Yolk Processing and Product Opportunities

Tong Wang, Ph.D., Professor
Department of Food Science and Human Nutrition
Iowa State University

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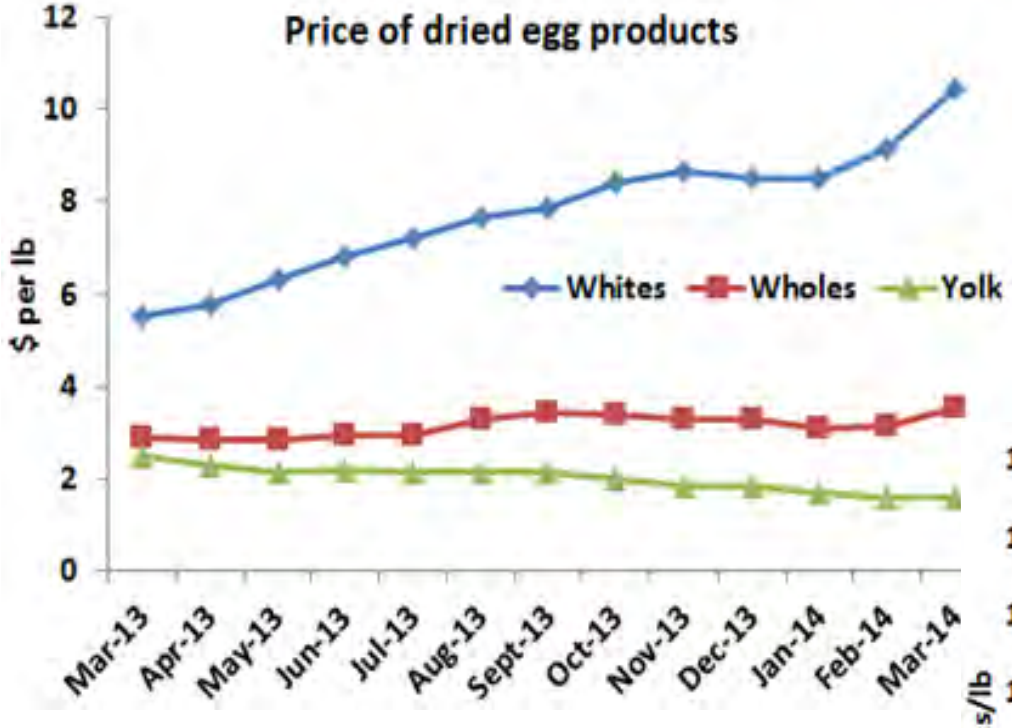
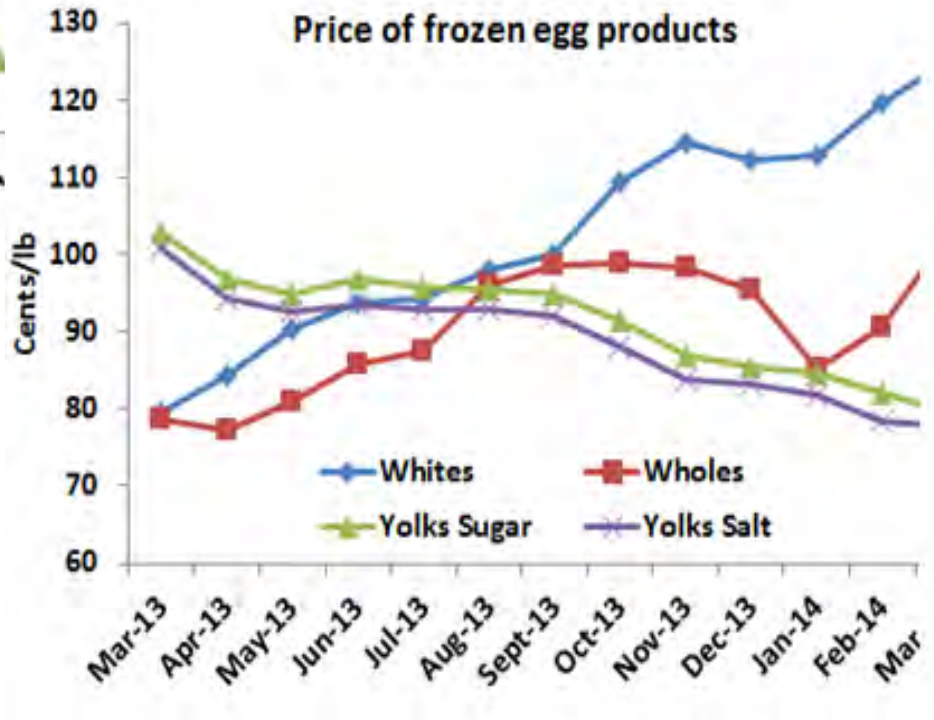
Taking the whole yolk apart

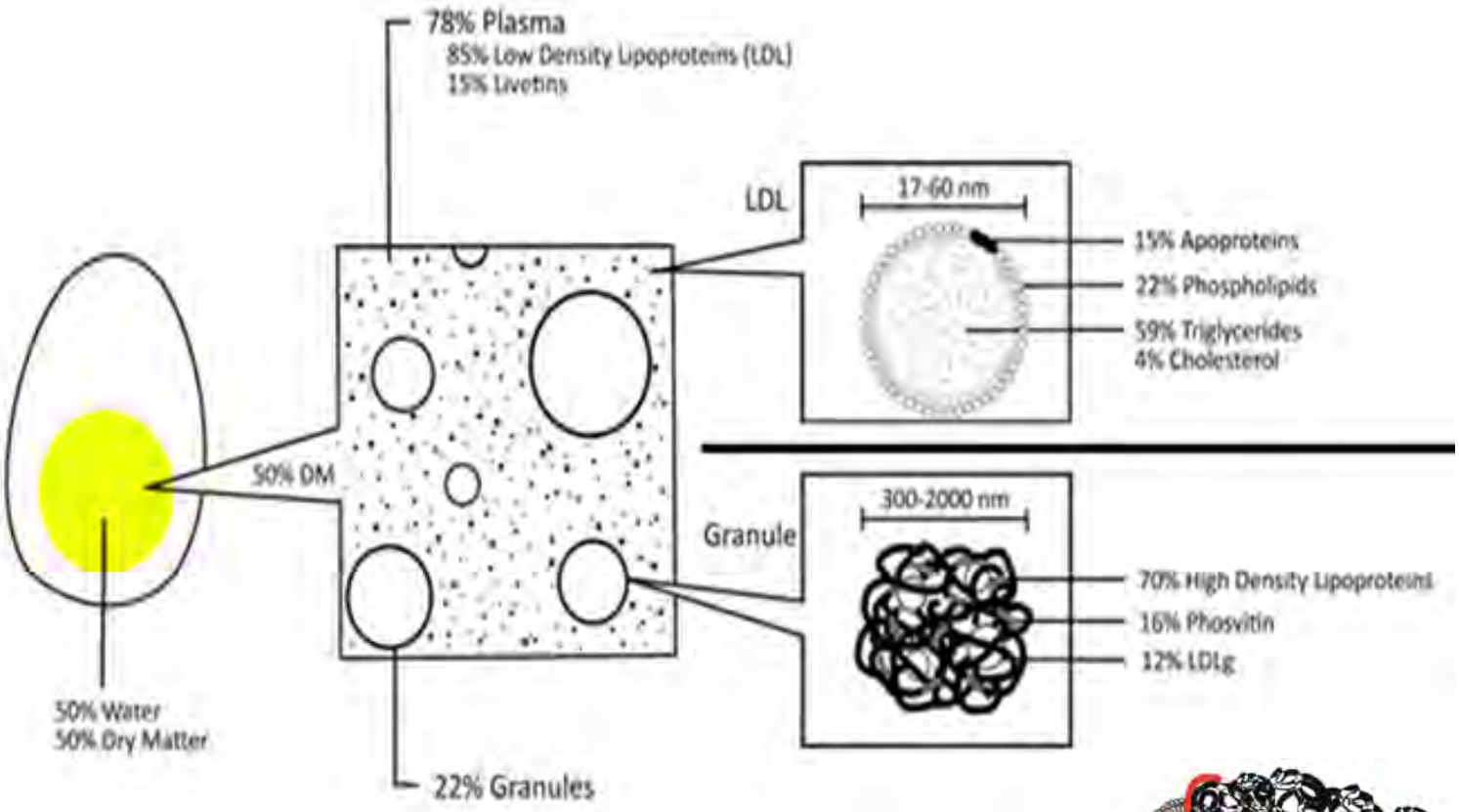
- Lipoproteins: LDL vs HDL
- Aqueous fractionation of yolk
- Defatting yolk by using solvents
- Lipids: neutral and polar lipids

Why yolk further processing?





Yolk Composition

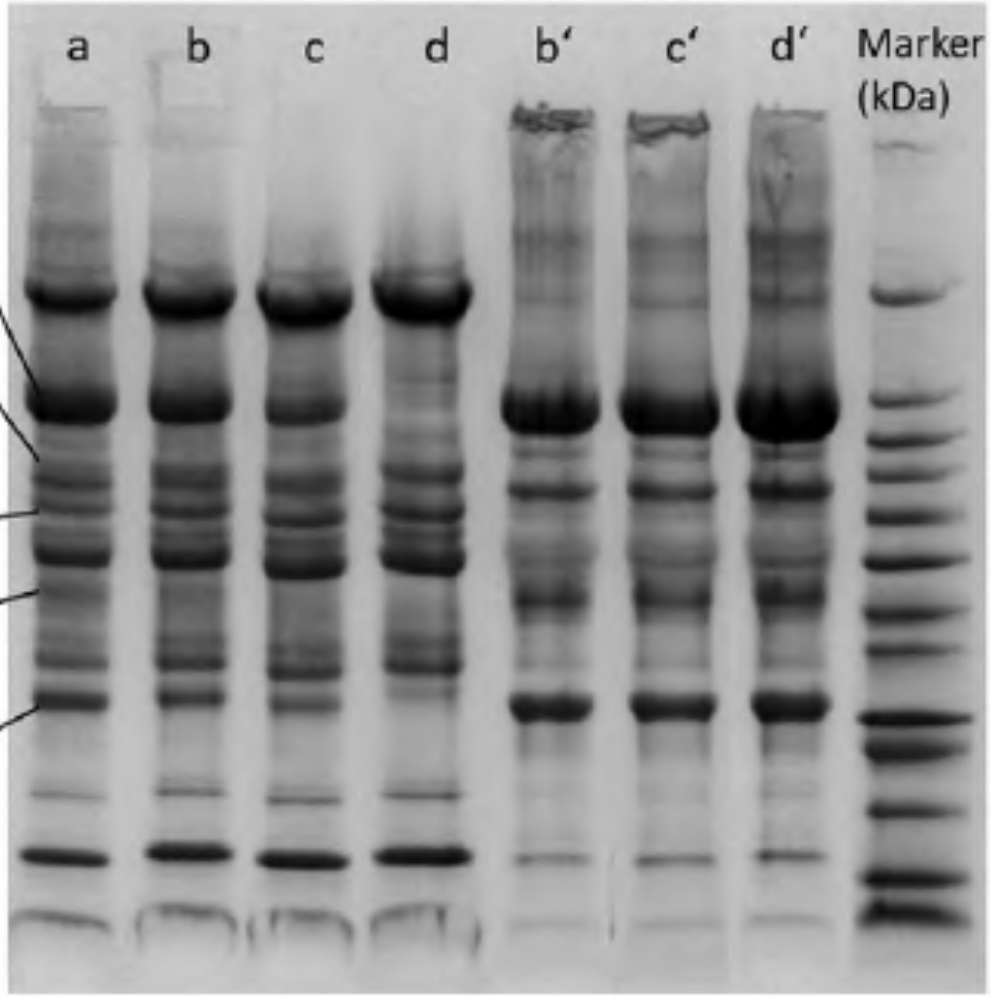


Strixner et al, 2014

Yolk has many types of proteins

(Strixner and Kulozik, 2013)

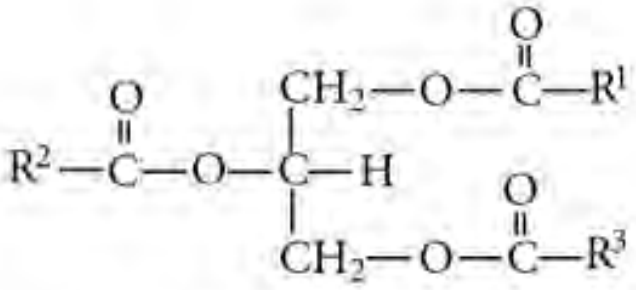
apovitellenin VIa	221
γ-livetin/apovitellenin VI	203
apo-LDL	122
apovitellin 3+4	110
apovitellenin Vb	93
apovitellenin V	85
apovitellin 5+6	78
α-livetin	72
apovitellenin IV	68
apovitellenin IIIa	62
phosvitin	59
α-livetin/ apovitellenin III	55
apovitellin 7	47
β-livetin	36
β-livetin I	33
apovitellin 8	31
apovitellenin IIa	21
apovitellenin II	20
apovitellenin I	17
apovitellenin Ia	5



- **IgY** – 160 -200 KDa, resistant to acid and heat, in immunotherapeutic applications
- **Phosvitin** – a yolk protein in the granule fraction, binds Fe^{+3}, and Ca^{+2}

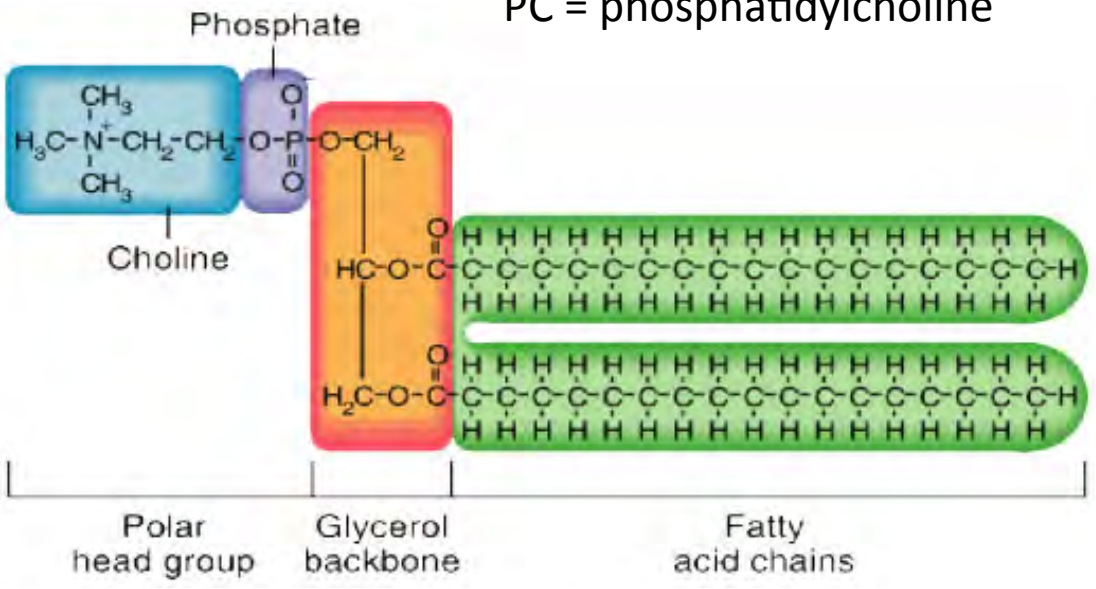
Yolk has a unique lipid composition

- Neutral vs. polar lipids
- The polar phospholipid has high PC content



1,2,3-triacyl-*sn*-glycerol (TAG)

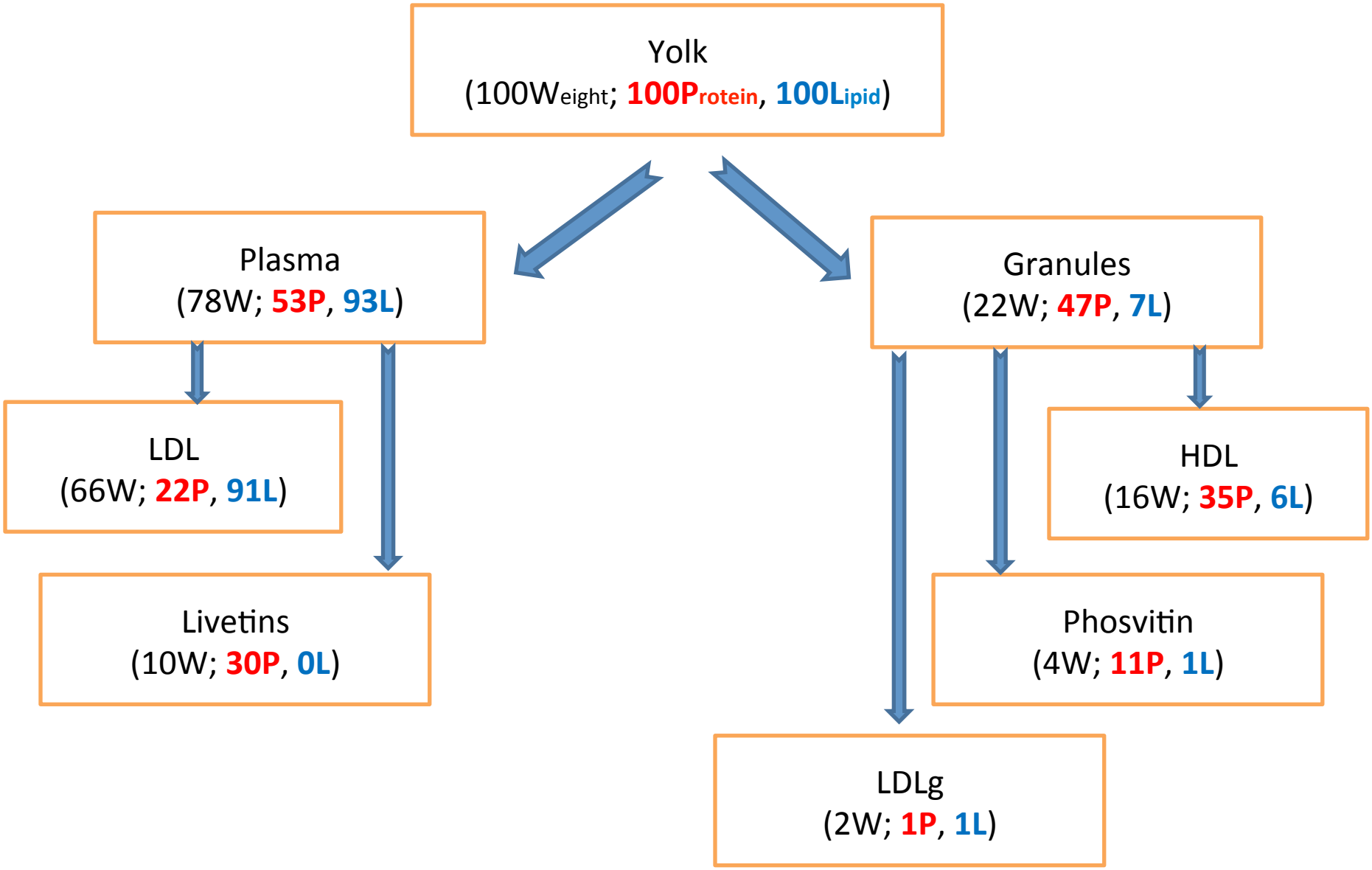
PC = phosphatidylcholine



Yolk fractionation technologies

- Lipid-protein separation by aqueous means
  - Plasma and granule fractions
    - Highly functional products
    - Reduced-fat granule product; used in salad dressings or as a main component of a new mayonnaise (Ulrichs and Ternes, 2010)
    - IgY as immunoglobulin concentrate
  - Keys factors affecting aqueous separation by centrifugation
    - Solid content
    - pH
  - The use of “chelating agents”, or of “the adhesive power of the hydrocolloid CMC to float the LDL”
- Lipid-protein separation by using solvents
  - Good total lipid yield
  - Proteins are denatured if using alcohols
  - Proteins are functional if using hexane, however,
    - Need dried material
    - Incomplete polar lipid extraction

Yolk lipoprotein fractions and mass balance



The Laca method (Laca et al. 2010. 2014)

100% mass
(31% protein,
69% lipids)

Egg yolk

Dilute
Add NaOH to pH 7
Held overnight at 4°C
Centrifuge

Precipitate
(Fraction 1: Granules)

29% mass
(59% protein,
41% lipid)

Supernatant
(Plasma)

Add sodium alginate
Centrifuge

Lipidic paste
(Fraction 2)

62% mass
(19% protein,
81% lipid)

Watery fraction
(Fraction 3)

9% mass
(61% protein,
10% lipid)

Native egg yolk without membranes

Dilute

Centrifuge

Precipitate
(Granules)

Supernatant
(Plasma)

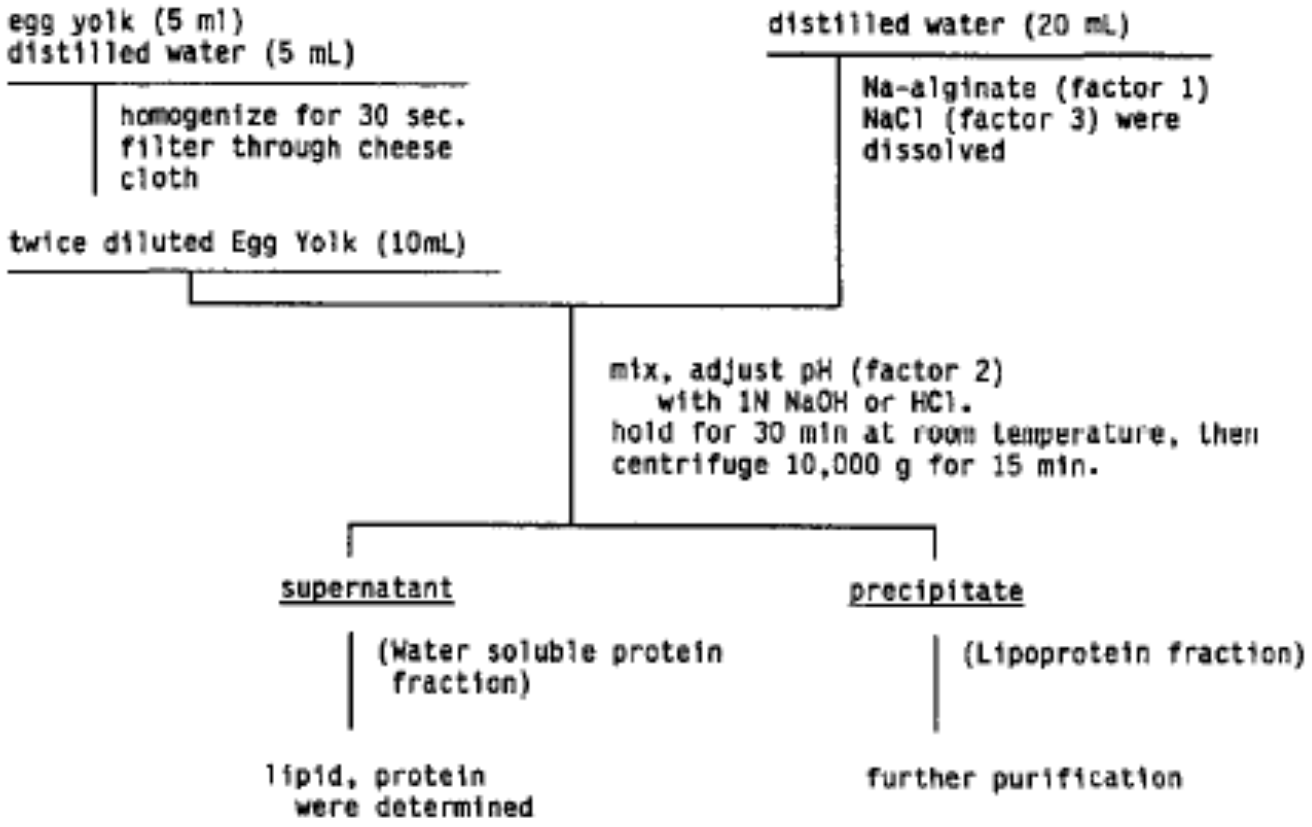
Composition of obtained fractions compared with yolk. Average values ± SD are reported.

	Yolk	Fraction 1 (granules)	Fraction 2 (lipidic paste)	Fraction 3 (watery fraction)
Dry matter (% w/w)	51[a]	41.4 ± 1.5	36.6 ± 0.8	3.1 ± 0.4
Proteins (% w/w)	16[a]	24.0 ± 0.9	6.9 ± 0.1	1.9 ± 0.1
Total lipids (% w/w)	36[a]	16.6 ± 2.8	28.8 ± 2.2	0.27 ± 0.19
Cholesterol (mg/100g sample)	1260[b]	291 ± 36	957 ± 58	50 ± 2
pH	6–6.4[c]	6.94 ± 0.01	7.03 ± 0.05	7.03 ± 0.02

Similar to the patent of Merkle and Ball, 2001

Many variations of separation methods

- Sodium alginate of 0 .1% (wb), pH at 5.8-6.4
- 20% of protein (α−, β−, γ-livetin) in the supernatant
- 99% lipids can be precipitated
- Phospholipids can be purified from the precipitated lipoproteins by 95% ethanol extraction



(Hatta et al, 1988)

Yolk plasma and granule separation by centrifugation

- A commercially viable procedure for separating egg yolk into three valuable fractions (Ulrichs and Terns, 2010)
  - Two fractions can be separated by centrifugation easily
  - The plasma can be further separated into two phases:
    - a semisolid, floating LDL-rich phase
    - an aqueous phase with high amounts of IgY-rich livetins
  - An industrial centrifugation (2800xg) method was developed to fractionate egg yolk into three fractions

- **Continuous centrifugal separation method is possible** (Strixner and Kulozik, 2013)
  - At 50C, pH 6.5, 0.15M NaCl, and dry matter < 29%, with 10,000 g-force
- IgY recovery from the LDL phase
  - lipid (90-93%) from the water-soluble fraction, with moderate (60%) recovery of IgY (Kwan et al, 1991)

Lipoprotein applications

- **Granule fraction**:
  - Better emulsion stability than plasma (Anton and Gandemer, 1997)
  - More resistant to heat treatment
  - Low cholesterol content

- Granules applications in food industry (Laca et al 2014)
  - Few studies have been carried out
  - Potentials in cosmetic or pharmaceutical industry

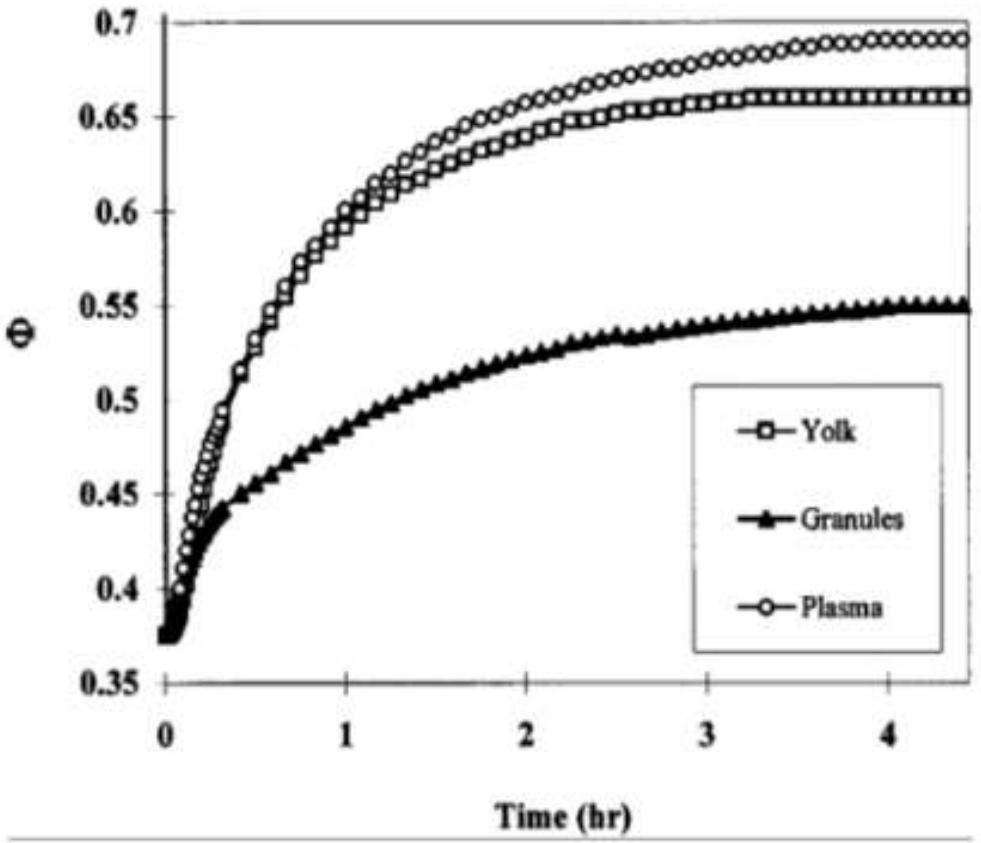
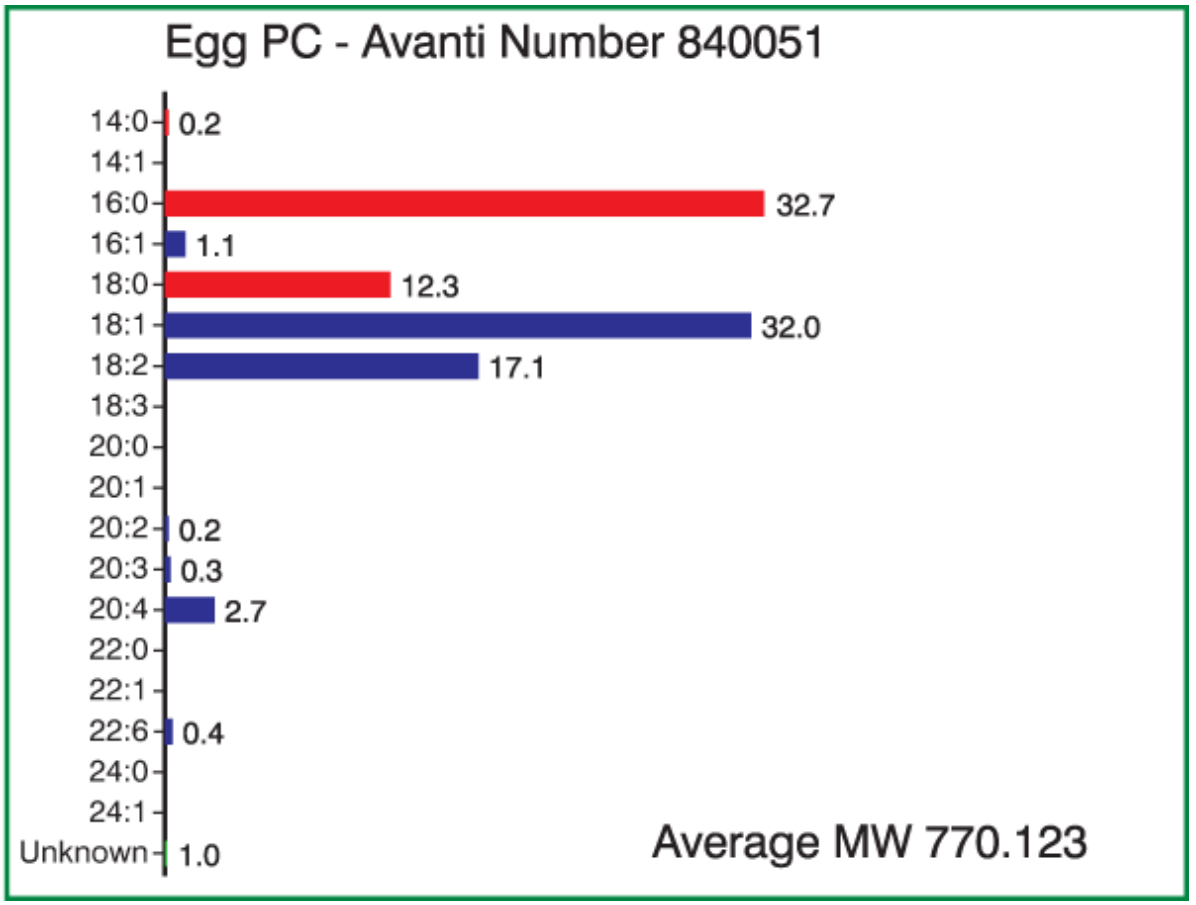


Fig. 3—Change in oil volume fraction (Φ) as related to time (hr); concentration (w/v) of yolk, granules and plasma = 0.1%. Each point is the mean ± S.D. of 10 determinations.

Maximize the extraction of yolk phospholipids – an essential nutrient



PC: maintain health of liver, nerve system, anti-cancer;
ARA and DHA: essential for infant eye, brain and nerve system development and support long term heart health.

Egg yolk lecithin

- Egg yolk is the richest source of phospholipids (PL)
- Egg yolk's polar lipid has unique compositions:
  - More saturated
  - Much higher phosphatidylcholine (PC) content
  - A good source of long-chain polyunsaturated fatty acids (PUFA) arachidonic acid (AA or ARA) and docosahexaenoic acid (DHA)
  - Contains minor PLs, such as sphingomyelin (SM)

Applications of egg yolk PL

- Nutritional supplements for infant formula;
  - FAO and WHO recommended the inclusion of LC-PUFA (DHA and ARA) to infant formula to a level close to that of human milk - **0.33-0.35% DHA 0.65-0.7% ARA**

- Purified PL for pharmaceutical and cosmetic applications (oxidation stability and free of soy allergens)

Yolk lecithin is a good source of choline!

Egg is nutrient dense and a good source of choline:

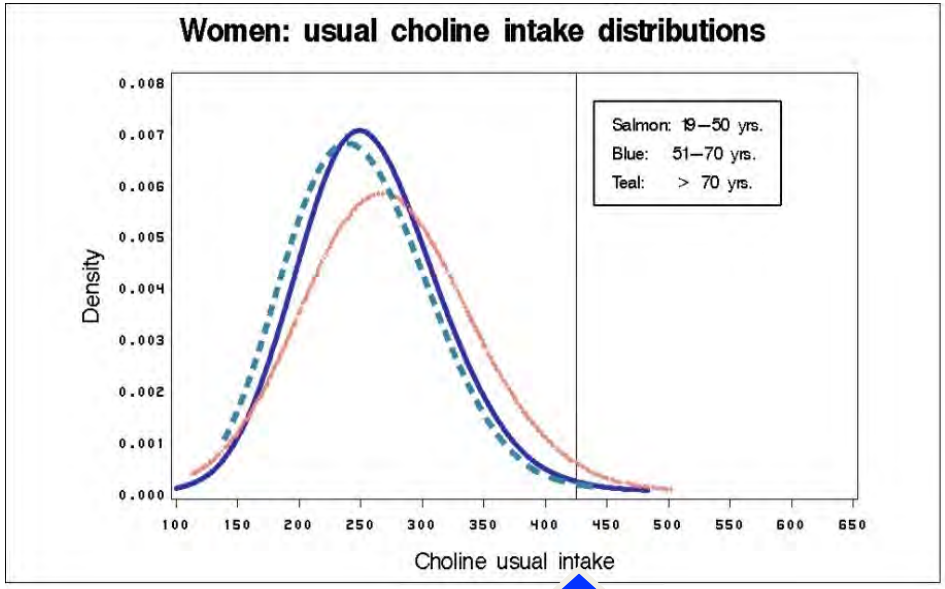
One Large Egg (whole, raw, fresh)		
Nutrient	**Amount**	**%DV**
Energy	72 kcal	*3.6*
Protein	6.28 g	*12.6*
Total Fat	4.75 g	*7.3*
Saturated Fat	1.56 g	*7.8*
Cholesterol	186 mg	*62.0*
Choline	126 mg	*23.0*
Riboflavin	0.23 mg	*13.5*
Folate, B_{12}, B_6	24, 0.45, 90 ug	*4.5-7.5*
Vitamin D	41 IU	*10.0*
Selenium	15.4 ug	*22.0*
Phosphorous	99 mg	*9.90*

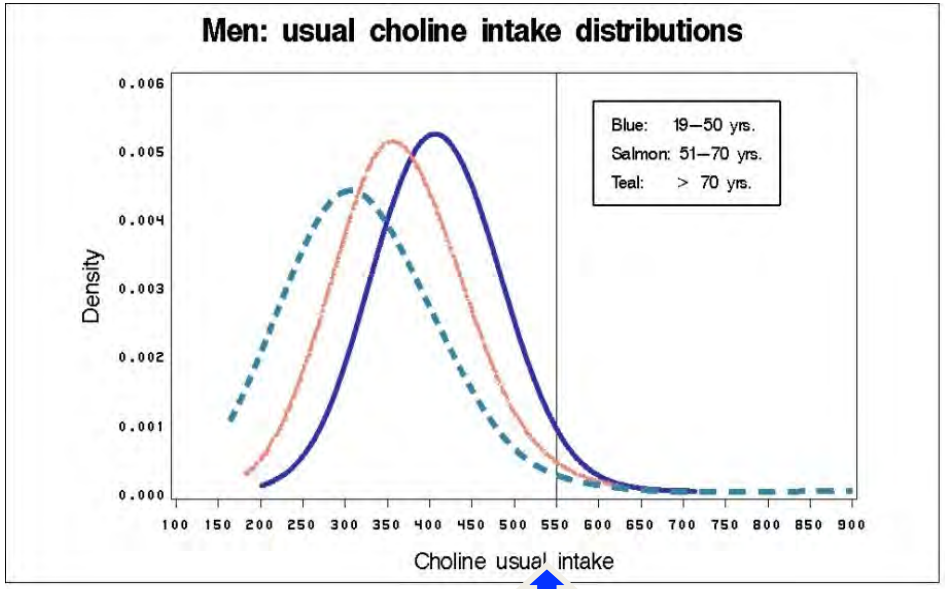
Choline and PC:

- Brain/cognitive function
- Liver health

USDA –ARS (2010)

Distribution of choline intake in men and women





425 ***Inadequate !!*** **550**

From Jensen, H., Iowa State University

Courtesy of Schalinske (ISU)

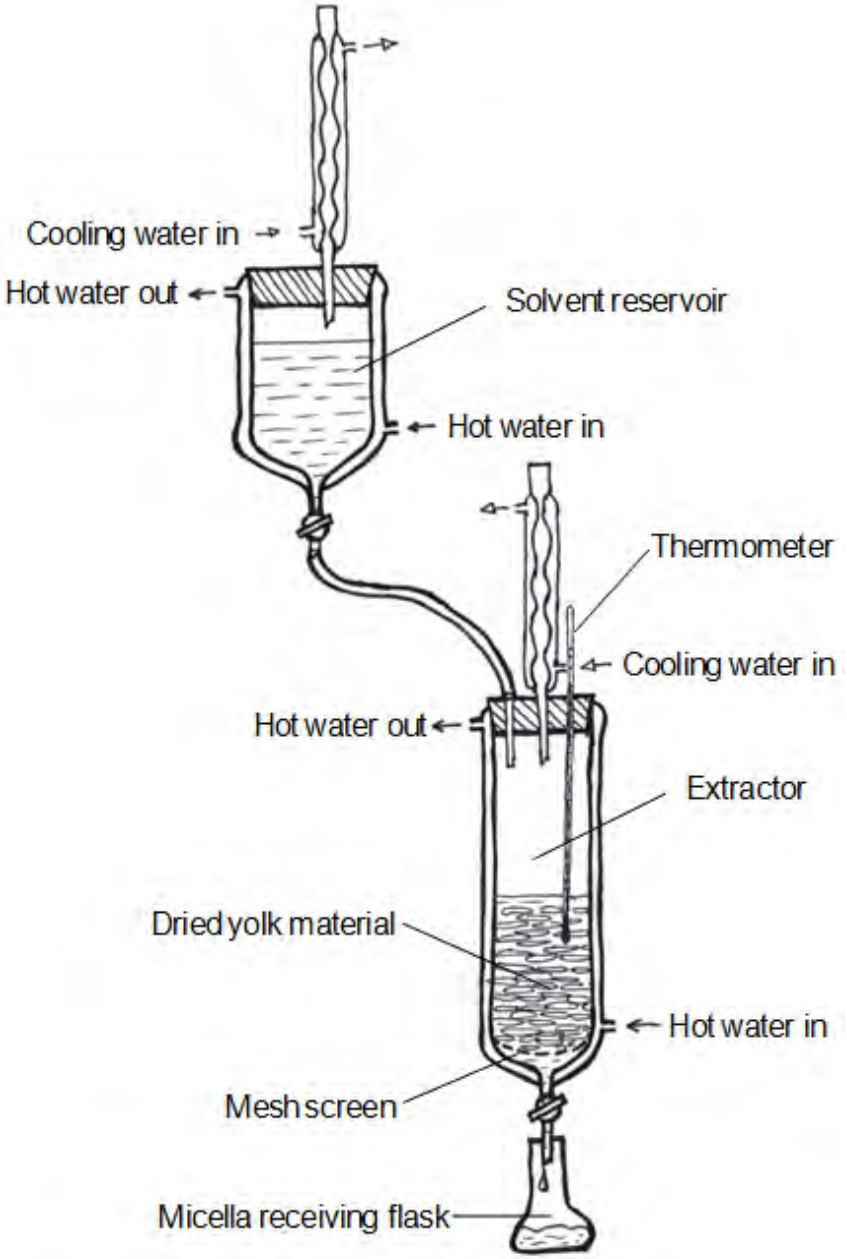
Using solvents for complete protein-lipid separation

- Using non-polar solvents
  - Dried yolk needed
  - "Muddy-cake" formation impacts solvent separation efficiency
- Using alcohols
  - Can work on liquid yolk
  - Proteins are denatured

Egg yolk preparation for total lipid extraction

- Our drum dried egg yolk flake is an ideal yolk material for yolk lipid extraction
- Yolk flakes can be directly used in commercial extractors (such as continuous percolation extractor for oilseeds)

Drying temperature: ~120°C
Flake thickness: 1-2 mm



Flaked yolk had much improved solvent drainage!


500 mL
PYREX
500 mL
PYREX
500 mL
PYREX

Simultaneous protein texturization and lipid extraction – an ISU technology

- Direct and complete lipid extraction from liquid yolk:
  - Simultaneous protein texturization
  - Excellent separation efficiency
  - Patent filing in progress

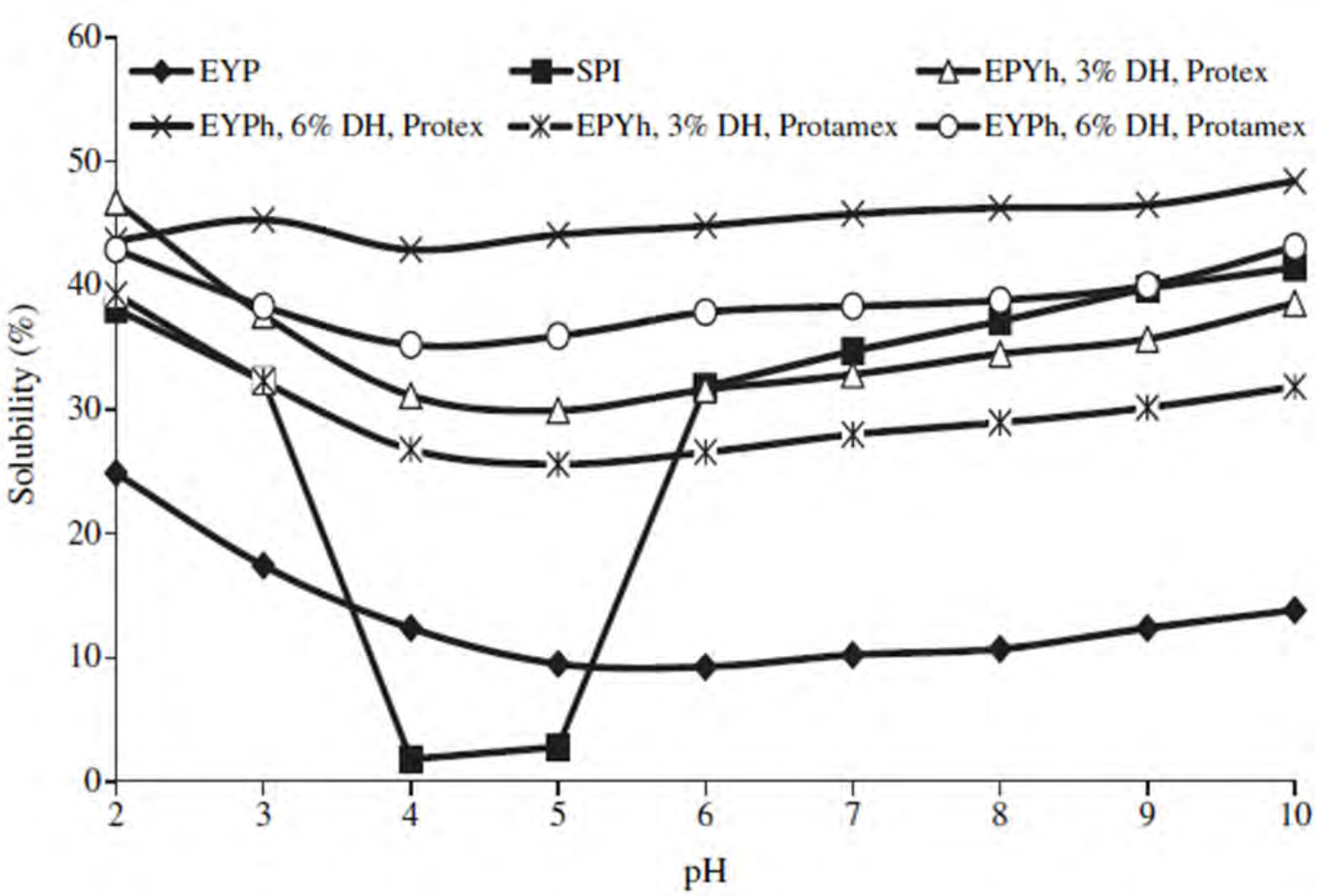
- Uniqueness:
  - Separation of protein and lipids without the use of high speed centrifugation
  - No need for yolk dilution and the cost related to further drying
  - No need for initial yolk drying
  - Complete separation
  - All proteins are denatured

Refunctionalize denatured yolk protein by partial hydrolysis

- Alcohol denatured egg yolk protein (EYP)
- Hydrolyzed by two endo-proteases
- EYP hydrolysates (EYPh) with 3% and 6% degrees of hydrolysis (DH)

Wang and Wang, 2008

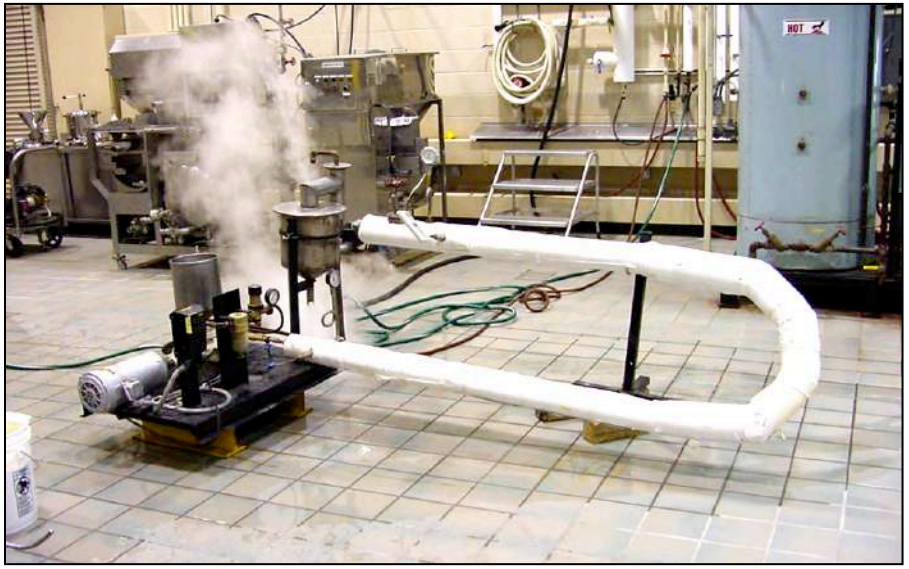
Solubility curves of egg yolk proteins

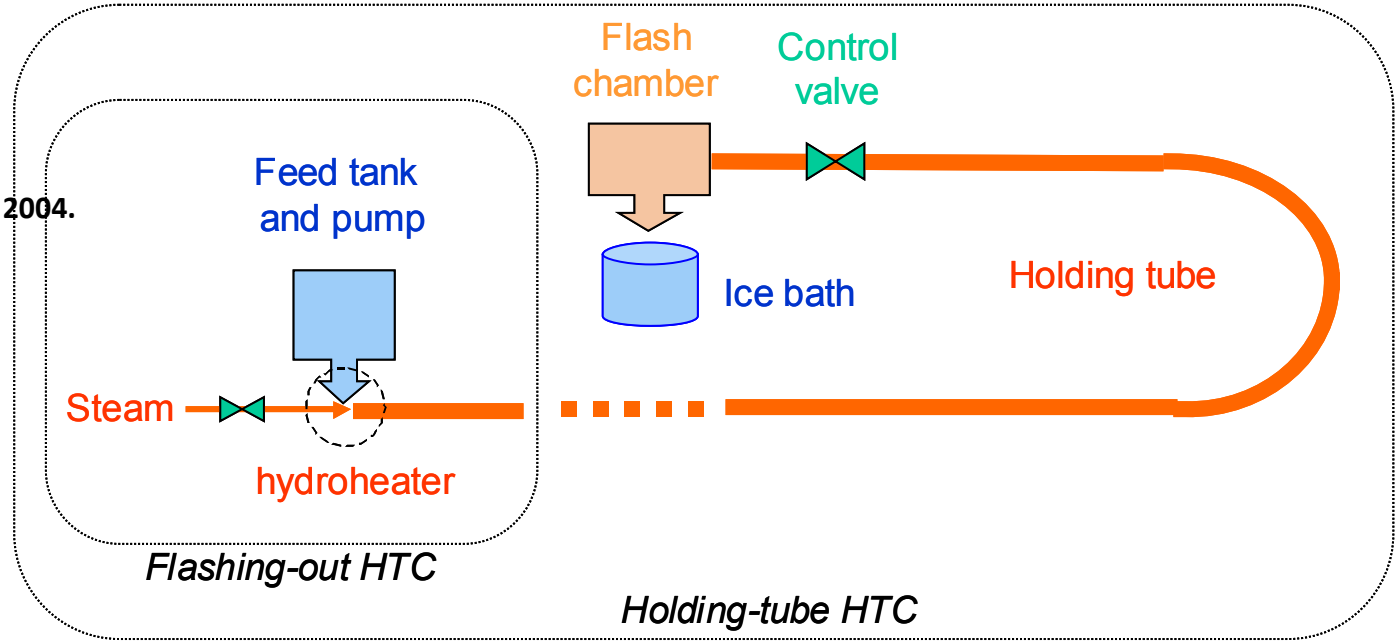


All protein functionalities are improved by hydrolysis treatment (Wang and Wang, 2008)

Re-functionalization by hydrothermal cooking (HTC)

- 90 psi at 154 and 104ºC, for with-holding-tube and flush-out, respectively
- 1.5 kg/min feed rate



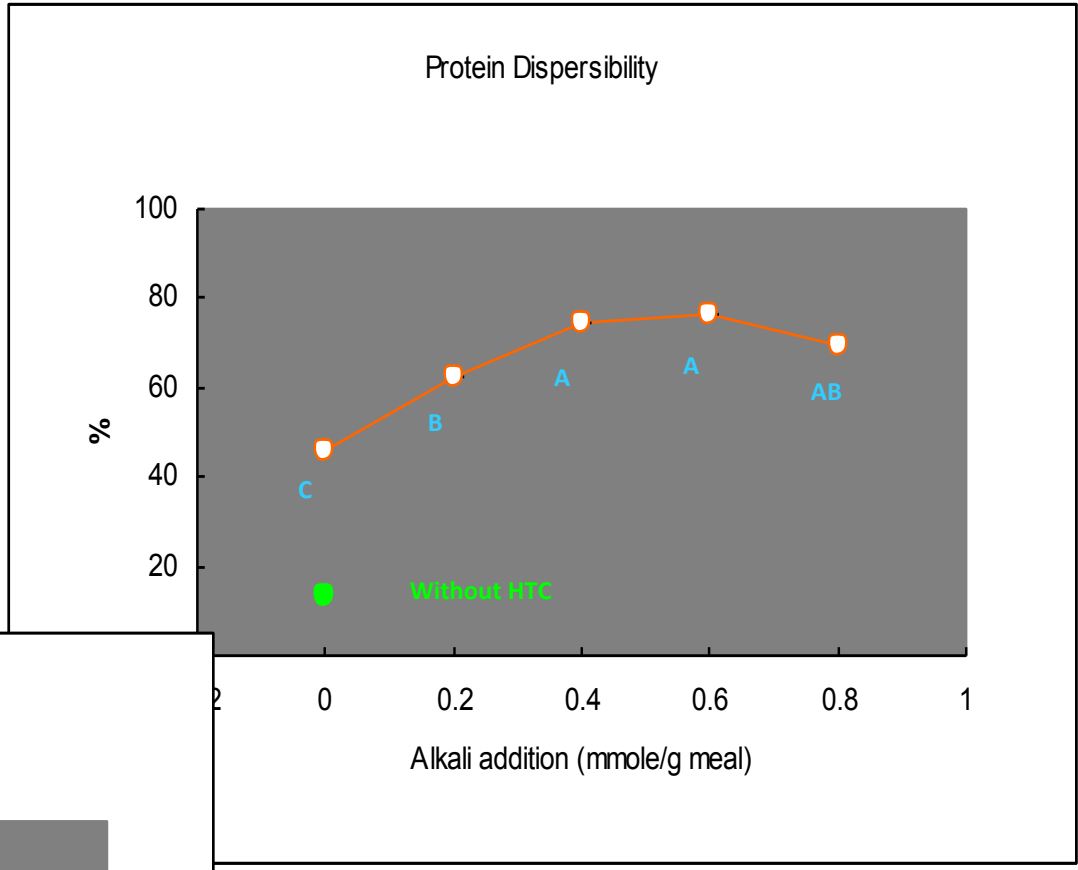


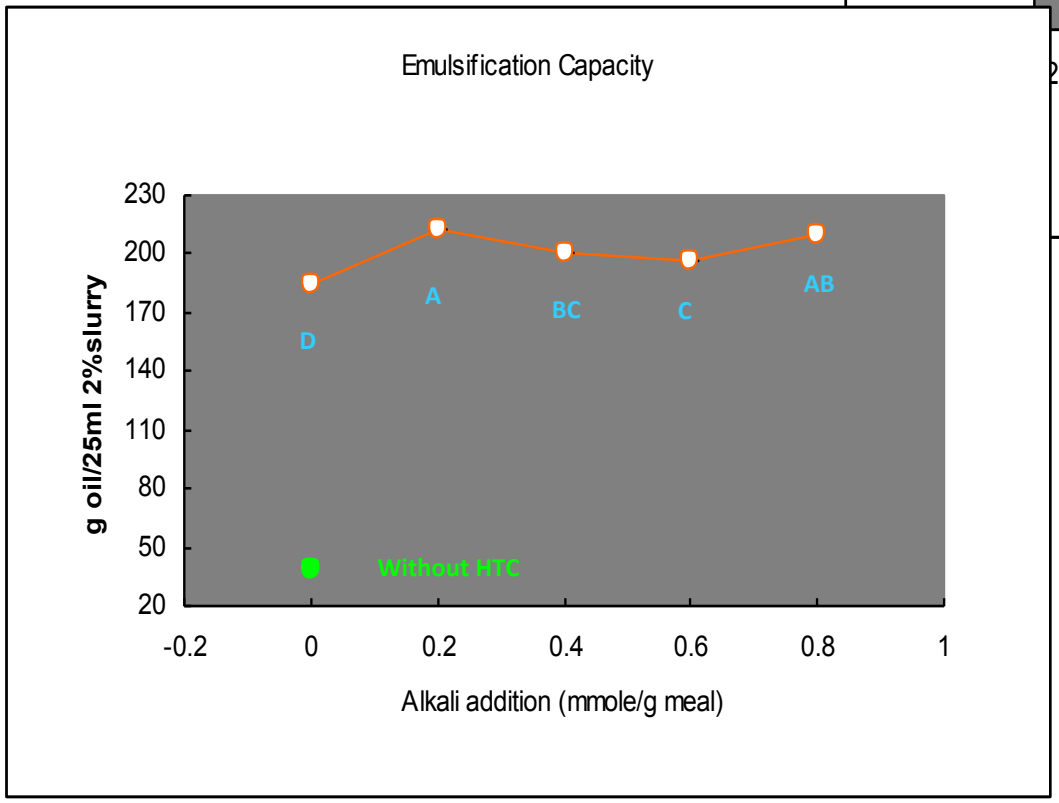
Wang, H., T. Wang, and L.A. Johnson. **2004.** *Journal of the American Oil Chemists' Society 81*:789-794.

Wang, H., T. Wang, and L.A. Johnson, **2005**. *Journal of the American Oil Chemists' Society 82:*451-456

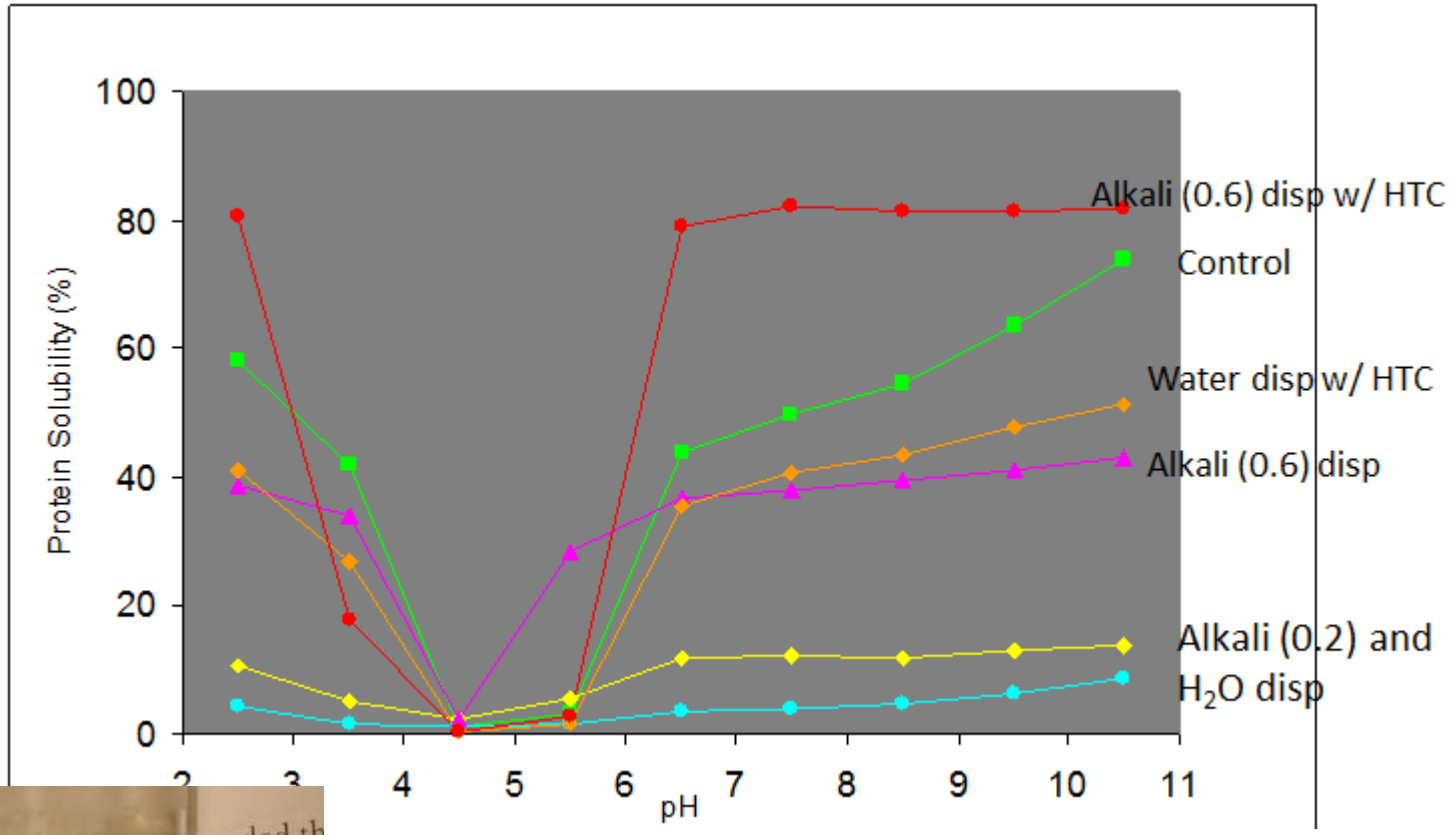
Wang, H., T. Wang, and L.A. Johnson. **2006**. *Journal of the American Oil Chemists' Society 83:*39-45.

Enhancing the effect of HTC refunctionalization using alkali





Re-functionalization with NaOH HTC

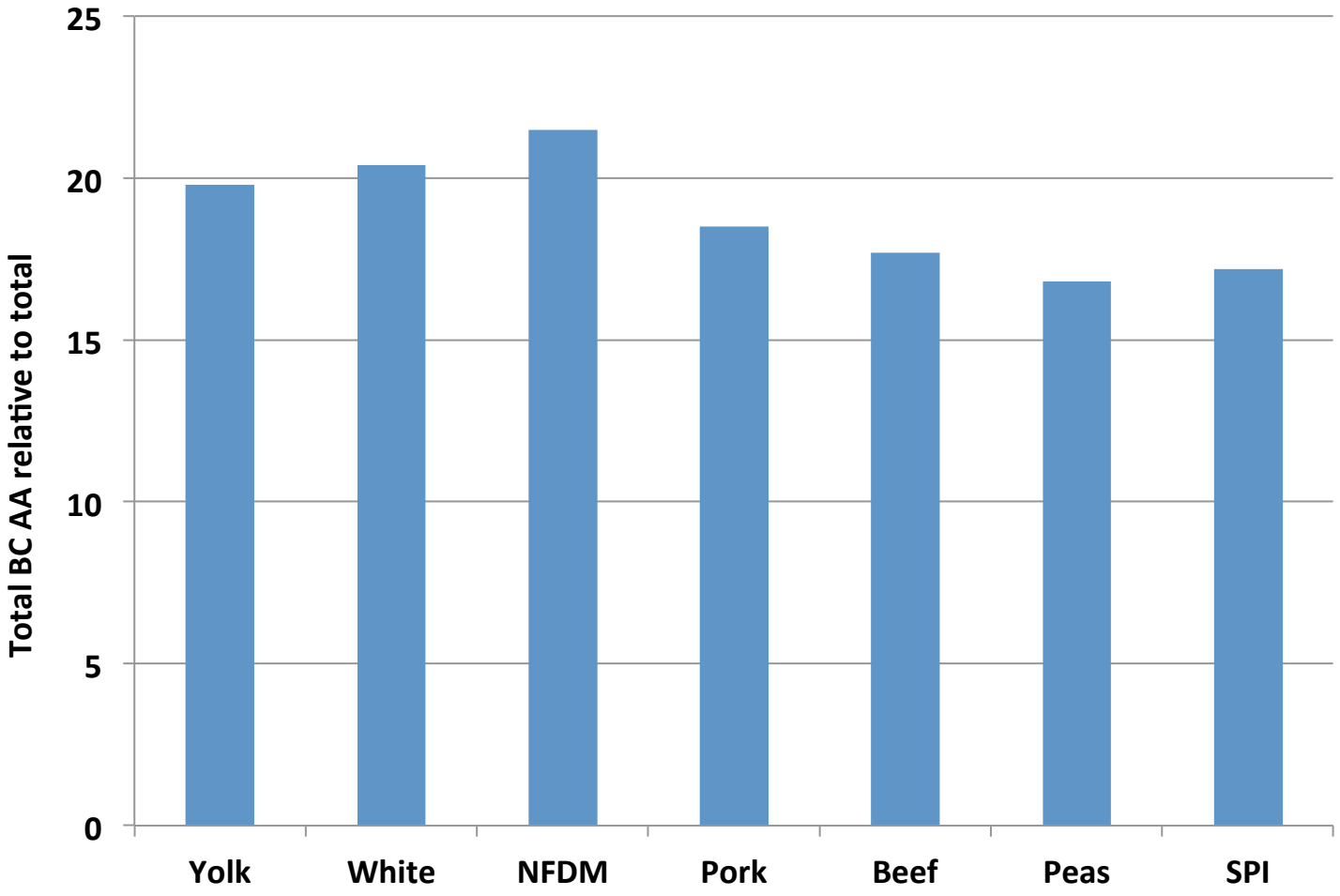


- solubility increased 20 times
- viscosity increased 100,000 times
- particle number decreased 100 times
- protein solution formed a translucent gel

Extruded high protein/fiber product from the fully defatted/de-cholesteroled yolk protein

- High protein snack bar
- Extruded product incorporating other nutritional carbohydrates
- Potential high-protein (and high quality protein) cereal, crispy bars, puffed baby food, etc

Is yolk protein better than other proteins?



Yolk protein has more branched chain amino acid than other animal and plant proteins.

BC AA attenuate muscle damage

- BCAA catabolism induced by exercise;
- BCAA supplementation before exercise attenuates the breakdown of muscle proteins during exercise (Shimomura et al 2006).

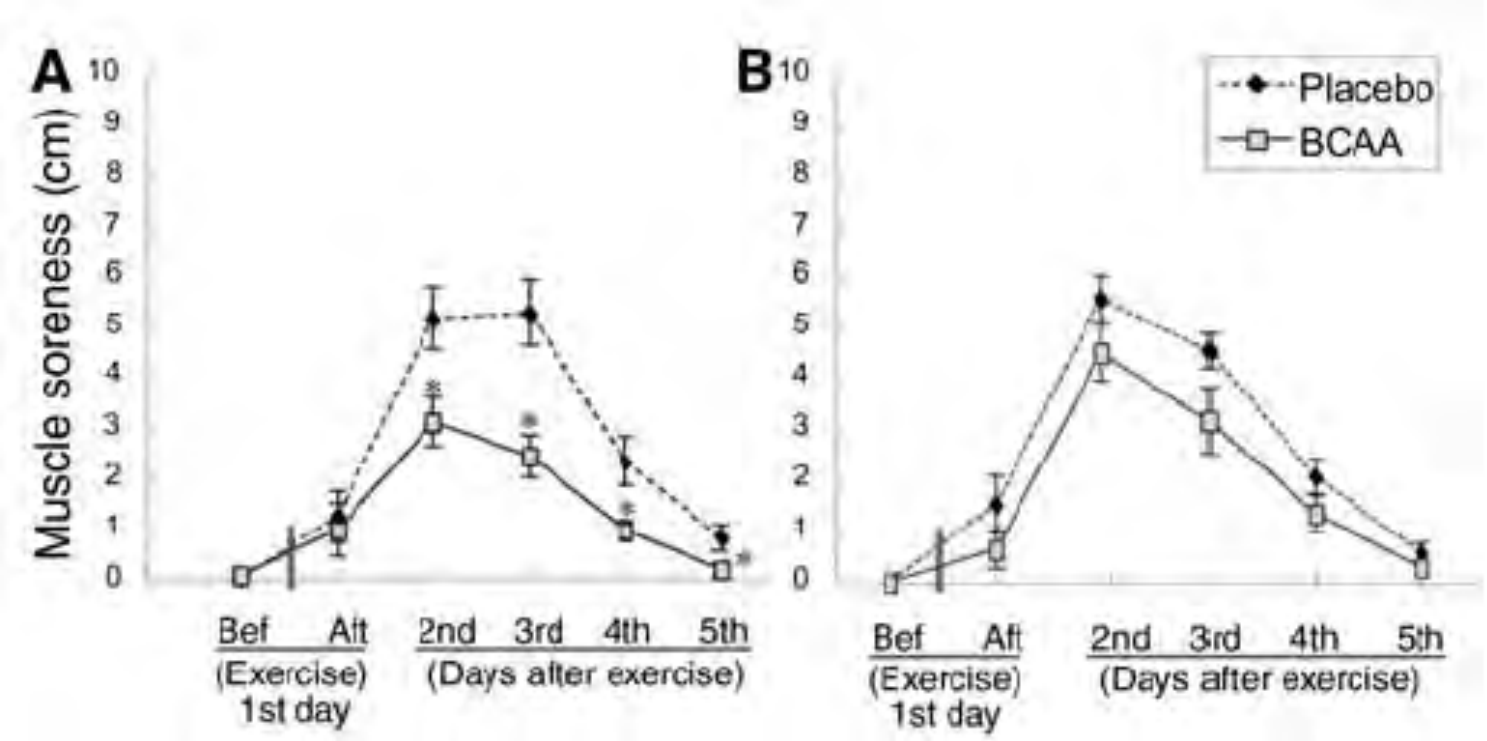
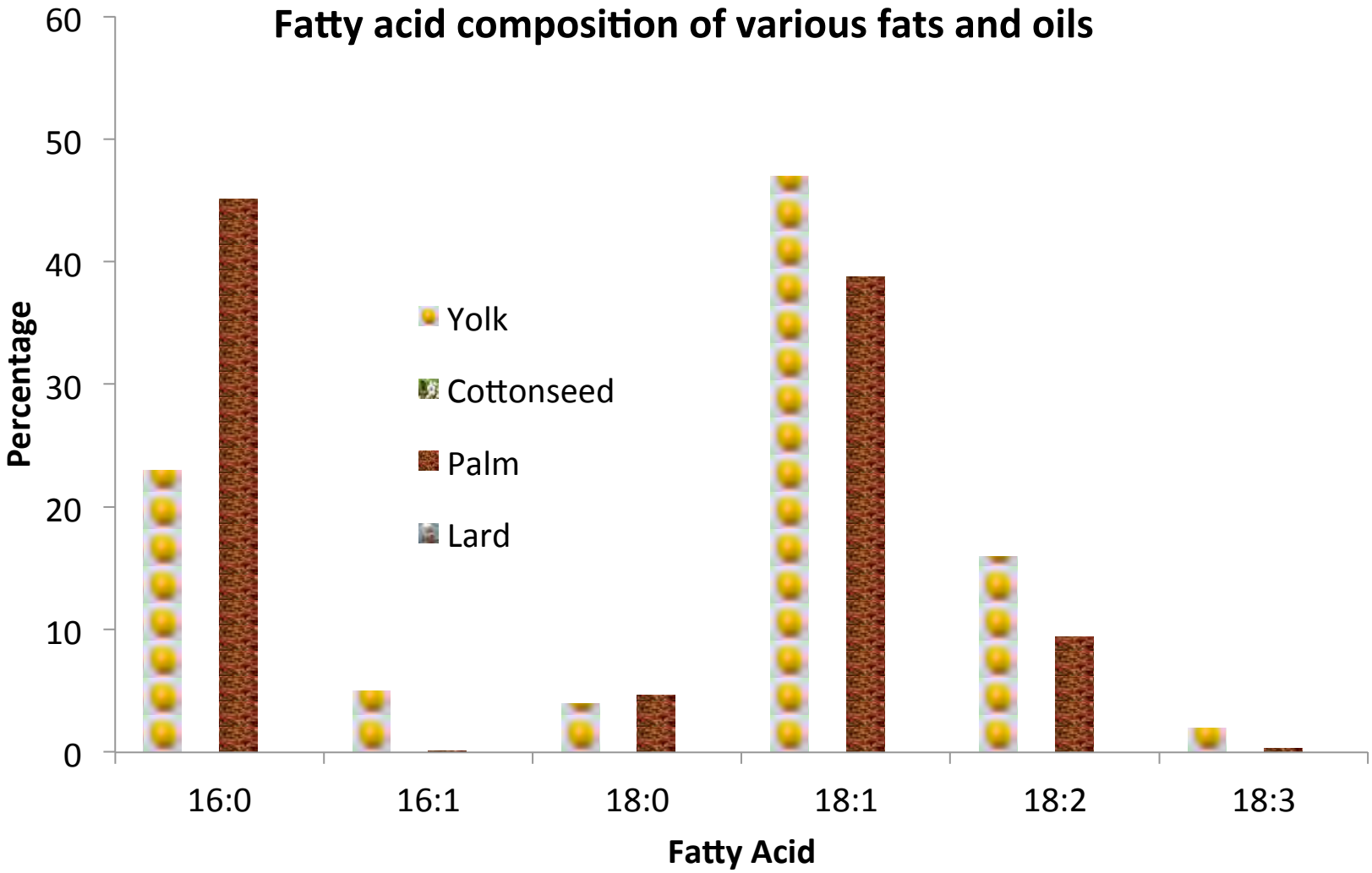


FIGURE 2 Effect of the BCAA supplement on DOMS induced by squat exercise. (*A*) females; (*B*) males. Values are means ± SEM for 16 females and 14 males. $^*P < 0.05$ to the corresponding placebo trial (Wilcoxon signed-rank test).

What to do with the neutral lipids?



Yolk lipid/oil is expected to behave as lard and can be ideal for baking!

The incredible yolk

- Yolk is the origin of life!
- Ideal lipids and proteins!
- Many potential opportunities for new products.