

**To Cite:** Ceylan, M M. (2023). Effect of high-pressure treatment on the stability of quinoa protein/canola oil nanoemulsions in terms of droplet size and lipid oxidation. *Journal of the Institute of Science and Technology*, 13(1), 298-304.

## Effect of High-Pressure Treatment on The Stability of Quinoa Protein/Canola Oil Nanoemulsions in Terms of Droplet Size and Lipid Oxidation

Mehmet Murat CEYLAN<sup>1\*</sup>

### Highlights:

- Quinoa protein mixed with canola oil
- A positive effect of quinoa protein on lipid oxidation of canola oil was determined.
- The potential of HPH method as an effective alternative to frequently used techniques for the modification of protein sources

### Keywords:

- Droplet size
- Quinoa protein isolate
- High-pressure homogenization
- Lipid oxidation
- Nanoemulsion

### ABSTRACT:

The stability characteristics of soluble nano-sized quinoa protein isolates prepared by different high-pressure homogenization in terms of droplet size and lipid oxidation were studied. Commercial quinoa protein isolates (QPI) were exposed to high-pressure homogenization (HPH) at various pressure (50, 100 and 200 MPa) and pressure cycles (one-cycle and two cycles). The quinoa isolate aggregates were utilized to produce oil-in-water nanoemulsions, which demonstrated the good stable characteristics for 28 day of storage at 4°C. While quinoa protein / canola oil nanoemulsions (QPCON) produced with untreated QPI and QPI samples by other HPH treatments were shown a significant increase in terms of droplet size and lipid oxidation in 28 days of storage, QPI samples treated with HPH at 100 MPa for a cycle (100 MPa-1) was found as the most efficient treatment in the stability of protein aggregate sizes and less oxidation level. The sample showed a slight increase from 98 nm to 117 nm for droplet size and from 58 to 102 mmol/kg for lipid oxidation from day 0 to day 28.

<sup>1</sup>Mehmet Murat CEYLAN ([Orcid ID: 0000-0002-8391-1680](https://orcid.org/0000-0002-8391-1680)), İğdır University, Faculty of Engineering, Department of Food Engineering, İğdır, Türkiye

\*Corresponding Author: Mehmet Murat CEYLAN, e-mail: m.murat.ceylan@igdir.edu.tr

## INTRODUCTION

Among plant-based protein sources, quinoas (*Chenopodium quinoa* wild.) are an annual herbaceous flower plant which show satisfactory nutritional and functional features because of their higher standard of protein quality with a broad amino acid composition, especially rich in lysine content. The mature quinoa seeds especially consist of 11S-type globulin defined as chenopodin, consisting of around 40% of the total protein content, and 2S-type albumin comprising about 35% of the seed proteins stabilized via disulfide bonds as well. In addition, prolamin content of quinoa seeds is comparatively low (0.5-7% of total proteins) which make them acceptable for patients who suffer from celiac disease (Dakhili et al., 2019). The predominant storage protein in quinoa is globulin, which is largely not soluble in H<sub>2</sub>O. The inadequate soluble contents of globulin in quinoa sources make it less efficient emulsifying agent compared to dairy protein sources, for instance, casein (Nishinari et al., 2014).

Although microorganisms and bacteria are effectively controlled by heat application, preservation methods that are less harmful to food are being researched since the applied heat treatments change the natural taste and aroma of the food and destroy the vitamins in food materials. The main purpose of new food processing methods is to produce higher-quality, less processed, more natural, healthier food products with less additives. One of these new methods is the high-pressure homogenization (HPH) (Angsupanich and Ledward, 1998; Anema et al., 2005a; Yildiz et al., 2017). Traditionally used thermal methods are usually utilized in the food sector for the purpose of extension of shelf life of food materials and to ensure food safety by inactivating pathogenic microorganisms (Anema et al., 2005b; Yıldız, 2022). This applied heat (energy) may lead to the formation of by-products or undesirable changes, resulting in undesirable reactions in food. Therefore, new methods that do less harm with less additives can be used in food without disturbing the flavor structure of the product.

Increasing demand not only for extending shelf life, but also for food quality and sensory needs has led to the use of non-thermal methods as food preservation methods (Arques et al., 2015; Jiang et al., 2019). Non-thermal treatments can preserve food effectively without reducing quality and require less processing time. The main purpose of food preservation with non-thermal processing methods is to produce products that are microbially safe, acceptable to the consumer, and with increased "fresh-like" quality and shelf life. Between the non-thermal techniques: high pressure homogenization (HPH), ultrasound, ultraviolet light (UV), microwave, pushed-electric field (PEF), high-intensity pulsed light, magnetic field and ozone applications. The high-pressure processing is perhaps the most widely used one. HPH is a process made by exposing liquid or solid foods to a pressure of 100–1000 MPa, with or without packaging. Although pressures of more than 1000 MPa can be applied to foods in research, HPH equipment designed today is produced with a feature that can apply a maximum pressure of 600 MPa (Minerich and Labuza, 2003).

Quinoa protein isolates (QPIs) are wonderful candidates as a food supplement and functional food. However, there are still needs more advanced researches in order to enhance and prove their functional characteristics to be appropriate for using in food additives and processing techniques. The purpose of the work was to improve quinoa protein nano-aggregates created by HPH technique and use as an alternative to animal-based proteins with improved functional properties.

## MATERIALS AND METHODS

### High-Pressure Homogenization and Nanoemulsion Production of QPI Samples

Nine grams of QPI was solved in 300 mL distilled water and agitated at ambient conditions for half an hour to gain protein solutions. Totally, 6 treatments were applied to QPI solutions. The samples names and treatments are shown in Table 1. In HPH treatment, the HPH process was employed via a high-pressure homogenizer (APV two step homogenizers; SX Liquid Technolog, Denmark) at 50, 100 and 200 MPa for one-cycle and two cycles along with 300 mL protein solutions consisting of 9 grams QPI. Subsequent to treatments, QPI solutions were exposed to centrifugation (Sorval ST 16R type of centrifugation, Thermo-Scientific, Waltham, MA, USA) at 14,000 rpm for 15 minutes. Supernatants were gathered as soluble quinoa proteins for additional analysis.

QPI nanoemulsions were prepared by using canola oil with untreated and HPH treated-QPI samples. Canola oils (0.25 g) were mixed with 50 mL soluble QPI samples and strongly stirred for 5 minutes using a magnet stirrer. For the achievement of desired homogenization of QPI nanoemulsions, the sample was exposed to ultrasound process for 5 min. Ultrasonication (US) application was employed by the help of ultrasonic probe at the frequency of 20 kHz (Sonics & Materials, Inc., Newtown, CT, USA).

**Table 1.** Sample names and treatments

Treatments	Explanation
Untreated QPI	QPI with no treatment
50 MPa-1	QPI samples (QPIs) treated with HPH at 50 MPa for a cycle
50 MPa-2	QPIs treated with HPH at 50 MPa for two cycles
100 MPa-1	QPIs treated with HPH at 100 MPa for a cycle
100 MPa-2	QPIs treated with HPH at 100 MPa for two cycles
200 MPa-1	QPIs treated with HPH at 200 MPa for a cycle
200 MPa-2	QPIs treated with HPH at 200 MPa for two cycles

### Stability of quinoa protein / canola oil nanoemulsions (QPCONs) (droplet size)

Droplet sizes of QPI-stabilized nanoemulsions, by using untreated QPI and QPI samples exposed to HPH treatments as a wall material, at an oil ratio of 0.25 (w/w) were measured. Droplet size of QPCON samples was determined by DLS according to the procedure figured out in the work of Lee et al. (2016). QPCON samples exposed to dilution step by 500-fold with distilled water earlier than analysis. The droplet size measurement of QPCON samples was performed at room temperature.

The stabilities of the QPCONs were determined via following the particle sizes of QPCON samples over a time span of 28 days under 4 °C.

### Lipid Oxidation of QPCONs

The occurrences of lipid hydroperoxides during storage of quinoa protein / canola oil nanoemulsions were measured according to the method declared in the study of Min et al. (2003). Quinoa protein / canola oil nanoemulsion (5 ml) was put in slightly sealed screw-cap experiment tubes and exposed to oxidation process under 37 °C in the dark environment, away from light. The formation of lipid hydroperoxide was determined via blending 0.5 ml of quinoa protein emulsion and 2.5 ml of isooctane and 2-propanol with the rate of 3:1 (v/v) by blending during 10 seconds (3 times in total) and getting solvent isolate thanks to centrifugation at 14.000 rpm for 2 minutes. The obtained isolated organic solvent (200 µl) were blended with 3 mL of metanol and butanol dispersion with the ratio of 2:1 (v/v), and sequenced by 15 µl of 3.97 M amonium thicyanate and 15 µl of ferous iron dispersion (made via blending 0.15 M BaCl<sub>2</sub> and 0.15 M FeSO<sub>4</sub>). Following to 20 minutes, the absorbance values of QPCONs were determined under the wavelengths of 510 nm. The measurement of lipid

hydroperoxide of quinoa protein / canola oil nanoemulsion was achieved at first, third, fifth, seventh, fourteenth, twenty-first and twenty-eight days.

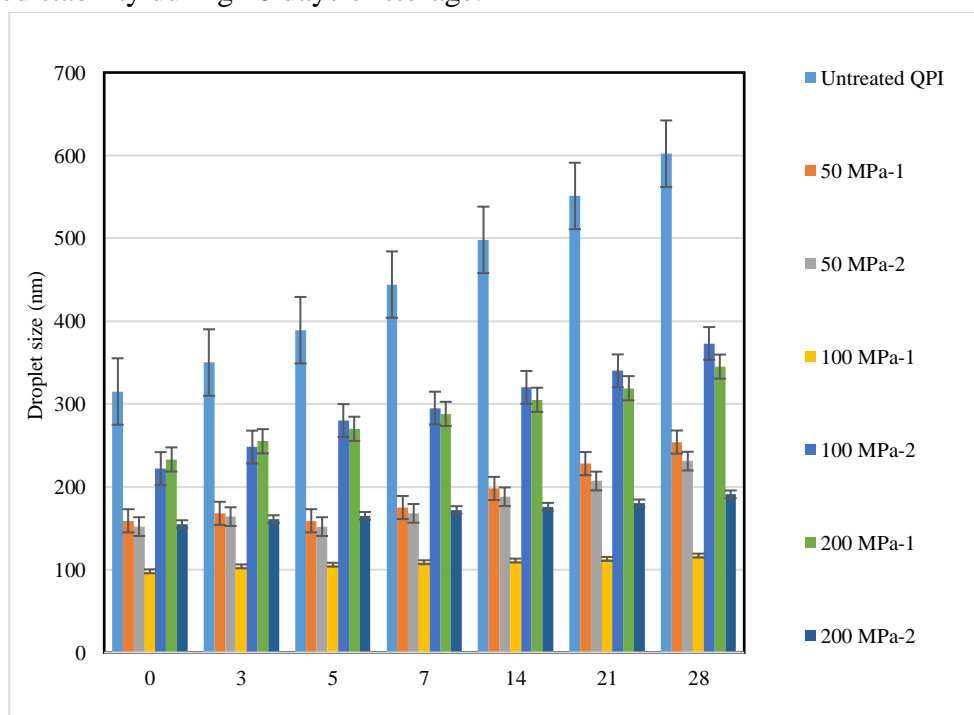
### Statistical Analysis

In total 3 different samples for every treatment were utilized for all above analysis measurements. The difference was determined by using General Linear Model in SAS programme (version 9.3, SAS Institute, Incorporation, North Carolina, USA). A significant difference between the average values was defined by LSD test (Least significant difference test) of  $<0.05$ .

## RESULTS AND DISCUSSION

### Droplet Size of Quinoa Protein / Canola Oil Nanoemulsions

Droplet sizes of the quinoa protein nanoemulsion, using untreated QPI and QPI samples made HPH process as wall materials, at an oil ratio of 0.25% were measured. The droplet sizes of all QPCON samples showed an increase with a time. The droplet sizes of QPCON samples at last day (28<sup>th</sup> days) were found bigger with the comparison of QPCON samples at first day (Day 0). The stabilities of QPCON samples were analyzed by measuring the droplet sizes of QPI samples during 28 d of period under 4 °C, as can see in Figure 1. The particle sizes of QPCON samples made with HPH at 100 MPa for a cycle (100 MPa-1) were shown a slight increase from 98 nm to 117 nm from first day to 28<sup>th</sup> days at 0.25% oil concentration (Figure 1). On the other hand, QPCON samples produced with untreated QPI and QPI samples by other HPH conditions were shown a significant increase at 0.25% concentrations in 28 d for especially QPCON samples made with untreated QPI samples. The droplet sizes of QPCON samples made with untreated QPI samples were shown a significant increase from 315 nm to 602 nm from first day to 28<sup>th</sup> days at 0.25% oil concentration. In overall, QPCON samples made with HPH treatment at 100 MPa for a cycle (100 MPa-1) at 0.25% oil concentration showed a relatively good stability during 28 days of storage.



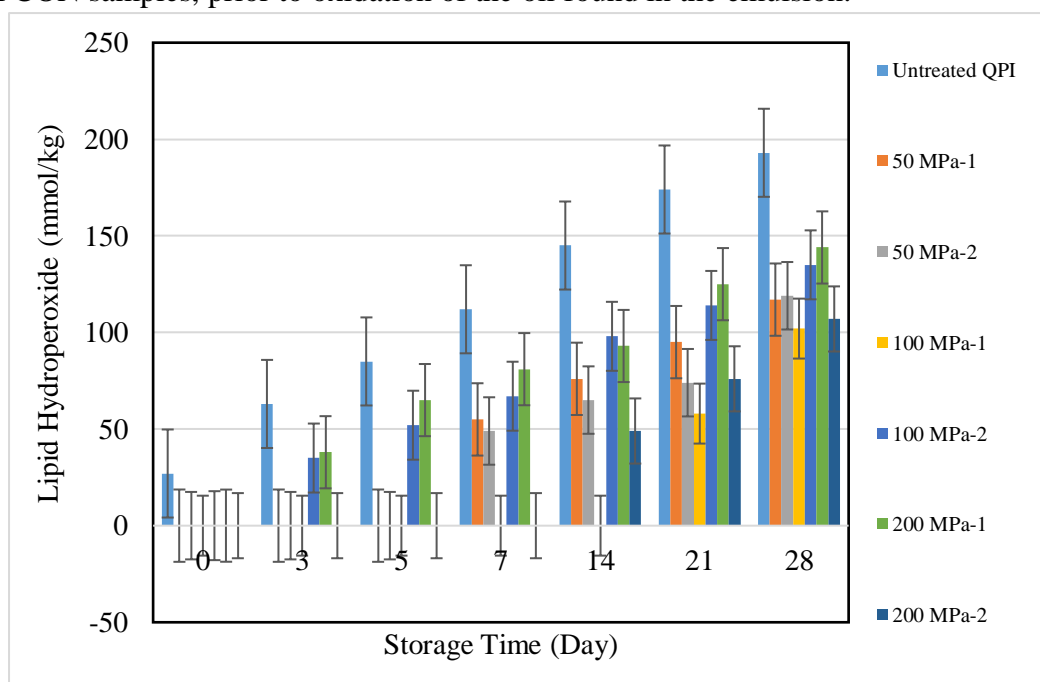
**Figure 1.** Droplet size of QPCONs with an oil concentration of 0.25% during storage at 4 °C for 28 days

Proteins, which have a very important place on the quality of food, are one of the most important elements of food. That's why the changes that may occur in the protein complex can also impact the

quality of the foodstuffs (Yildiz et al., 2018). The influence of HPH on proteins relies on the amount of pressure applied, the structure of the protein and the environment (temperature, pH, ionic strength) of the protein (Su et al., 2021). The employment of HPH may lead variations in the particle size of proteins. While HPH application accelerates the reactions and processes that accompany the decrease in the volume of the protein, it also inhibits the reactions that cause the increase in particle sizes. It has been suggested that the smaller particle size is due to either the breaking or formation of non-covalent bond and the rearrangement of solvent molecules (Wan et al., 2022; Luo et al., 2022).

### Lipid Oxidation of Quinoa Protein / Canola Oil Nanoemulsions

Lipid hydroperoxides of QPCONs at an oil concentration of 0.25% for four weeks (28 days, 672 hours in total) kept at 37 °C are demonstrated in Figure 2. No and/or negligible lipid oxidation was determined in the QPCON samples till the day of 21 for the QPCON samples prepared with HPH at 100 MPa for a cycle (100 MPa-1) at 0.25% oil concentration. A significantly higher lipid oxidation values starting from 21<sup>st</sup> days to 28<sup>th</sup> days for the QPCON samples were observed. It can be stated that the capability of quinoa protein which acts as a chemical obstruction in order to postpone the lipid oxidation occurrence is proved in the first 504 hours of storage period. In addition, no lipid oxidation was determined up till 7<sup>th</sup> days for the QPCON samples prepared with HPH at 200 MPa for two cycles (200 MPa-2) with the oil concentration of 0.25%. Similarly, no lipid oxidation was determined for 50 MPa-1 and 50 MPa-2 samples until day 5. On the other hand, QPCON samples produced with untreated QPI and QPI samples by other HPH treatments were shown a significant increase at 0.25% oil concentration in 28 days for especially QPCON samples made with untreated QPI samples (Figure2). Ceylan (2022), reported similar results with present study. It is well-known that amino acid molecules including cysteine in proteins could be oxidized more than lipid compounds (Gulseren et al., 2007; Yildiz et al., 2017). The relatively higher free SH bonds in the quinoa protein samples exposed to HPH treatment could postpone the lipid oxidation process. So, encapsulating the secondary metabolites by quinoa protein nanoemulsions needed to be accomplished within 504 h following the making of QPCON samples, prior to oxidation of the oil found in the emulsion.



**Figure 2.** Lipid hydroperoxide values of QPCONs with an oil concentration of 0.25% during storage at 37 °C for 28 days (672 h)

## CONCLUSION

A novel technology, high-pressure homogenization (HPH), was offered and analyzed in current work to make functionalized nano-sized quinoa protein isolates. QPI samples treated with HPH at 100 MPa for a cycle (100 MPa-1) was found as the most efficient treatment in the stability of protein aggregate sizes and less oxidation level. The sample showed a slight increase from 98 nm to 117 nm for droplet size and from 58 to 102 mmol/kg for lipid oxidation from day 0 to day 28. The results from this research showed the potential of HPH method as an effective alternative to frequently used techniques for the modification of protein sources. The improved quinoa proteins created by current method could be preferred as an alternative to animal-based proteins with improved physicochemical characteristics.

## Conflict of Interest

The author declared that there is no conflict of interest.

## Author's Contributions

Planned and designed the study, collected and analyzed data, made statistical analyzes and wrote the article.

## REFERENCES

- Angsupanich, K., & Ledward, D.A. (1998). High pressure treatment effects on cod (*Gadus morhua*) muscle. *Food Chemistry*, 63 (1), 39–50. [https://doi.org/10.1016/S0308-8146\(97\)00234-3](https://doi.org/10.1016/S0308-8146(97)00234-3)
- Anema, S. G., Lowe, E. K., & Stockmann, R. (2005a). Particle size changes and casein solubilisation in high-pressure-treated skim milk. *Food Hydrocolloids*, 19, 257–267. <https://doi.org/10.1016/j.foodhyd.2004.04.025>
- Anema, S. G., Lauber, S., Lee, S. K., Henle, T., & Klostermeyer, H. (2005b). Rheological properties of acid gels prepared from pressure and transglutaminase-treated skim milk. *Food Hydrocolloids*, 19, 879–887. <https://doi.org/10.1016/j.foodhyd.2004.12.001>
- Arques, J. L., Rodriguez, E., Gaya, P., Medina, M., Guamis, B., & Nunez, M. (2005). Inactivation of *Staphylococcus aureus* in raw milk cheese by combinations of high-pressure treatments and bacteriocin producing lactic acid bacteria. *Journal of Applied Microbiology*, 98, 254–260. <https://doi.org/10.1111/j.1365-2672.2004.02507.x>
- Ceylan, M.M., (2022). The influence of high pressure homogenization and high- intensity ultrasound on the functional properties of whey-protein/canola oil nanoemulsions during storage. *Harran Tarım ve Gıda Bilimleri Dergisi*, 26(2): 237-243. DOI:10.29050/harranziraat.1079031
- Dakhili, S., Abdolalizadeh, L., Hosseini, S.M., Shojaee-Aliabadi, S., & Mirmoghtadaie, L. (2019). Quinoa protein: Composition, structure and functional properties. *Food Chemistry*, 299, 125161. <https://doi.org/10.1016/j.foodchem.2019.125161>
- Gulseren, I., Guzey, D., Bruce, B.D., & Weiss, J. (2007). Structural and functional changes in ultrasonicated bovine serum albumin solutions. *Ultrasonics Sonochemistry*, 14, 173–183. <https://doi.org/10.1016/j.ultsonch.2005.07.006>
- Jiang, S., Yildiz, G., Ding, J., Andrade, J., Rababah, T.M., Almajwal, A., Abulmeatyc, M.M., & Feng, H. (2019). Pea Protein Nanoemulsion and Nanocomplex as Carriers for Protection of Cholecalciferol (vitamin D3). *Food and Bioprocess Technology*, 12 (6), 1031-1040. <https://doi.org/10.1007/s11947-019-02276-0>
- Lee, H., Yildiz, G., Dos Santos, L.C., Jiang, S., Andrade, J., Engeseth, N.C., & Feng, H. (2016). Soy protein nano-aggregates with improved functional properties prepared by sequential pH treatment and ultrasonication. *Food Hydrocolloids*, 55, 200–209. <https://doi.org/10.1016/j.foodhyd.2015.11.022>

- Luo, L., Wang, Z., Deng, Y., Wei, Z., Zhang, Y., Tang, X., Liu, G., Zhou, P., Zhao, Z., Zhang, M., & Li, P. (2022). High-pressure homogenization: A potential technique for transforming insoluble pea protein isolates into soluble aggregates. *Food Chemistry*, 397, 133684. <https://doi.org/10.1016/j.foodchem.2022.133684>
- Min, H., McClements, D.J., & Decker, E.A. (2003). Lipid oxidation in corn oil-in-water emulsions stabilized by casein, whey protein isolate, and soy protein isolate. *Journal of Agricultural and Food Chemistry*, 51, 1696–1700. <https://doi.org/10.1021/jf020952j>
- Minerich, P.L., & Labuza, T.P. (2003). Development of a Pressure Indicator for High Hydrostatic Pressure Processing of Foods. *Innovative Food Science and Emerging Technologies*, 4, 235–243. [https://doi.org/10.1016/S1466-8564\(03\)00023-7](https://doi.org/10.1016/S1466-8564(03)00023-7)
- Nishinari, K., Fang, Y., Guo, S., & Phillips, G.O. (2014). Soy proteins: a review on composition, aggregation and emulsification. *Food Hydrocolloids*, 39, 301–318. <https://doi.org/10.1016/j.foodhyd.2014.01.013>
- Su, C., He, Z., Wang, Z., Zhang, D., & Li, H. (2021). Aggregation and deaggregation: The effect of high-pressure homogenization cycles on myofibrillar proteins aqueous solution. *International Journal of Biological Macromolecules*, 189, 567-576. <https://doi.org/10.1016/j.ijbiomac.2021.08.133>
- Wang, Y.L., Yang, J.J., Dai, S.C., Tong, X.H., Tian, T., Liang, C.C., Li, L., Wang, H., Jiang, L.Z. (2022). Formation of soybean protein isolate-hawthorn flavonoids non-covalent complexes: Linking the physicochemical properties and emulsifying properties. *Ultrasonics Sonochemistry*, 84, 105961. <https://doi.org/10.1016/j.ultsonch.2022.105961>
- Yildiz, G. (2022). Changes in Emulsifying Properties, Droplet Size, Turbidity and Lipid Oxidation of Pea Protein Nanoemulsions Exposed to High-Intensity Ultrasound and High-Pressure Homogenization during Storage. *Akademik Gıda*, 20 (4), 336-342. <https://doi.org/10.24323/akademik-gida.1224299>
- Yildiz, G., Andrade, J., Engeseth, N. E., & Feng, H. (2017). Functionalizing soy protein nano-aggregates with pH shifting and mano-thermo-sonication. *Journal of Colloid and Interface Science*, 505, 836–846. <https://doi.org/10.1016/j.jcis.2017.06.088>
- Yildiz, G., Ding, J., Andrade, J., Engeseth, N.J., & Feng, H. (2018). Effect of plant protein-polysaccharide complexes produced by mano-thermo-sonication and pH-shifting on the structure and stability of oil-in-water emulsions. *Innovative Food Science and Emerging Technologies*, 47, 317-325. <https://doi.org/10.1016/j.ifset.2018.03.005>