Evaluation of Lipid Peroxidation of Liposomes and Cooking Oils in Various Conditions

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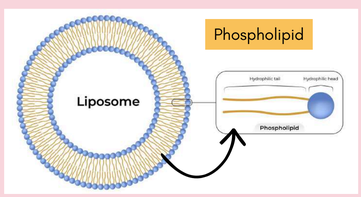
Abstract

Lipid peroxidation is a major oxidative degradation process affecting lipid-rich systems such as liposomes and cooking oils, leading to reduced stability and functionality. Therefore, reliable evaluation of lipid oxidative stability is crucial. This study aimed to evaluate lipid peroxidation in liposomes and cooking oils under various conditions using the thiobarbituric acid reactive substances (TBARS) assay, which measures malondialdehyde (MDA) as an indicator of lipid peroxidation. In this study, liposomes composed of soy phosphatidylcholine (SoyPC) and cholesterol at different molar ratios were prepared and subjected to varying storage durations, NaCl supplementation, and freeze-thaw treatments, and stability was assessed using the TBARS assay together with particle size, zeta potential, and polydispersity index (PDI). Cooking oils, including soybean oil and lard, were analyzed under storage conditions, air exposure, and heat treatment. The results demonstrated that lipid composition and preparation conditions significantly influenced oxidative stability in liposomes, and liposomes with different SoyPC:cholesterol ratios showed increased MDA production over time. The SoyPC:cholesterol (10:1) liposomes exhibited the greatest susceptibility to oxidation during prolonged storage but had the highest physical stability, based on its zeta potential. Freeze-thaw treatment increased MDA formation, indicating reduced membrane stability. In cooking oils, lard exhibited higher MDA levels than soybean oil during storage, whereas air exposure reduced MDA levels, possibly due to the loss of volatile oxidation products, and heat treatment induced greater lipid peroxidation in soybean oil due to its higher polyunsaturated fatty acid content. Overall, these findings underscore the necessity of optimizing lipid composition and storage environments to minimize oxidative damage. This study provides a foundational for improving the shelf-life of pharmaceutical liposomal formulations and enhancing the quality control of cooking oils in the food industry.

Introduction

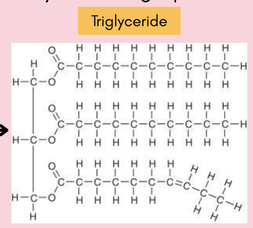
- **Liposome**: A phospholipid nanovesicle for drug/gene delivery that protects therapeutic agents from degradation.
- **Cooking oil**: A triglyceride-based lipid from plant/animal sources used as a major dietary fat.

Both **liposomes** and **cooking oils** contain polyunsaturated fatty acids (PUFAs), which are highly prone to oxidation. This susceptibility leads to a process known as Lipid Peroxidation, a major oxidative degradation pathway affecting lipid-rich systems.



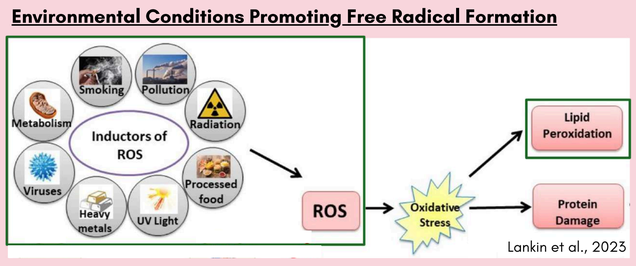


Triglyceride

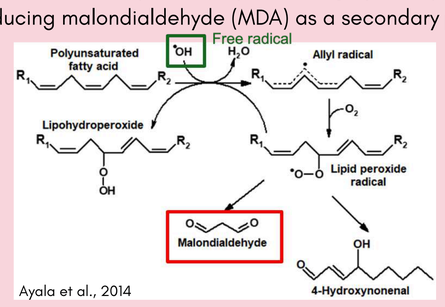


- **Stability Challenges**: High PUFA content in liposomes and cooking oils makes them vulnerable to free radical attack. This triggers lipid peroxidation, leading to malondialdehyde (MDA) formation, which compromises system integrity and quality .
- **Lipid peroxidation** involves the oxidative degradation of PUFAs, producing malondialdehyde (MDA) as a secondary product.

Environmental Conditions Promoting Free Radical Formation

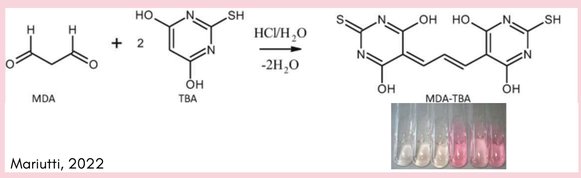


Lankin et al., 2023

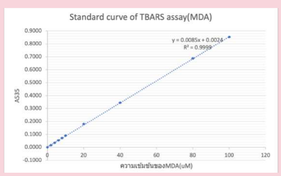


Ayala et al., 2014

- The **TBARS assay** is widely used to quantify MDA as an indicator of lipid peroxidation.



Mariutti, 2022

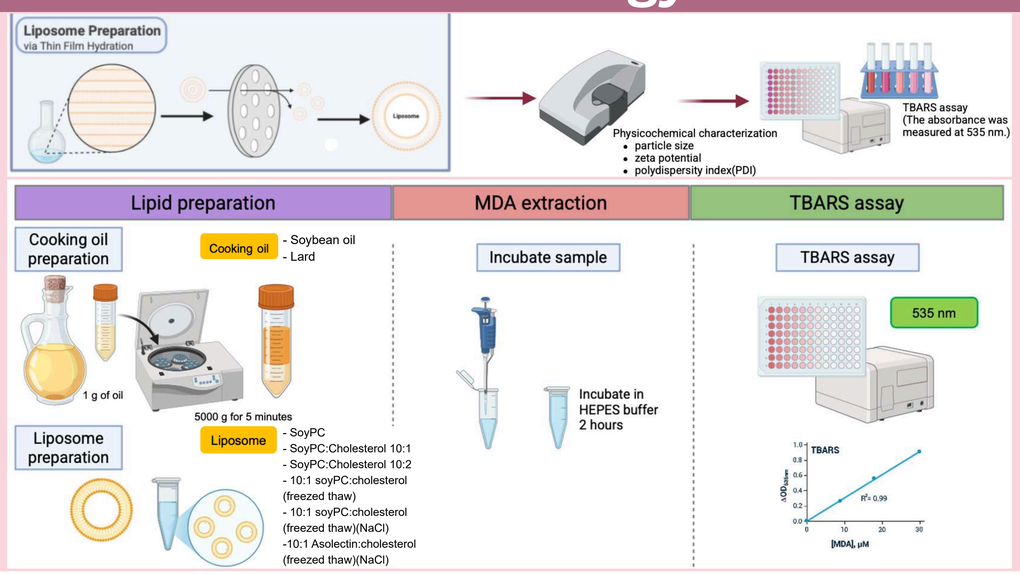


This study evaluated lipid peroxidation in liposomes and cooking oils in various conditions.

Objectives

- To validate TBARS assay protocol for evaluation lipid peroxidation.
- To compare the physicochemical stability of liposomes under different conditions and evaluate lipid peroxidation as an indicator of oxidative degradation.
- To assess oxidative degradation in cooking oils under different conditions.

Methodology



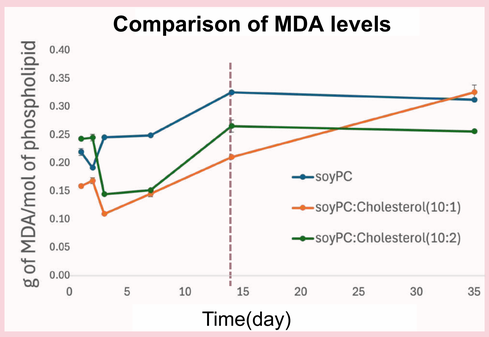
Results & Discussion

Liposomes

- **Vary time points and lipid composition**

Samples	Particle size (nm)	Zeta potential (mV)	PDI
10:0 soyPC:cholesterol	124.5 ± 2.93	-26.9 ± 2.5	0.142 ± 0.029
10:1 soyPC:cholesterol	117.0 ± 4.80	-49.9 ± 4.5	0.180 ± 0.040
10:2 soyPC:cholesterol	133.1 ± 5.22	-31.0 ± 3.2	0.117 ± 0.037
± Standard Deviation (SD), n=3			

10:1 SoyPC:Cholesterol was chosen because it showed the best physicochemical properties, including particle size closest to 100 nm and and the highest absolute zeta potential.



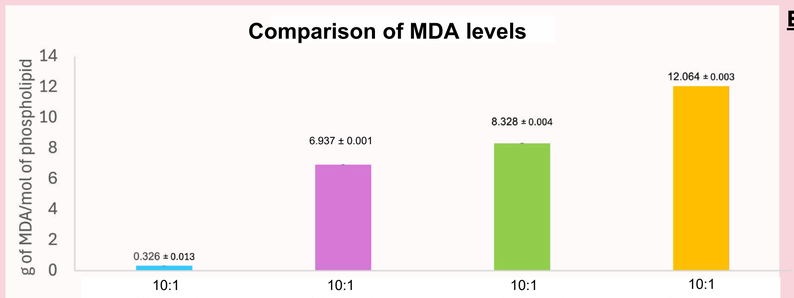
TBARS assay (Lipid Peroxidation)
Malondialdehyde(MDA) levels accelerated after two weeks, indicating progressive lipid oxidation during storage.

Results & Discussion

Liposomes

- **Comparison in different preparation conditions**

Samples	Lipid peroxidation	Physicochemical properties		
	g of MDA/mol of Phospholipid	Particle size (nm)	Zeta potential (mV)	PDI
10:1 soyPC:cholesterol	0.326 ± 0.013	117.0 ± 4.80	-49.9 ± 4.5	0.180 ± 0.040
10:1 soyPC:cholesterol (freezed thaw)	12.064 ± 0.003	100.5 ± 2.13	-64.1 ± 7.3	0.195 ± 0.033
10:1 soyPC:cholesterol (freezed thaw)(NaCl)	6.937 ± 0.001	88.2 ± 2.12	2.2 ± 1.9	0.070 ± 0.058
10:1 Asolectin:cholesterol (freezed thaw)(NaCl)	8.328 ± 0.004	105.6 ± 1.56	-40.7 ± 11.9	0.180 ± 0.033
± Standard Deviation (SD), n=3				



Overall, freeze-thaw improves the physical stability of liposomes, while salt mainly helps moderate oxidative degradation.

Effect of Freeze-Thaw Treatment

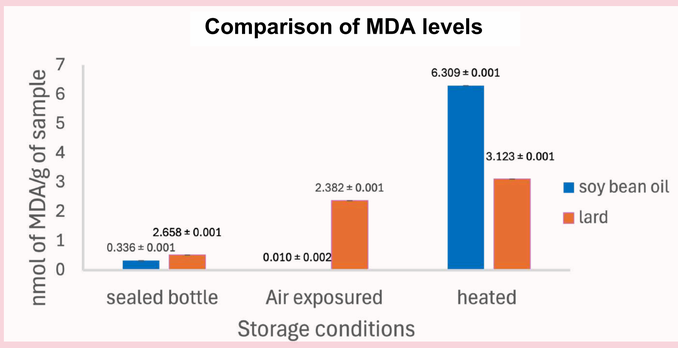
- 10:1 soyPC:cholesterol (freezed thaw) produced the highest MDA level, indicating the greatest lipid peroxidation, likely due to membrane stress during freezing and thawing.
- Despite increased oxidation, this condition also showed the best physicochemical stability, with particle size closest to 100 nm and the highest absolute zeta potential.

Effect of Sodium Chloride (NaCl)

- NaCl addition during freeze-thaw reduced MDA levels, suggesting a partial stabilization against lipid oxidation.

Cooking oils

Conditions	Lipid peroxidation (g of MDA/g of Sample)	
	Soybean oil	Lard
sealed bottle	0.336 ± 0.001	2.658 ± 0.001
Air exposured	0.010 ± 0.002	2.382 ± 0.001
Heated	6.309 ± 0.001	3.123 ± 0.001
± Standard Deviation (SD), n=3		



Comparison among the same type of oil

- Soy bean oil : Heated > sealed bottle > Air exposured
- Lard : Heated > Air exposure > sealed bottle

- Heating produced the highest MDA levels, whereas air exposure showed lower measured MDA, possibly due to partial loss of MDA into the air.

Conclusion

TBARS assay

- Suitable for evaluating lipid peroxidation in both liposomes and cooking oils.
- Effective for comparative assessment of lipid oxidation trends.

Lipid Peroxidation in Different Lipid Systems

Liposome

- Lipid peroxidation is strongly influenced by lipid type, composition, and environmental conditions.
- In the liposome system, the SoyPC:Cholesterol ratio affected both physical stability and oxidative behavior.
  - The 10:1 SoyPC:Cholesterol formulation showed good particle stability.
  - The 10:2 SoyPC:Cholesterol formulation exhibited the lowest long-term increase in MDA.
- Freeze-thaw cycles and NaCl addition enhanced lipid oxidation due to membrane stress.

Cooking oil

- In soy bean oil, heating was the most significant factor promoting lipid oxidation, followed by air exposure.
- In lard, heating was the most significant factor promoting lipid oxidation, followed by sealed bottle.

Acknowledgements

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