Dairy proteins as color stabilizers

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2/26/26

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Natural and Functional Foods

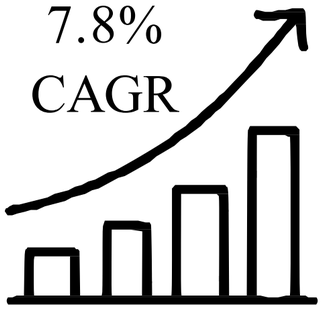
↑ concern of artificial ingredients, colorants, and additives

(Chang & Chen, 2022)

Natural :

- simple
- less processed
- “green”

(Lusk, 2019.)



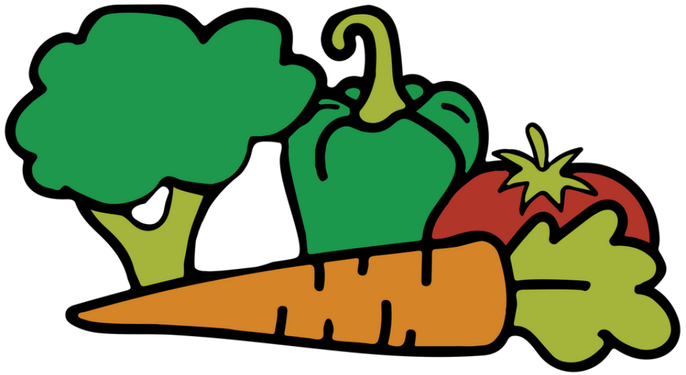
(Natural Food and Drinks Market [2033] Trends, Scope & Growth Report, n.d.)

Functional:

- bioactive compounds
- beyond nutrition
- health benefits

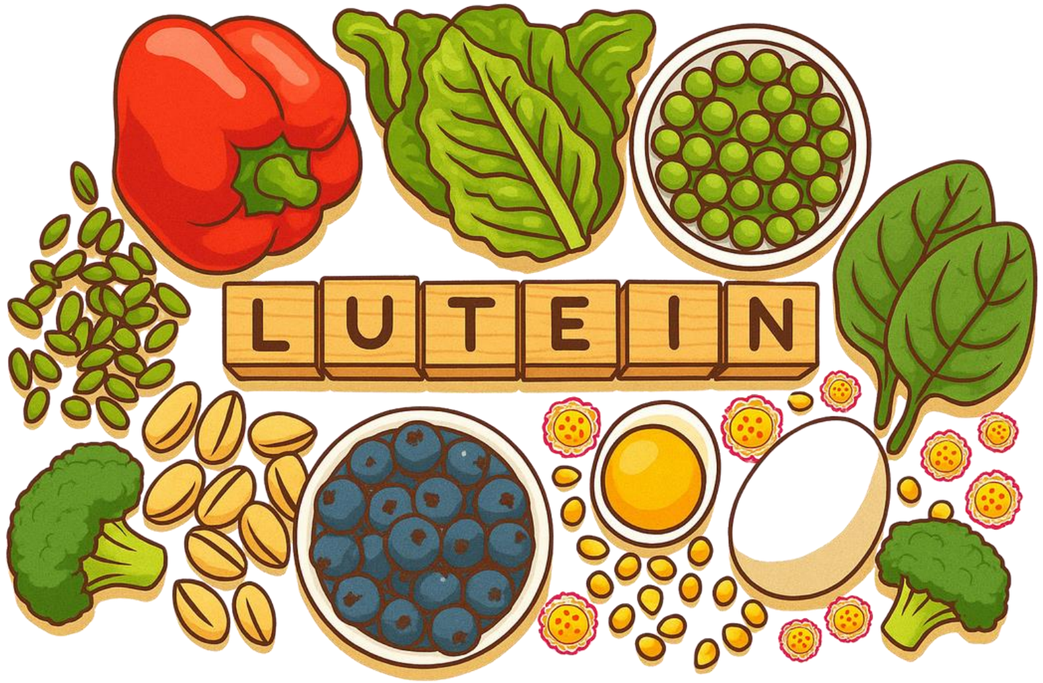
(USDA, n.d.)

(USDA Functional Foods Market Size & Trends Analysis Report, 2024, n.d.)



Lutein

- Carotenoid found in egg yolks, marigolds, and dark leafy vegetables
- Concentrated **yellow-gold pigment**
- Decreases cataracts and age-related macular degeneration
- ↓ solubility, instability

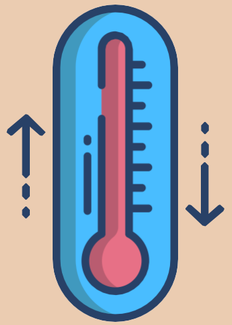


(Gumus et al., 2016; Bazana et al., 2019)

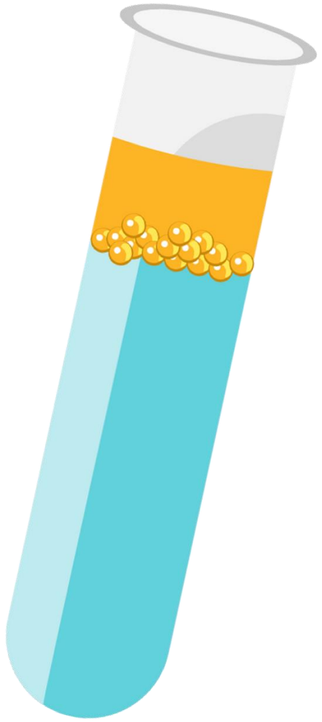
Emulsion-based Delivery Systems

One immiscible liquid dispersed as small droplets in another immiscible liquid (O/W)

Increase solubility



Thermodynamically unstable



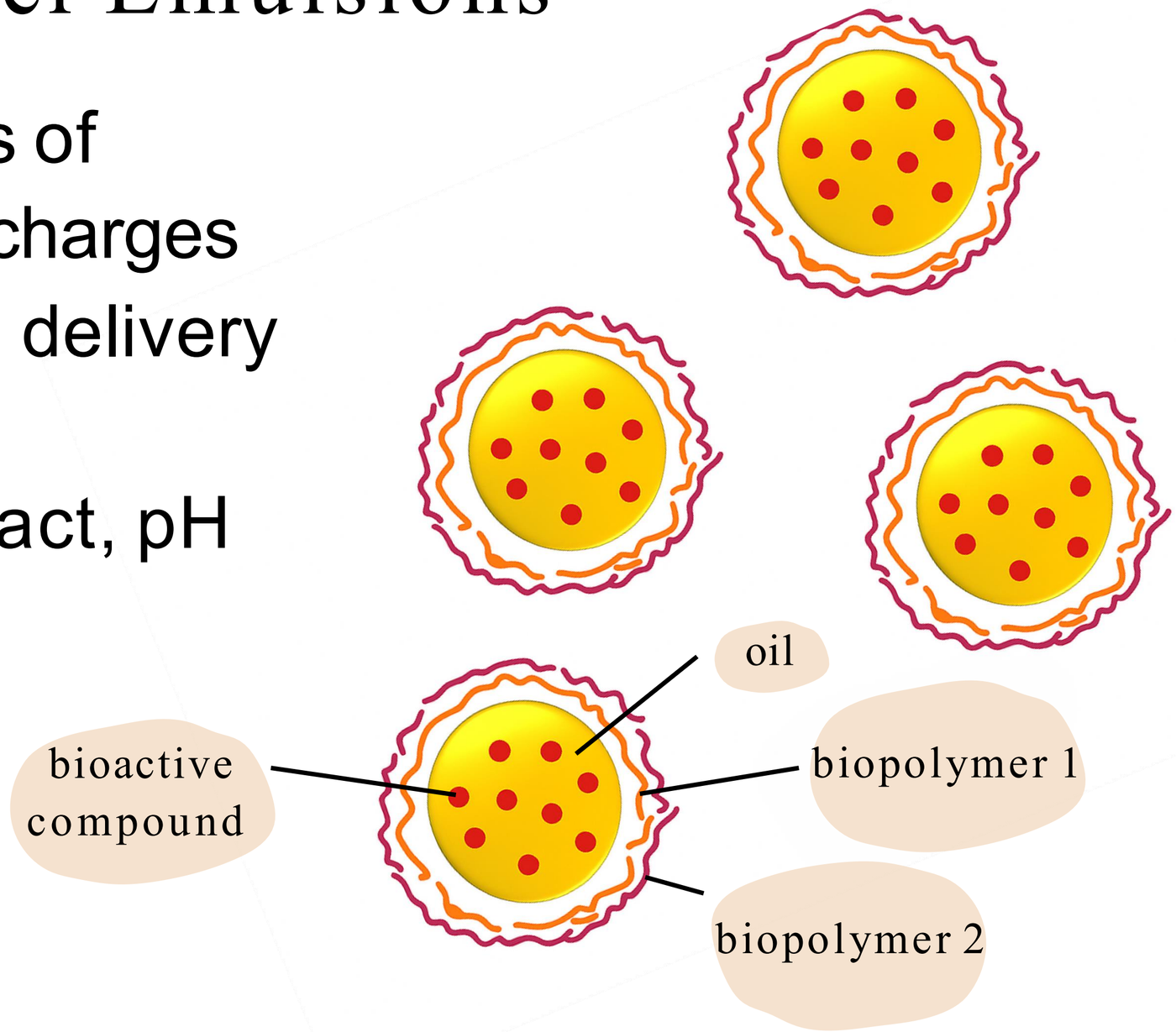
(de Carvalho-Guimarães et al., 2022)

Multilayer Emulsions

Oil droplets coated by layers of biopolymers of alternating charges

- **High physical stability**, delivery of bioactive
- Composition = high impact, pH dependent

(Mukherjee et al., 2009; Tan & McClements, 2021)



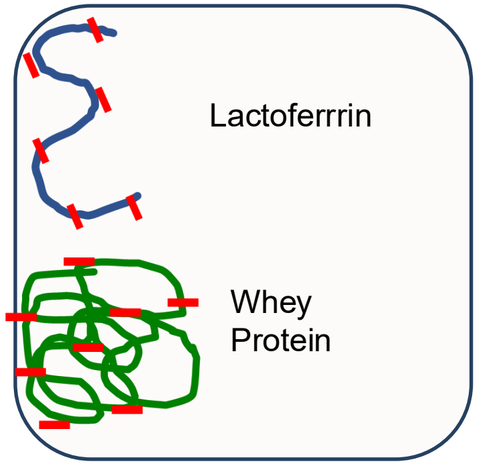
Proteins

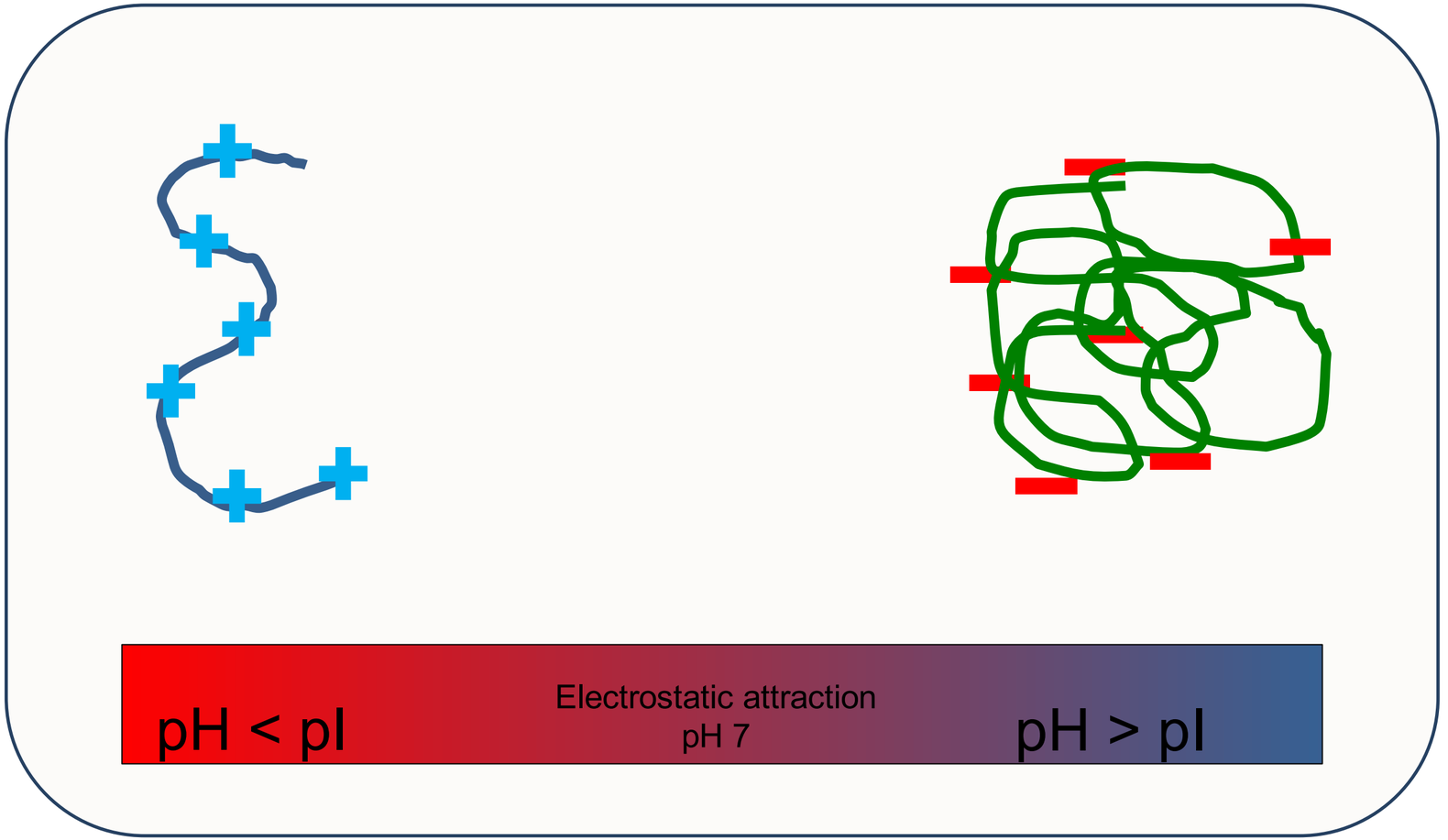
- Dairy Proteins: **whey protein isolate (WPI) & lactoferrin (LF)**
  - natural emulsifiers
  - byproducts of cheese production
- Lactoferrin
  - **pI= 8.0-9.0**
  - (+) at neutral pH
  - affinity for iron
- WPI
  - **pI= 4.0-6.0**

(Elzoghby et al., 2020; Matsuyama et al., 2021; Pires et al., 2021; Ye & Singh, 2007; Zhong et al., 2021)

WPI- Lactoferrin Complexes

- Spontaneous
- Low-energy
- All-natural





Research Question

Will the use of multiple protein layers improve the stability of carotenoid-enriched emulsions?

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Objective 1

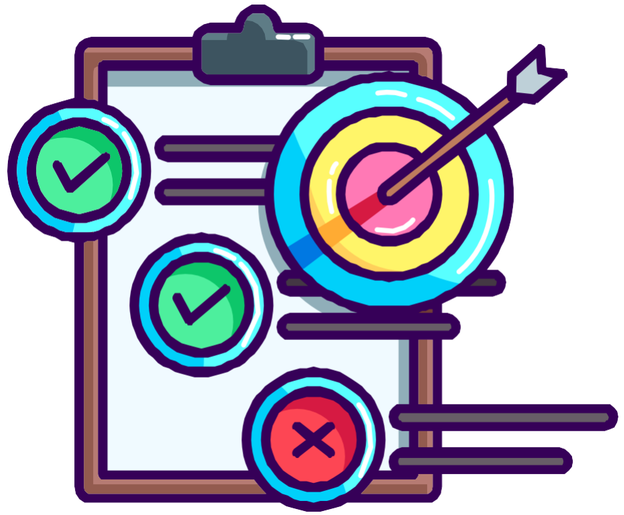
Determine the optimal conditions to form multilayer emulsions.

Independent Variables

- Emulsifier
  - WPI
  - LF
- Concentration of proteins
  - 1-2%
  - 0-2%
- Storage Temperature
  - 4℃
  - 25℃
  - 37℃

Dependent Variables

- Zeta Potential
- Particle Diameter & Distribution



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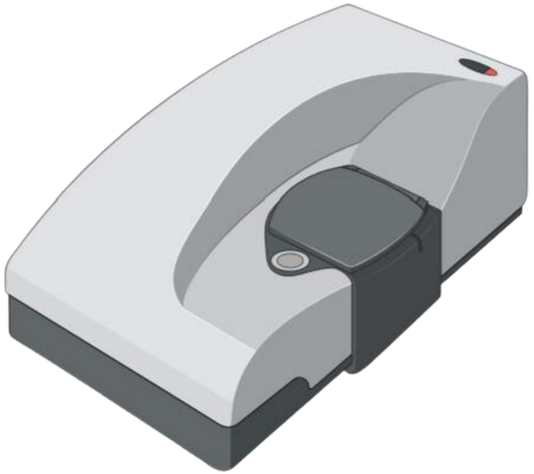
Zeta Potential Profile of Proteins

2% (w/w) protein solutions were made

- WPI & LF 2% (w/w) dissolved in DI water
- magnetically stirred overnight in 4˚C storage

pH was adjusted from 2-12

- solutions were split
  - half was adjusted down
  - half was adjusted up



Zeta potential was measured and assessed at pH=1 increments

(Caballero et al., 2022)

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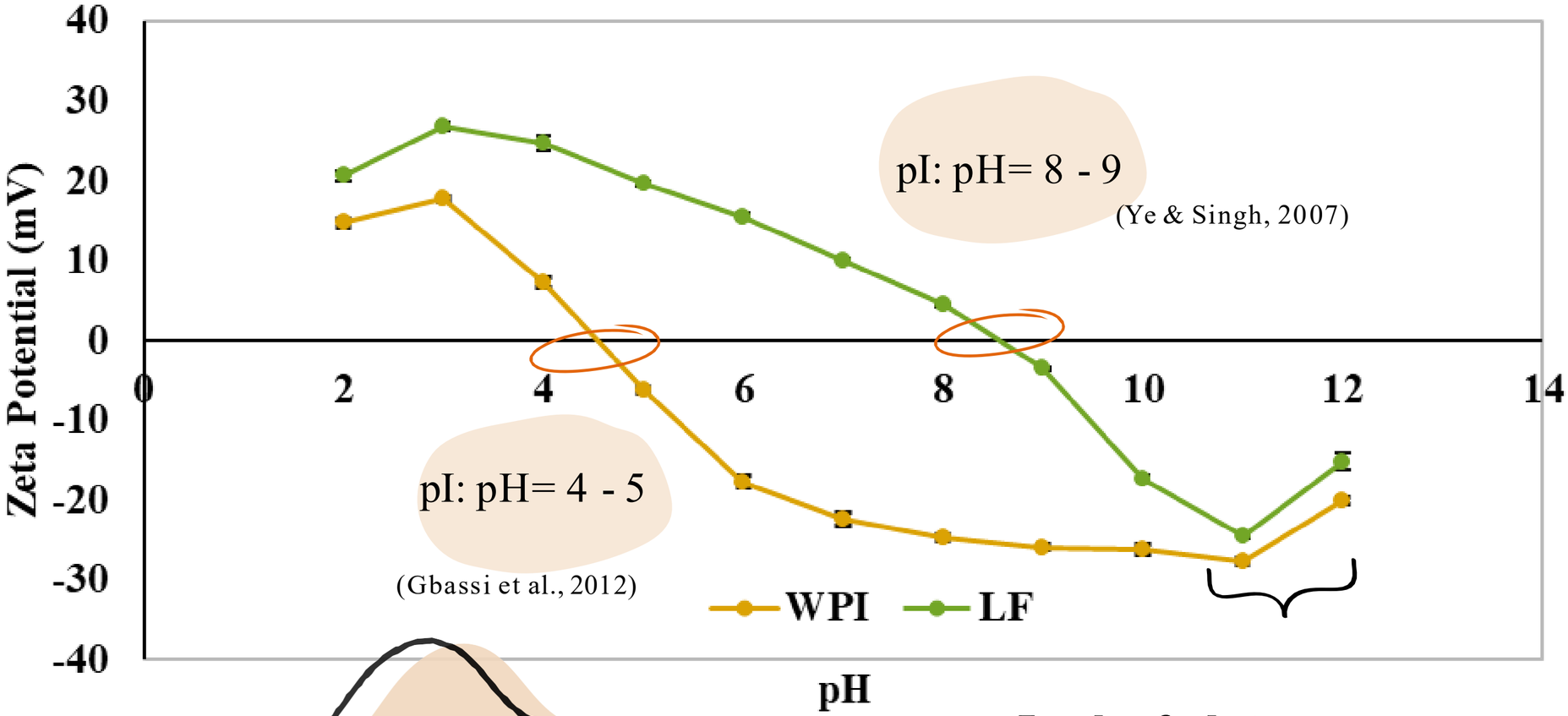
Results

Zeta potential becomes more (-) as pH ↑

- carboxyl groups are ionized → balance in charge → plateau is reached when all groups are fully charged

(Ravindran et al., 2018)



pH 7

- proteins oppositely charged
- neutral

Lack of plateau

- protein unfolding at extreme pH
- exposes underlying charged groups

(Guckeisen et al., 2021)

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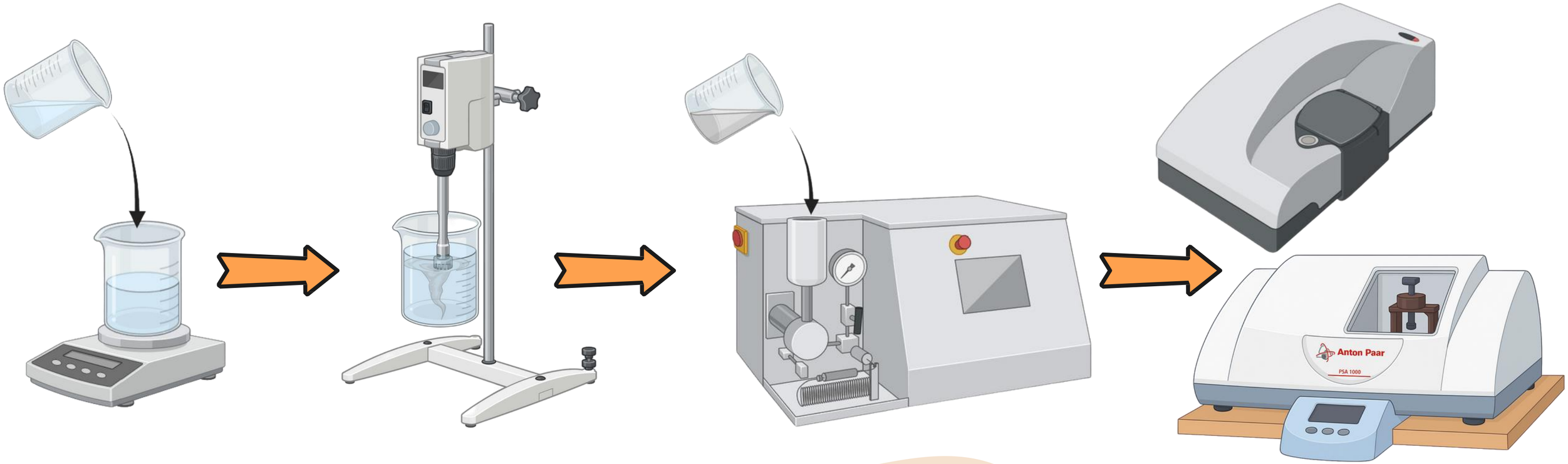
Protein Layer Order Determination



Added 10% (w/w) MCT to 1% (w/w) protein solutions

Emulsified using high-shear homogenizer at 6970 rpm for 5 minutes

Emulsified further with microfluidizer for 5 passes at 20,000 PSI

Assessed zeta potential, particle diameter & distribution

1

Results

Primary Emulsion Layer	Particle Diameter (nm)	Zeta Potential (mV)
WPI	338.0 ±6.8	-40.78 ±1.19
LF	7,290 ±481	-3.77 ±0.36

Zeta potential (±30 mV) is a standard for emulsion stability due to higher electrostatic repulsion

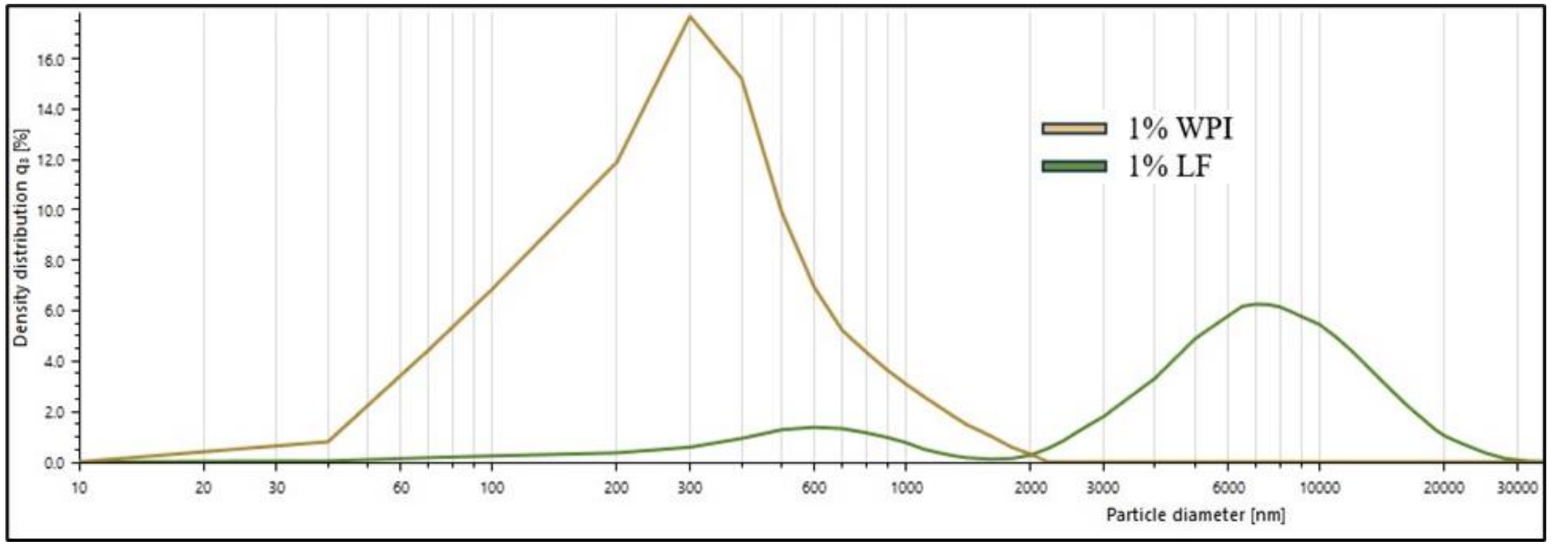
- minimizes droplet coalescence & flocculation
- electrostatic repulsion

Less physical stability

- higher chance of coalescence

(Querol et al., 2017)

(Kupikowska-Stobba et al., 2024)

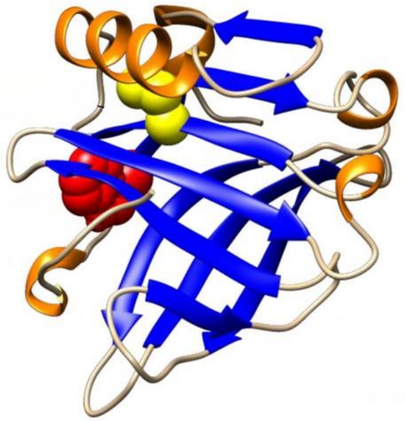


Multimodal broad peaks
=particle diameter variation

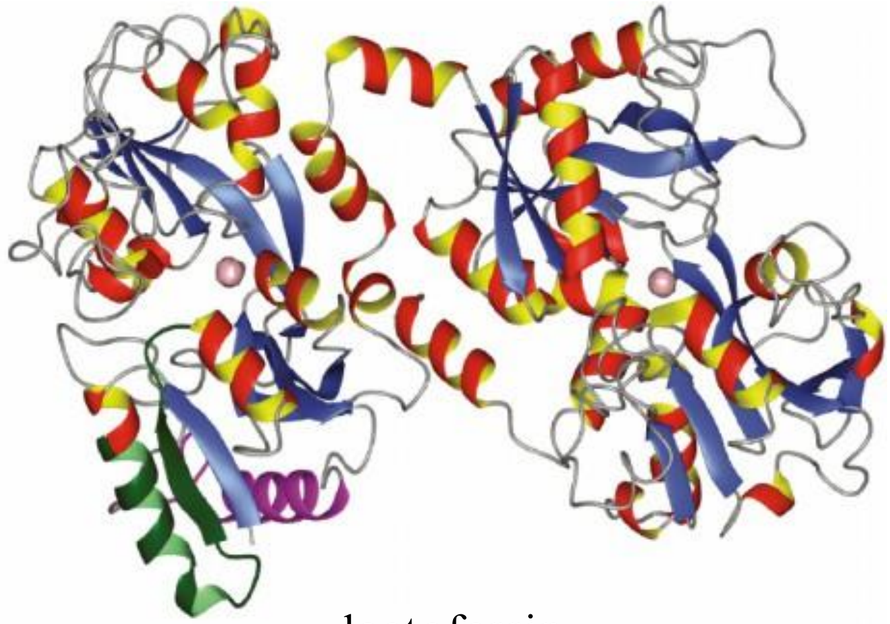
1

Differences in particle diameter can be due to molecular sizes and structures

- lactoferrin: **~80 kDa**
- β-lactoglobulin: **~18.28 kDa**
  - faster adsorption
- lactoferrin is more rigid
  - 3 turn helix that links its 2 lobes
  - less unfolding &**less efficient coverage of droplets**



β-lactoglobulin
(Barbiroli et al., 2022)



lactoferrin
(Vogel, 2012)

WPI

- smaller particle diameter
- narrow unimodal distribution
- stronger zeta potential

1

LF Concentration Determination

0%

0.25%

0.5%

1%

1.5%

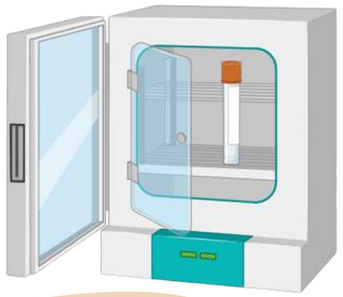
2%

Made emulsions with 1% (w/w) WPI & mixed with 0-2% (w/w) LF solutions at a 1:1 ratio for 30 mins.

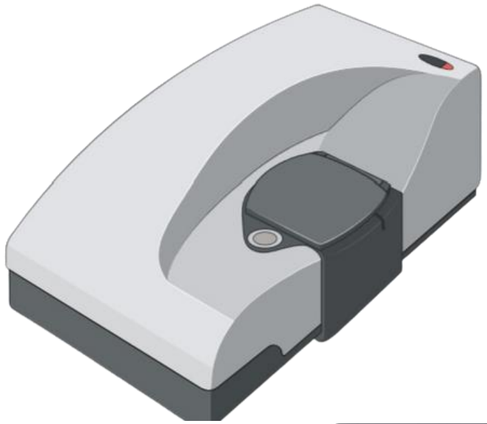
4°C

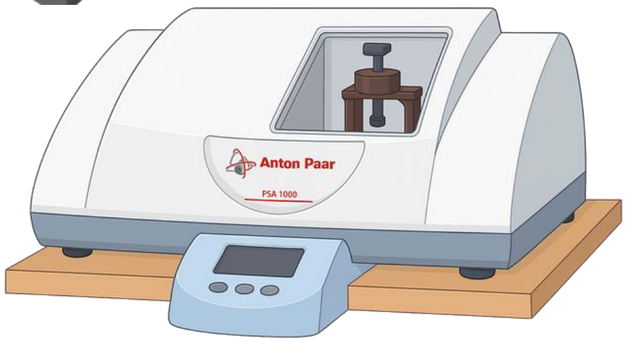
25°C

37°C



Held in storage for 3 weeks

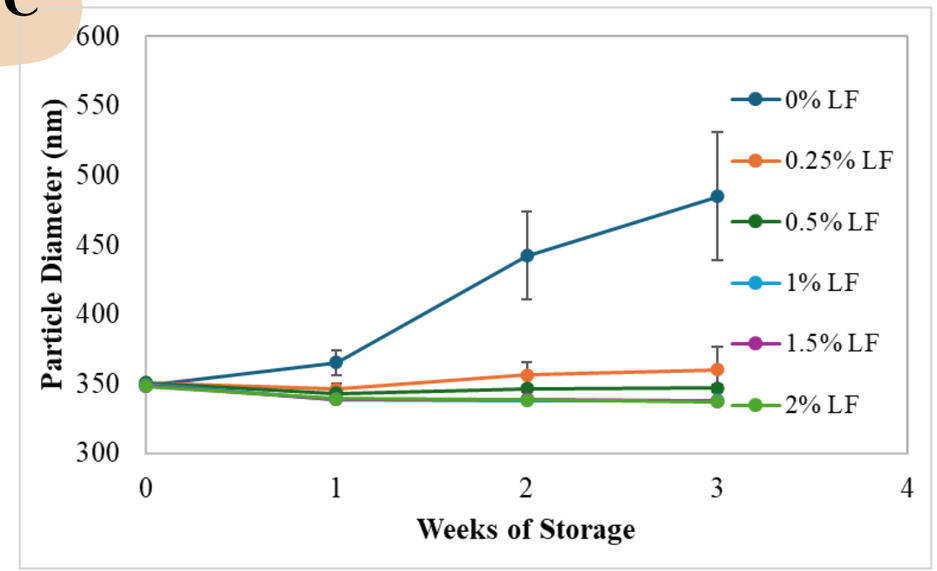


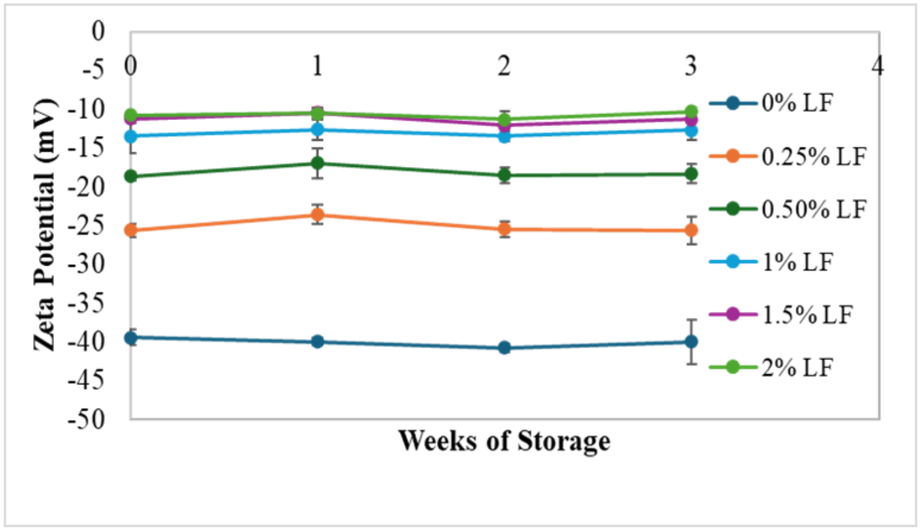


Assessed zeta potential, particle diameter

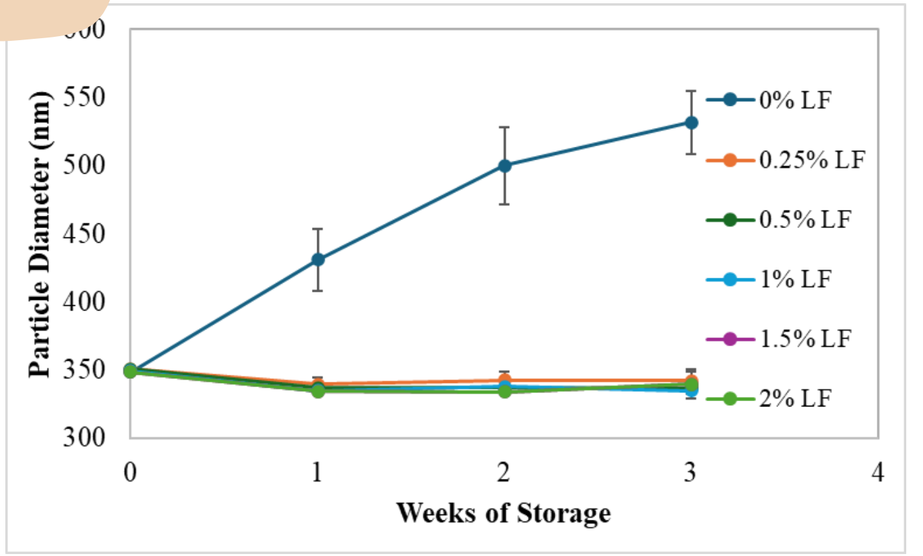
1 Results

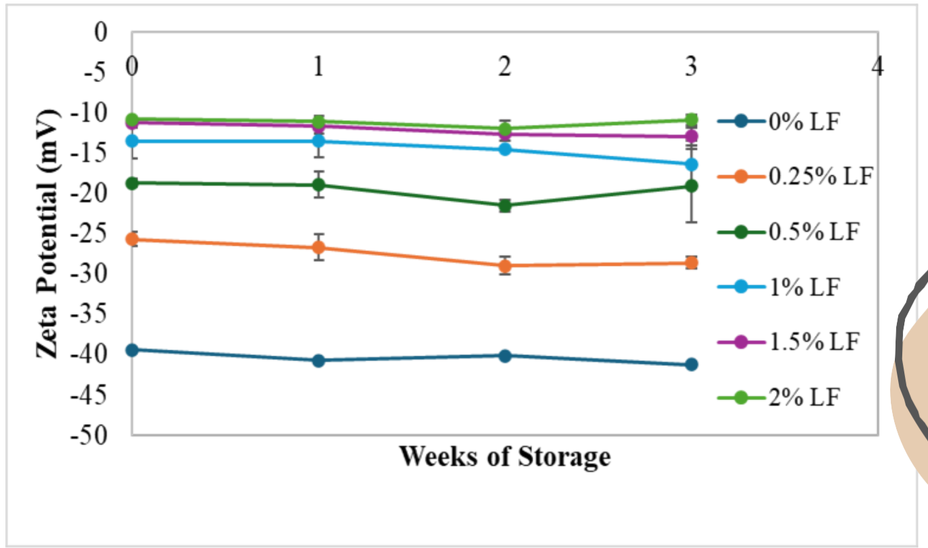
25°C





37°C





↓ viscosity of continuous phase
↑ droplet mobility
(Bera et al., 2021)

partial unfolding of proteins exposing charged residues
(Zhang et al., 2023)

1% (w/w)

- stability across storage
- same concentration as WPI
- limits excess protein

2

Objective 2

Assess the effect of the multilayer emulsions on the stability of lutein-enriched emulsions.

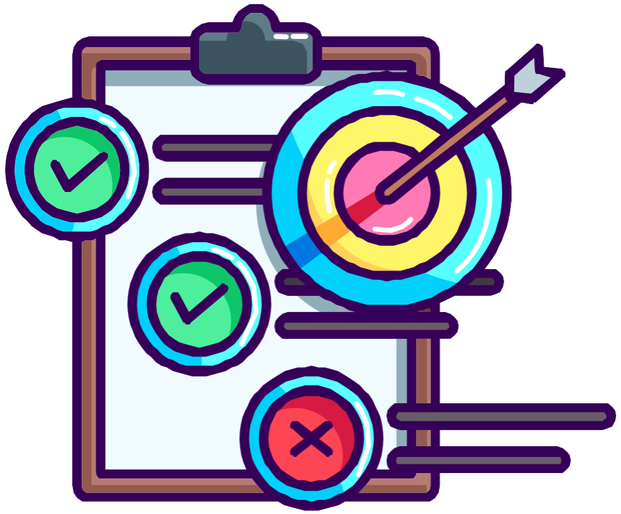
Independent Variables

- Composition of Emulsions
  - WPI+Buffer
  - WPI+LF

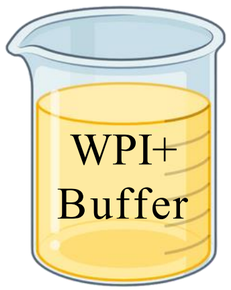
  Storage Temperature
  - 25℃
- 50℃
  -
  -

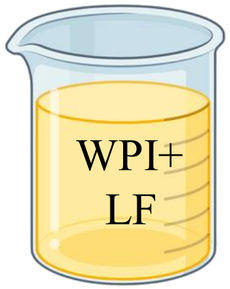
Dependent Variables

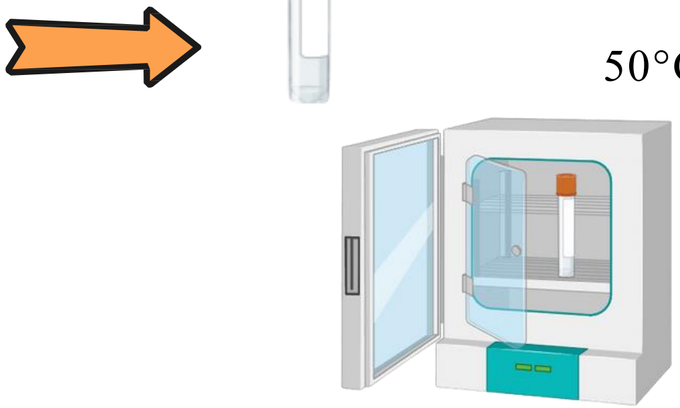
- Zeta potential
- Particle diameter
- Color of lutein
- Radical scavenging



2 Physical & Chemical Stability of Final Emulsions







$$\Delta E* = \sqrt{(L^* - L_i^*)^2 + (a^* - a_i^*)^2 + (b^* - b_i^*)^2}$$

2 types of emulsions were made using 10% lutein-enriched (0.1%) MCT oil

Samples were held in storage for 2 weeks

Samples were assessed initially by zeta potential, particle diameter weekly & L*a*b* values daily

(Caballero et al., 2022)

2 Results

Protein layers had an apparent impact on physical stability across storage temperatures

Emulsion Type	Temperature (℃)	Particle Diameter (nm)		
		Day 0	Day 7	Day 14
WPI+Buffer	25°C	341.70 ±4.23a	348.47 ±9.07ab	356.1 ±8.27bc
WPI+LF	25°C	347.78 ±6.03a	334.90 ±7.32a	339.55 ±5.09a
WPI+Buffer	50°C	341.70 ±4.23a	537.8 ±8.23b	598.9 ±49.23c
WPI+LF	50°C	347.78 ±6.03a	333.3 ±5.10a	334.6± 4.85a

(a–c) Groups sharing the same letter are not significantly different from each other on the same day within the same storage temperature, based on a one-way ANOVA followed by Tukey's post hoc test ($p \leq 0.05$).

Double-layer emulsions ↓ in particle diameter in storage

Interfacial rearrangement of proteins over time

(Hinderink et al., 2022)

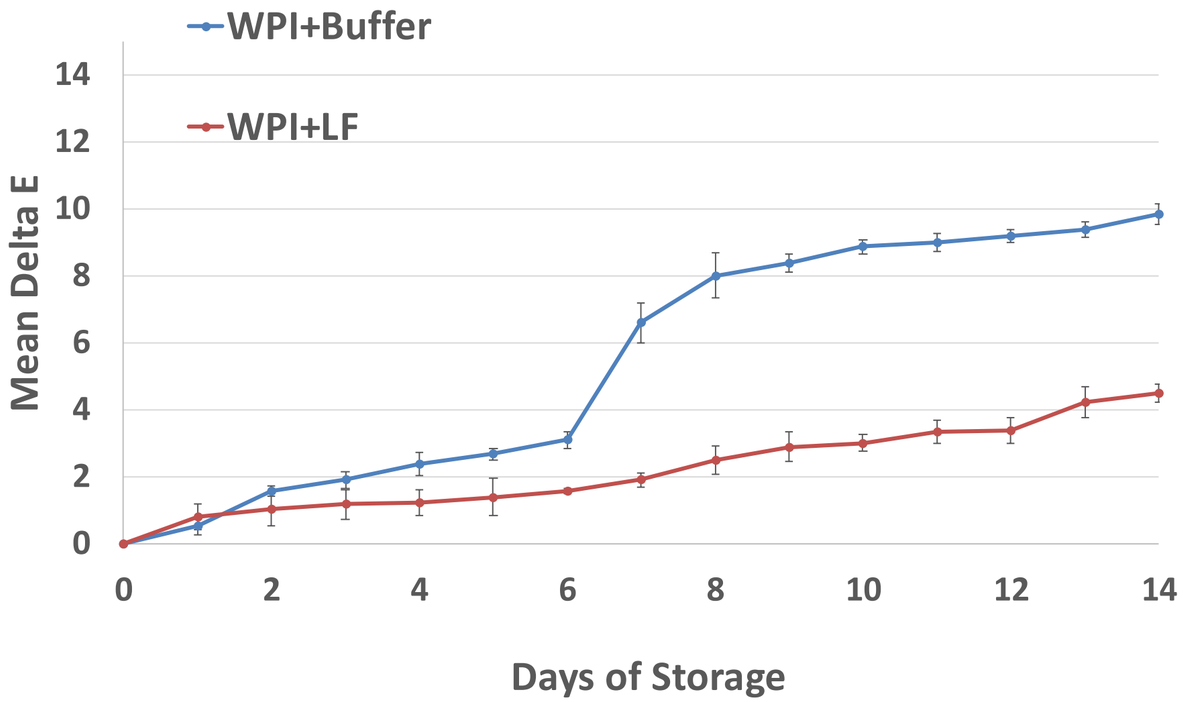
↑particle diameter at ↑ temperature

Decreased viscosity → collisions & coalescence

(Bera et al., 2021)

2 Results

Emulsions held at 25°C



Peroxyl biradicals → development of additional oxidative compounds

(Boon et al., 2010; Martínez-Delgado et al., 2017)

WPI +Buffer emulsions showed the largest ↑ ΔE, followed by:

- WPI +LF
- WPI-Res +Buffer
- WPI-Res+ LF-Hes

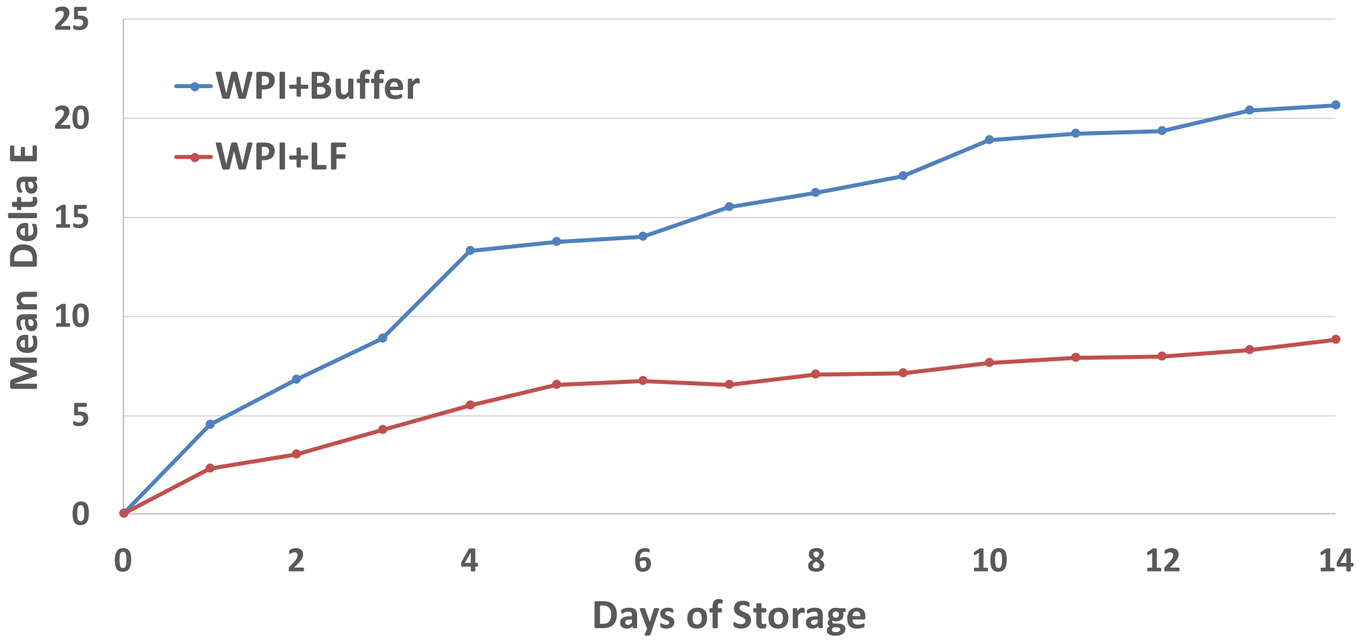
LF has iron-binding capacity that can prevent the formation of radicals

(González-Chávez et al., 2009)

WPI -Res+LF-Hes emulsions showed the highest oxidative stability

2 Results

Emulsions held at 50°C



The same trend is seen with more advanced color degradation

WPI +Buffer → WPI +LF →
WPI-Res +Buffer → WPI-Res+ LF-Hes

WPI -Res+LF-Hes emulsions showed the highest oxidative stability across all storage temperatures

↑ temperature = ↑ color fading

heating in the presence of oxygen → volatile and non-volatile degradation compounds such as epoxides → accelerated oxidation

(Boon et al., 2010; Martínez-Delgado et al., 2017)

2 Results

Emulsions held at 25°C

WPI +Buffer emulsions showed the most visually apparent color fading

Day 0

Day 7

Day 14

Atmospheric oxygen promotes formation of peroxyl biradicals and other reactive species → structural changes in lutein's double bond system → visible color fading

(Boon et al., 2010; Martínez-Delgado et al., 2017)

2 Results

Emulsions held at 50°C

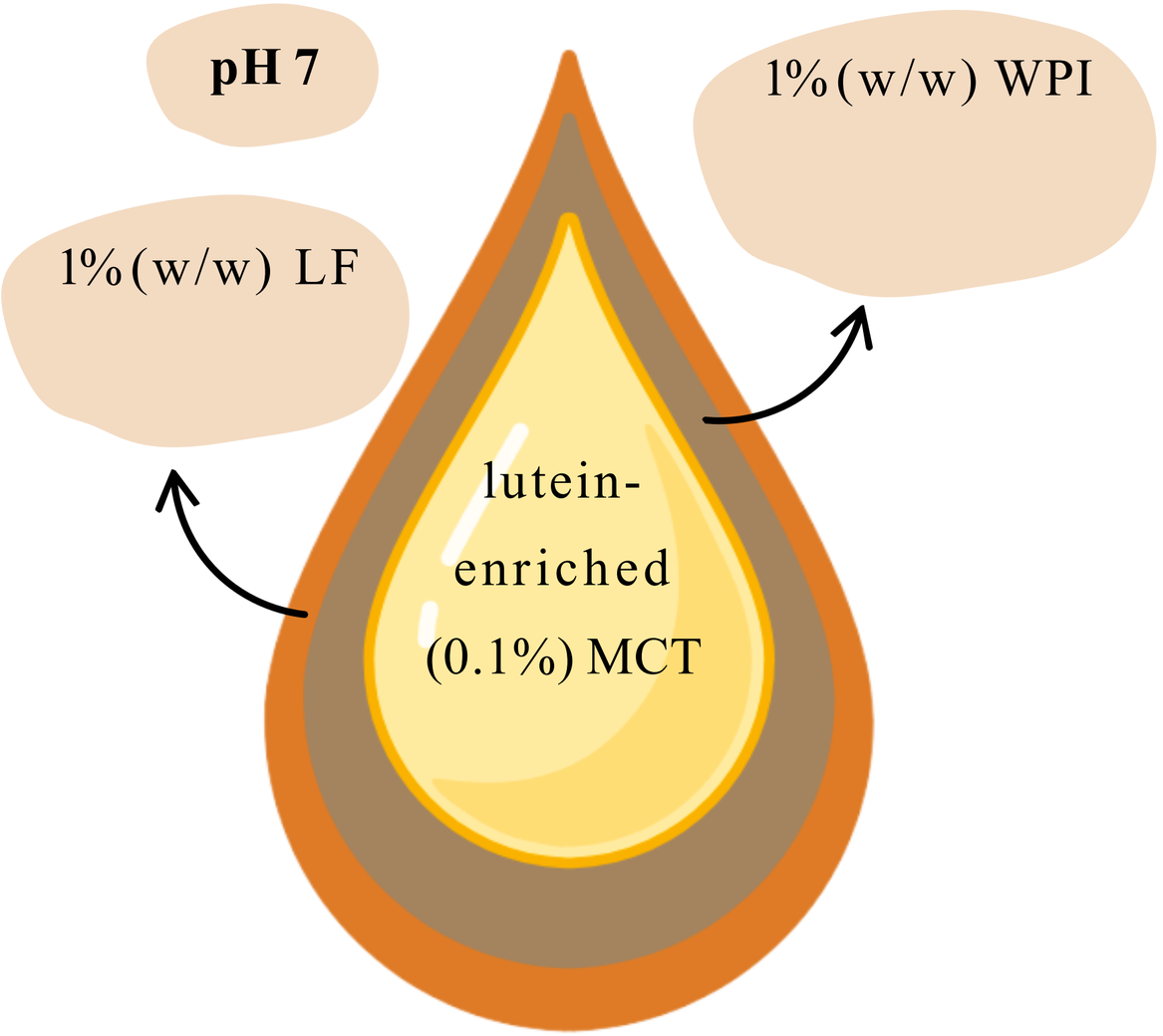
Again, WPI +Buffer emulsions showed the most visually apparent color fading

Day 0

Day 7

Day 14

Conclusions



Ideal parameters

Physical Stability

Multilayer emulsions >Single layer emulsions

Chemical Stability

WPI-LF emulsions = ↑oxidative stability

Consistent with ABTS+ results

Results suggest that combining proteins produces a synergistic effect that ↑ the physical and chemical stability of lutein in emulsion systems

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Thank You!

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