 5 °C after processing. Since only liquid oil was present in the system, no phase transition related to melting of oil was anticipated. Consequently, OB proteins were the primary set of molecules at a relatively high concentration level that could undergo significant thermal transitions and DSC peaks were attributed to protein denaturation. In addition, denaturation took place over a large temperature range for both samples possibly owing to the presence of multiple oleosins in the system [24]. As also observed by the slight changes in IR spectra, the structure of proteins and their interactions with water might have been slightly altered due to the freeze-drying and reconstitution. Furthermore, the presence and interactions of phospholipids are critical for OB stability [22]. The altered interactions of phospholipids with water or oleosins could also have a bearing on the stability of emulsions. Consequently, the interactions of olesins with water, phospholipids and components of the oil phases could have a bearing on their thermal stability. Potentially freeze-drying caused alterations on the intensity of oil-protein interactions (i.e., reduced water and comparatively increased oil content during freeze-drying) which could lead to altered thermal stability of oleosins. Thus, a slight shift in the denaturation point was monitored (Fig. 3). However, these changes potentially lead to reduced emulsion stability upon reconstitution causing changes in particle size spectrum (Fig. 2).

The influence of combined heating and freeze-drying treatments

In the previous section, the influence of freeze-drying was found to be relatively small on OB stability. Consequently, the influence of a combined heating and lyophilization treatment was carried out. Firstly, the unprocessed emulsions were heated in a water-bath, aliquots were periodically removed and lyophilized immediately afterwards. FT-IR spectroscopic analyses were carried out for all samples (Fig. 4).

The IR spectrum for the heated and lyophilized samples was significantly altered by heating treatments and the extent of change was clearly a function of the heating duration (Fig. 4a). The changes demonstrated by C-O bending (900–1150 cm^{-1}) (Fig. 4b), or C–H stretch vibrations, around 2920–2930 cm^{-1} accounted for changes in triacylglyceride (TAG) related signals [31]. Similarly, potential changes in protein structure such as Amide II related secondary structural changes (Fig. 4c), Amide I related (Fig. 4d) or Amide A related changes (especially around 3295 cm^{-1}) have been observed [31]. Since the signal intensity increased with the intensity of the heating processes, gradual denaturation of the native OB proteins can be hypothesized. In addition, increasing signal

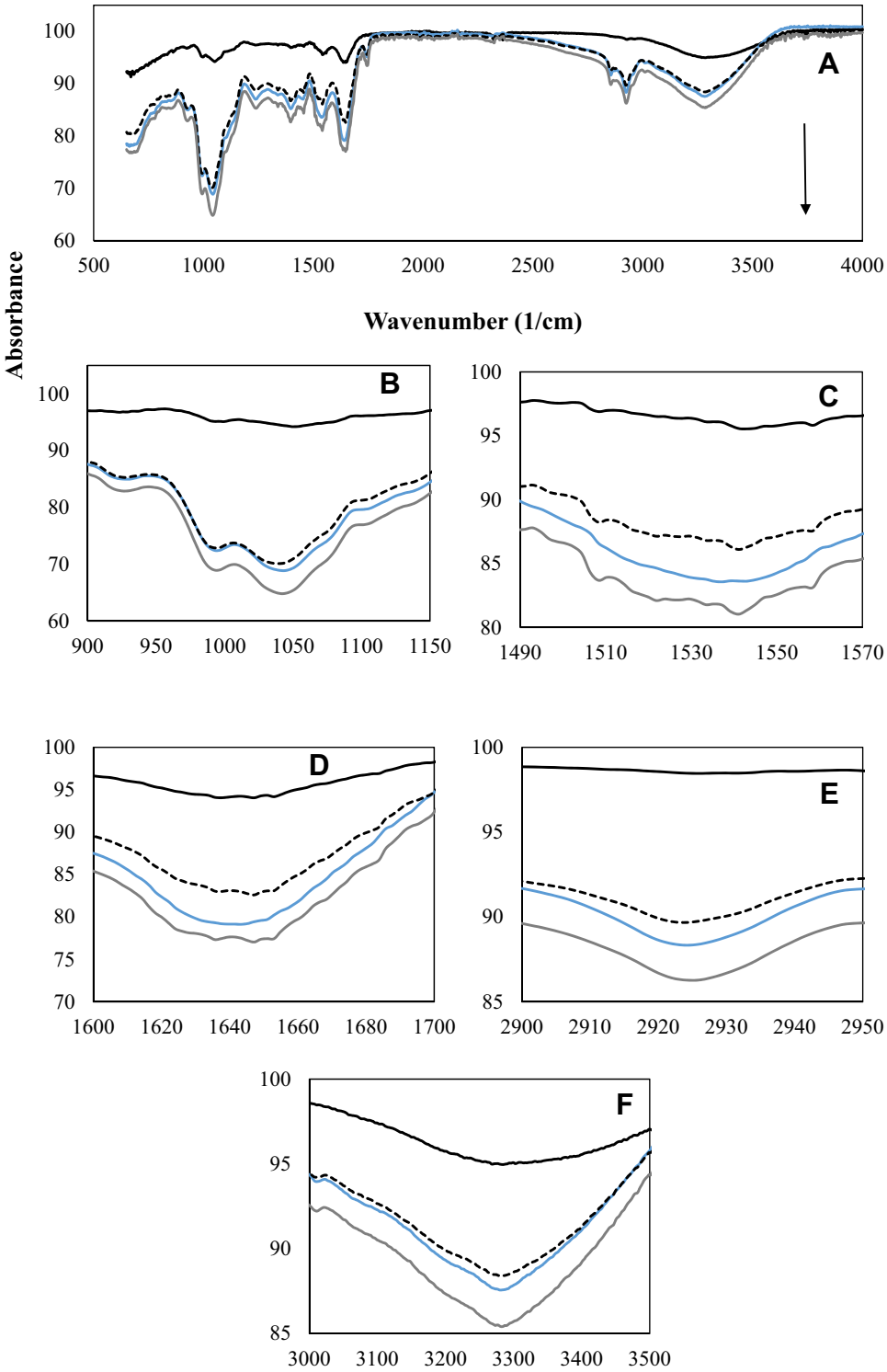


Fig. 4 FT-IR spectra and their corresponding details for heated and freeze-dried OB emulsion systems. Using a boiling water-bath, a 30 min heating process was carried out, and the sample temperatures increased from ambient temperature to approx. 80 °C, while one sample was withdrawn every 10 min. The downwards arrow indicates the increasing process duration and temperature. **a** Full FT-IR spectra, **b** 900–1150 cm^{-1} wavenumber range, **c** 1490–1570 cm^{-1} wavenumber range, **d** 1600–1700 cm^{-1} wavenumber range, **e** 2900–2950 cm^{-1} wavenumber range, **f** 3050–3500 cm^{-1} wavenumber range

intensity around 1650 cm^{-1} could possibly be attributed to the increases in the abundance of random coils [31]. Furthermore, around 3286 cm^{-1}, the amplification of amide (i.e., N–H stretching) bands were also coupled to the denaturation of oleosins [32] (Fig. 4f). In digestion studies, loss of secondary structural attributes were shown to reduce the stability of oleosomes [33]. Current findings were coherent with these observations.

Potentially the peptide bonds were increasingly more exposed upon heating treatments. The changes in protein structure lead to increases in the intensities of TAG related signals, which implied partial destabilization of OB

emulsions due to the "undesirable" changes in protein structure. Consequently, unlike the findings on freeze-drying, heating experiments caused net changes in the stability of OB emulsion systems.

The influence of combined acidification, freeze-drying and heating treatments

In this section, combination of acidification treatments with immediate freeze-drying was utilized in the processing of OB emulsions. While acidification increased the magnitude of all TAG and protein related peaks mentioned in the previous sections, the extent of magnitude change was a function of pH reduction in all cases (Fig. 5a–e). In the case of soybean oleosomes, acidification was characterized by decreasing stability of the system as well [34], which was coherent with the current findings. Once again, the signals corresponding to random coils and amide stretching were also found to increase with the current treatments (Fig. 5).

Finally, when the acidified systems were heated for 30 min in a water-bath (Fig. 6a–e), although the trends and the changes in structural attributes were comparable to Fig. 5, the behavior of pH 7 samples significantly differed. Consequently, it was clear that the structural attributes attained at pH 7 reduced the thermal sensitivity of OB emulsions, although unprocessed samples (Fig. 1) represented the most stable system. The relative stability of pH 7 samples could be attributed in part to the changes in protein structure as well as to their electrostatic charges. Further DSC and particle size data were not included here for brevity as various extents of destabilization promptly took place. Essentially an attempt was made here to investigate the stability limits for wheat germ OB emulsions, while further processing could significantly enhance such limits.

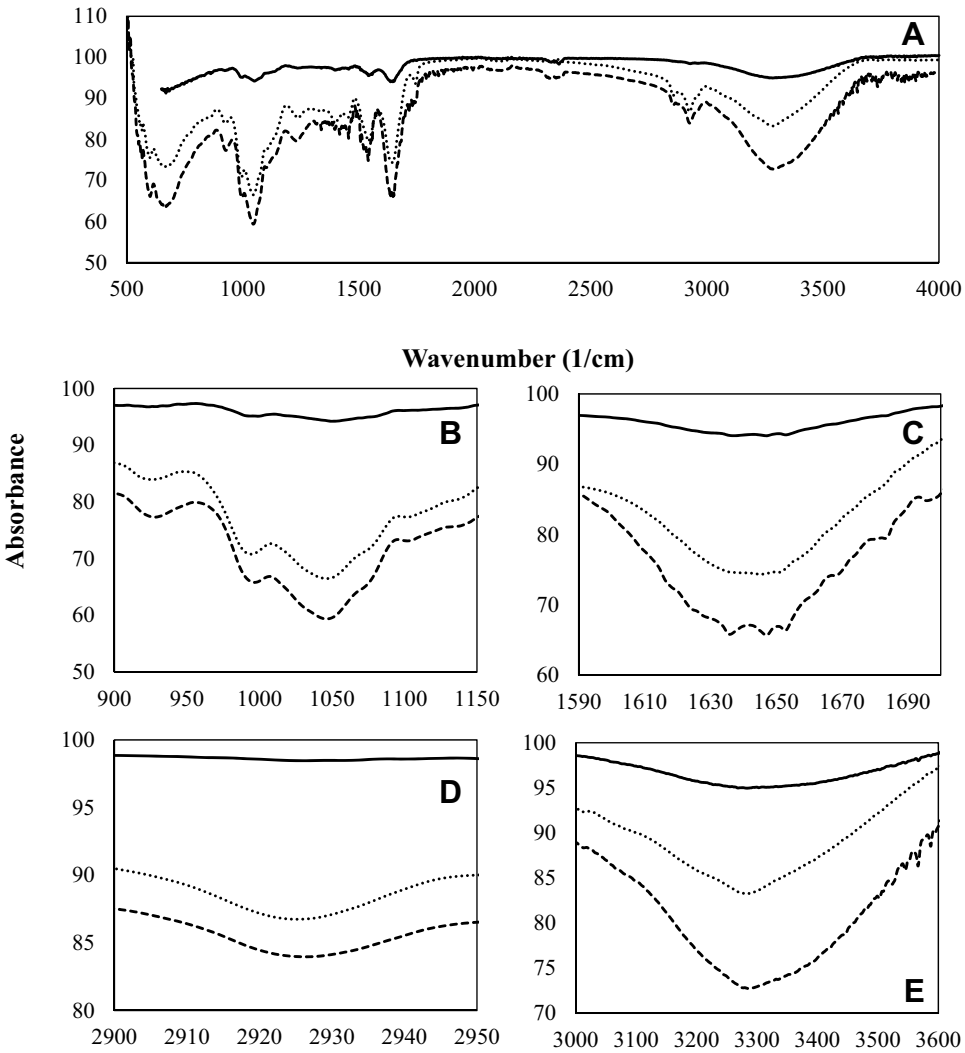


Fig. 5 FT-IR spectra and their corresponding details for pH adjusted (pH 3, 7 and 9) emulsion systems kept at ambient conditions. **a** Full FT-IR spectra, **b** 900–1150 cm^{-1} wavenumber range, **c** 1590–1690 cm^{-1} wavenumber range, **d** 2900–2950 cm^{-1} wavenumber range, **f** 3000–3600 cm^{-1} wavenumber range. Dashed lines and dots represent adjustment to pH 3 and 7, respectively, whereas the solid lines stand for the original pH value

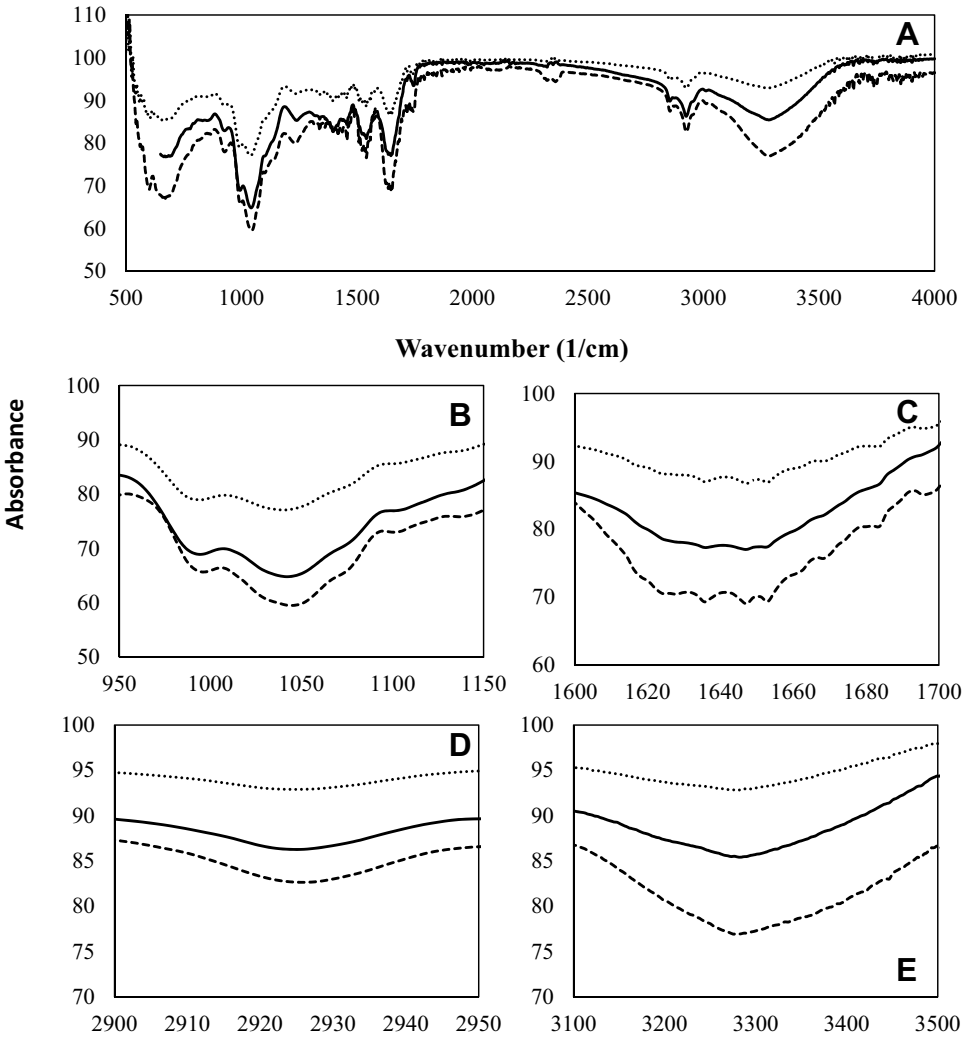


Fig. 6 FT-IR spectra and their corresponding details for pH adjusted (pH 3, 7 and 9) emulsion systems heated using a water-bath. **a** Full FT-IR spectra, **b** 900–1150 cm^{-1} wavenumber range, **c** 1590–1690 cm^{-1} wavenumber range, **d** 2900–2950 cm^{-1} wavenumber range, **f** 3000–3600 cm^{-1} wavenumber range. Dashed lines and dots represent adjustment to pH 3 and 7, respectively, whereas the solid lines stand for the original pH value

Conclusions

Wheat germ is a highly nutritive natural material that is being increasingly utilized in functional food formulations. Here, we present a simple extraction scheme where natural OB emulsions could be generated from wheat germ. Wheat germ OB emulsions were resistant to refrigerated storage and freeze-drying, which extend their utilization potential. Meanwhile acidification and heating operations rapidly alter the structural attributes of native OB proteins and TAGs, which could limit their utilization. To further exploit this technology in food processing, simple precautions might need to be taken such as the usage of other hydrocolloids and/or emulsifiers and further homogenization. Based on these enhancements, wheat germ could potentially be utilized in functional food formulations along with other commercial products that can exploit the nutrient-dense nature of wheat germ OB emulsions. While the stability limits for a simple aqueous extraction scheme was investigated here, the global capacity in wheat germ generation could justify utilization of wheat germ OB emulsions. In our future studies, our group will make an effort to further stabilize OB systems and analyze their performance in model products and in simulated gastrointestinal digestion studies.

Acknowledgements The authors would like to express their gratitude to Sinangil Flour Company, Turkey for the donation of wheat germ samples. Ant Teknik, Turkey is also acknowledged for support in the temporary loan of the particle sizer system (SALD-2300, Shimadzu) used in the current study.

Funding None.

Declarations

Conflicts of interest The authors declare that they have no conflict of interest.

References

1. D.J. McClements, E.A. Decker, Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical

reactions in heterogeneous food systems. J. Food Sci. **65**(8), 1270–1282 (2000). https://doi.org/10.1111/j.1365-2621.2000.tb10596.x
2. B. Chen, D.J. McClements, D.A. Gray, E.A. Decker, Physical and oxidative stability of pre-emulsified oil bodies extracted from soybeans. Food Chem. **132**, 1514–1520 (2012). https://doi.org/10.1016/j.foodchem.2011.11.144
3. Y. Cao, L. Zhao, Y. Ying, X. Kong, Y. Hua, Y. Chen, The characterization of soybean oil body integral oleosin isoforms and the effects of alkaline pH on them. Food Chem. **177**, 288–294 (2015). https://doi.org/10.1016/j.foodchem.2015.01.052
4. J.T.C. Tzen, Y.Z. Cao, P. Laurent, C. Ratnayake, A.H.C. Huang, Lipids, proteins and structure of seed oil bodies from diverse species. Plant Physiol. **101**(1), 267–276 (1993). https://doi.org/10.1104/pp.101.1.267
5. G.I. Frandsen, J. Mundy, J.T.C. Tzen, Oil bodies and their associated proteins, oleosin and caleosin. Physiol. Plantarum. **112**(3), 301–307 (2001). https://doi.org/10.1034/j.1399-3054.2001.1120301.x
6. D.A. White, I.D. Fisk, J.R. Mitchell, B. Wolf, S.E. Hill, D.A. Gray, Sunflower-seed oil body emulsions: rheology and stability assessment of a natural emulsion. Food Hydrocoll. **22**(7), 1224–1232 (2008). https://doi.org/10.1016/j.foodhyd.2007.07.004
7. A.H.C. Huang, Oil bodies and oleosins in seeds. Annu. Rev. Plant Physiol. Plant Mol. Biol **43**, 177–200 (1992). https://doi.org/10.1146/annurev.pp.43.060192.001141
8. T.L. Shimada, I. Hara-Nishimura, Oil-body-membrane proteins and their physiological functions in plants. Biol. Pharm. Bull. **33**(3), 360–363 (2010). https://doi.org/10.1248/bpb.33.360
9. E.C.F. Chen, S.S.K. Tai, C.C. Peng, J.T.C. Tzen, Identification of three novel unique proteins in seed oil bodies of sesame. Plant Cell Physiol. **39**, 935–941 (1998). https://doi.org/10.1093/oxfordjournals.pcp.a029457
10. L.J. Lin, S.S. Tai, C.C. Peng, J.T. Tzen, Steroleosin, a sterol-binding dehydrogenase in seed oil bodies. Plant Physiol. **128**, 1200–1211 (2002). https://doi.org/10.1104/pp.010982
11. I.D. Fisk, D.A. White, M. Lad, D. Gray, Oxidative stability of sunflower oil bodies. Eur. J. Lipid Sci. Technol. **110**(10), 962–968 (2008). https://doi.org/10.1002/ejlt.200800051
12. C.V. Nikiforidis, O.A. Karkani, V. Kiosseoglou, Exploitation of maize germ for the preparation of a stable oil-body nanoemulsion using a combined aqueous extraction ultrafiltration method. Food Hydrocoll. **25**, 1122–1127 (2011). https://doi.org/10.1016/j.foodhyd.2010.10.009
13. A. Moure, J. Sineiro, H. Dominguez, J.C. Parajo, Functionality of oilseed protein products: a review. Food Res. Int. **39**(9), 945–963 (2006). https://doi.org/10.1016/j.foodres.2006.07.002
14. A. Rosenthal, D.L. Pyle, K. Niranjan, Aqueous and enzymatic processes for edible oil extraction. Enzyme Microb. Technol. **19**(6), 402–420 (1996). https://doi.org/10.1016/S0141-0229(96)80004-F
15. C.V. Nikiforidis, C.G. Biliaderis, V. Kiosseoglou, Rheological characteristics and physicochemical stability of dressing-type emulsions made of oil bodies–egg yolk blends. Food Chem. **134**, 64–73 (2012). https://doi.org/10.1016/j.foodchem.2012.02.058
16. V.N. Kapchie, D. Wei, C. Hauck, P.A. Murphy, Enzyme-assisted aqueous extraction of oleosomes from soybeans (*Glycine max*). J. Agric. Food Chem. **56**(5), 1766–1771 (2008). https://doi.org/10.1021/jf0721390
17. B. Chen, D.J. McClements, D.A. Gray, E.A. Decker, Stabilization of soybean oil bodies by enzyme (laccase) cross-linking of adsorbed beet pectin coatings. J. Agric. Food Chem. **58**, 9259–9265 (2010). https://doi.org/10.1021/jf102082u
18. J.M. Abdullah, J. Weiss, H. Zhang, Recent advances in the composition, extraction and food applications of plant-derived oleosomes. Trends Food Sci. Technol **106**, 322–332 (2020). https://doi.org/10.1016/j.tifs.2020.10.029
19. C.V. Nikiforidis, Structure and functions of oleosomes (oil bodies). Adv. Colloid Interface Sci **274**, 102039 (2019). https://doi.org/10.1016/j.cis.2019.102039
20. J. Ding, J. Wen, J. Wang, R. Tian, R.L. Yu et al., The physicochemical properties and gastrointestinal fate of oleosomes from non-heated and heated soymilk. Food Hydrocoll **100**, 105418 (2020). https://doi.org/10.1016/j.foodhyd.2019.105418
21. M. Kirimlidou, A. Matsakidou, E. Scholten, C.V. Nikiforidis, V. Kiosseoglou, Composite gels structured by a gelatin protein matrix filled with oil bodies. Food Struct **14**, 46–51 (2017). https://doi.org/10.1016/j.foostr.2017.06.003
22. F. Zaaboul, H. Raza, C. Cao, L. Yuanfa, The impact of roasting, high pressure homogenization and sterilization on peanut milk and its oil bodies. Food Chem **280**, 270–277 (2019). https://doi.org/10.1016/j.foodchem.2018.12.047
23. K. Ghafoor, M.M. Özcan, F.A.L. Juhaımı, E.E. Babıker, Z.I. Sarker, I.A.M. Ahmed, M.A. Ahmed, Nutritional composition, extraction, and utilization of wheat germ oil: a review. Eur J Lipid Sci Technol **119**(7), 1600160 (2017). https://doi.org/10.1002/ejlt.201600160
24. J.T. Tzen, A.H. Huang, Surface structure and properties of plant seed oil bodies. J. Cell Biol **117**(2), 327–335 (1992). https://doi.org/10.1083/jcb.117.2.327
25. J. Zhao, X. Yang, W. Wang, J. Liang, Y. Orooji, C. Dai et al., Efficient sorbitol producing process through glucose hydrogenation catalyzed by ru supported amino poly (styrene-co-maleic) polymer (ASMA) encapsulated on γ-Al_2O_3. Catalysts **10**(9), 1068 (2020). https://doi.org/10.3390/catal10091068
26. Y. Orooji, H. Khojasteh, O. Amiri, M. Amiri, S. Hasanifard et al., A combination of hydrothermal, intercalation and electrochemical methods for the preparation of high-quality graphene: Characterization and using to prepare graphene-polyurethane nanocomposite. J. Alloys Compd **848**, 156495 (2020). https://doi.org/10.1016/j.jallcom.2020.156495
27. Y. Orooji, A. Movahedi, Z. Liu, M. Asadnia, E. Ghasali et al., Luminescent film: biofouling investigation of tetraphenylethylene blended polyethersulfone ultrafiltration membrane. Chemosphere **267**, 128871 (2021). https://doi.org/10.1016/j.chemosphere.2020.128871
28. İ Gülseren, A. Guri, M. Corredig, Effect of interfacial composition on uptake of curcumin–piperine mixtures in oil in water emulsions by Caco-2 cells. Food Funct. **5**(6), 1218–1223 (2014). https://doi.org/10.1039/C3FO60554J
29. Ö. Coşkun, B. Çakır, B. Vahapoğlu, İ Gülseren, Influence of extraction conditions on structural and functional characteristics of black cumin protein concentrates and ACE-inhibition in their hydrolyzates. J. Food Meas. Charact. **13**(3), 2328–2338 (2019). https://doi.org/10.1007/s11694-019-00152-1
30. L. Delmotte, C. Ganne-Chedeville, J.M. Leban, A. Pizzi, F. Pichelin, CP-MAS 13C NMR and FT-IR investigation of the degradation reactions of polymer constituents in wood welding. Polym. Degrad. Stab. **93**(2), 406–412 (2008). https://doi.org/10.1016/j.polymdegradstab.2007.11.020
31. D.J. Lacey, N. Wellner, F. Beaudoin, J.A. Napier, P.R. Shewry, Secondary structure of oleosins in oil bodies isolated from seeds of safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.). Biochem. J. **334**(2), 469–477 (1998). https://doi.org/10.1042/bj3340469
32. T. Riaz, N. Zeeshan, F. Zarif, K. Ilyas, N. Muhammad et al., FTIR analysis of natural and synthetic collagen. Appl. Spectrosc. Rev. **53**(9), 703–746 (2018)

33. S. Maurer, G. Waschatko, D. Schach, B.I. Zielbauer, J. Dahl et al., The role of intact oleosin for stabilization and function of oleosomes. J. Phys. Chem. B **117**(44), 13872–13883 (2013). https://doi.org/10.1021/jp403893n
34. B. Qi, J. Ding, Z. Wang, Y. Li, C. Ma et al., Deciphering the characteristics of soybean oleosome-associated protein in maintaining the stability of oleosomes as affected by pH. Food Res. Int. **100**, 551–557 (2017). https://doi.org/10.1016/j.foodres.2017.07.053