Recent Developments on Emulsification Techniques: Formulation of Nanoscale Antioxidant Food Materials

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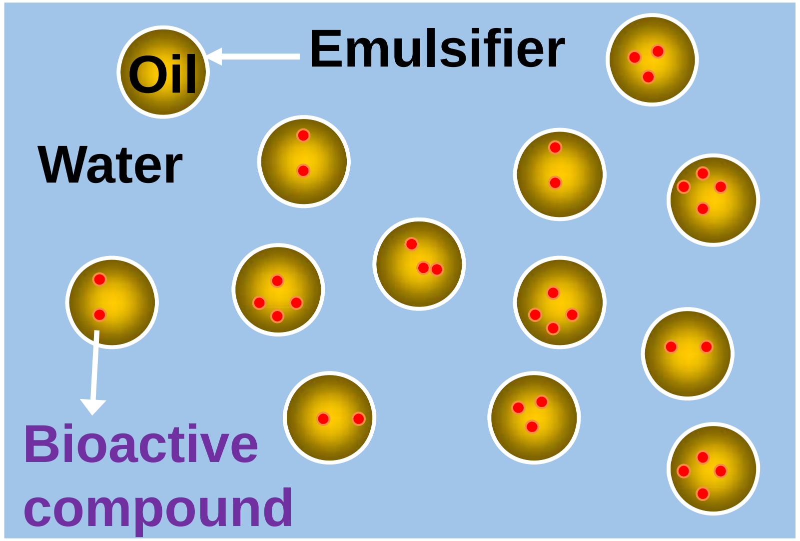
Contents

- **Introduction**
- **Emulsification processes**
- **Formulation of monodisperse emulsions using Microchannel emulsification**
- **β-carotene nanoemulsions passing through an *in vitro digestion model***
- **Food nanoemulsions containing bioactive compounds**
- **Investigation of functional molecules from medicinal plants**
- **Food nanotechnology Project**

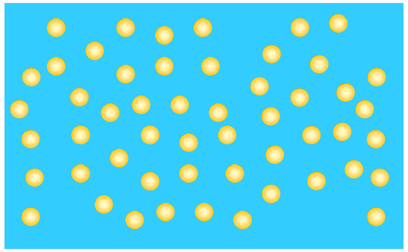
Introduction

Emulsion

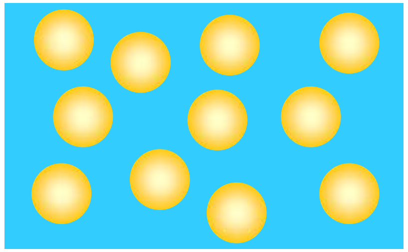
"Small, spherical droplets of one of two immiscible liquids in the continuous phase of another"



Classification



Nano-emulsion
(<500 nm)



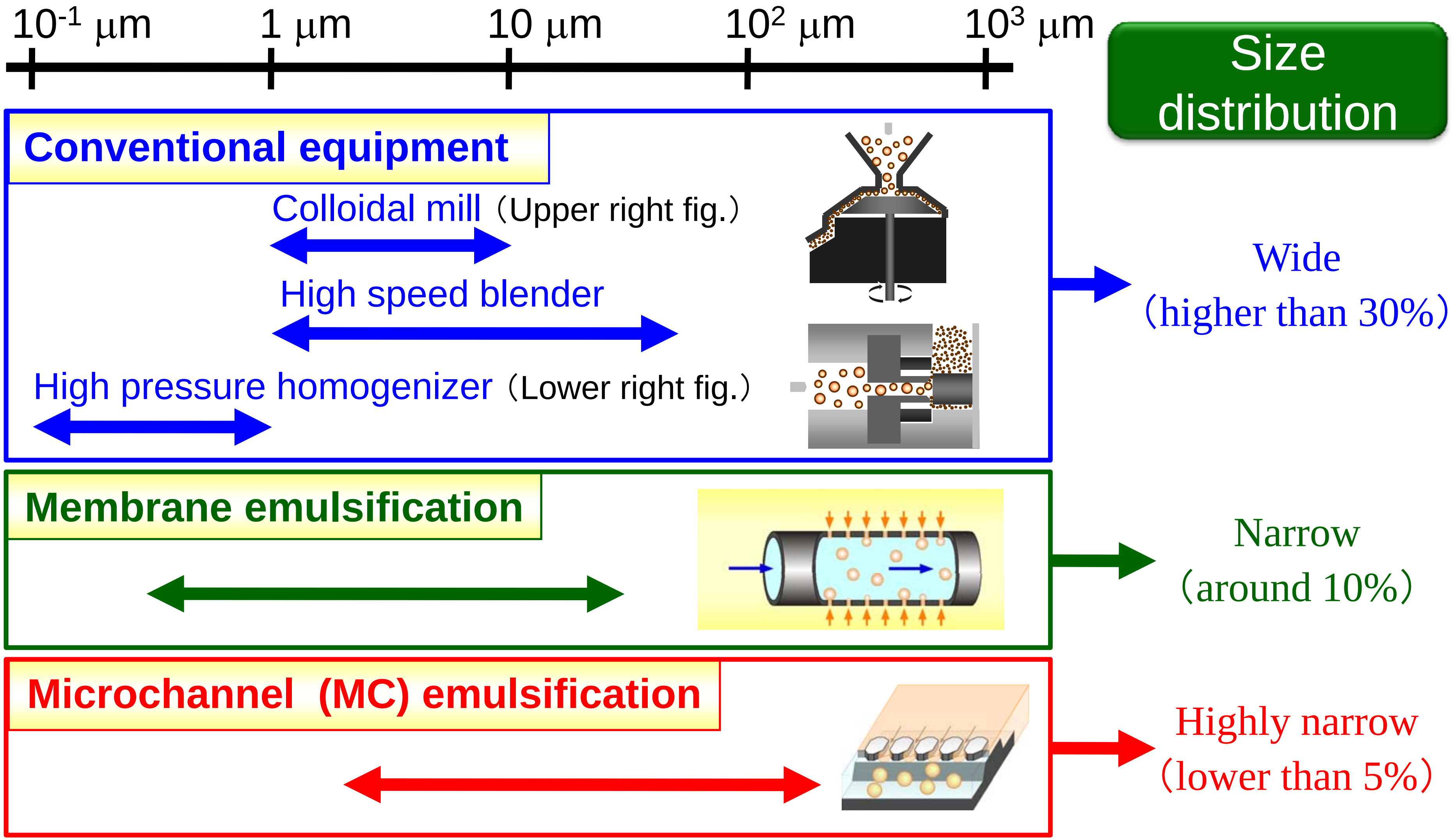
Macro-emulsion
(0.5-100 μm)

Food Formulation Using Oil-in-Water Emulsions

- Increased bioavailability
  - ✓ Lipophilic bioactive compounds dissolved in oil
- Wide application in food industry
  - ✓ Water dispersible system

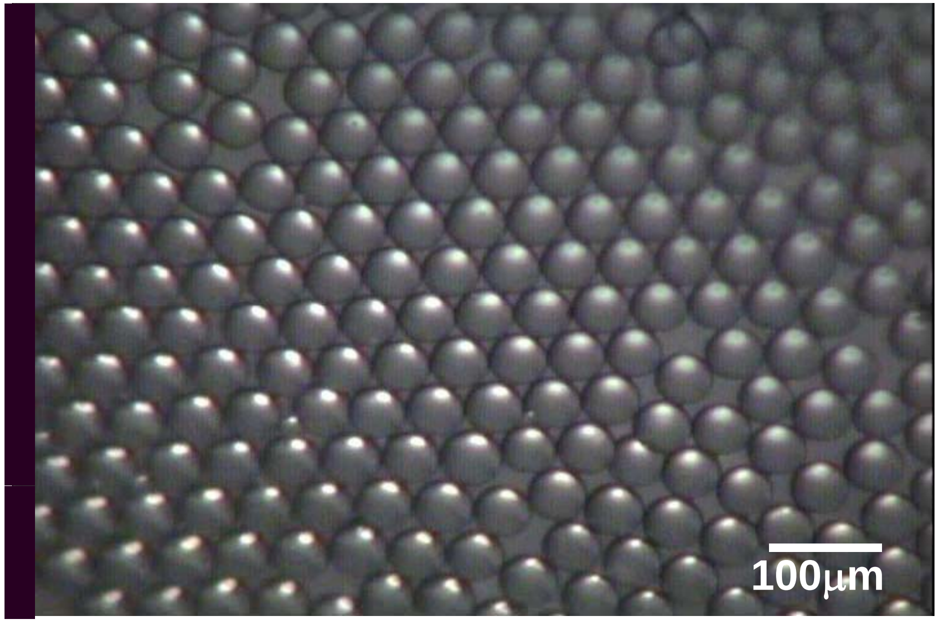
Comparison of emulsification processes

Relation between the type of process and size distribution

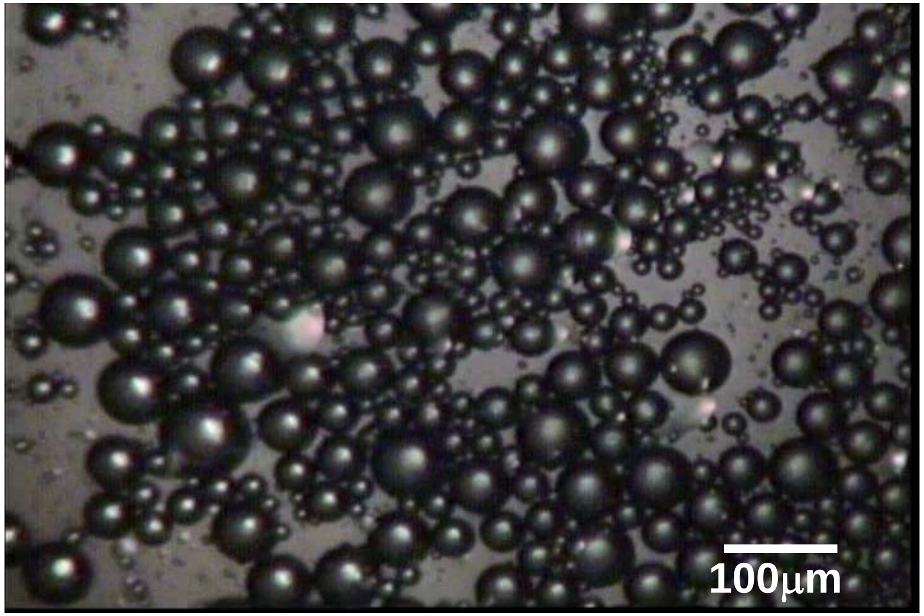


Protein-stabilized* O/W emulsions prepared by different methods

MC emulsification
(Straight-through MC)



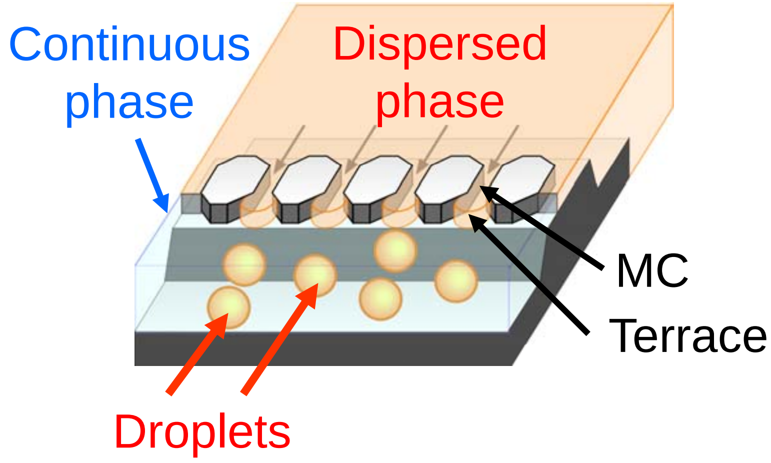
Homogenization
(Polytron)

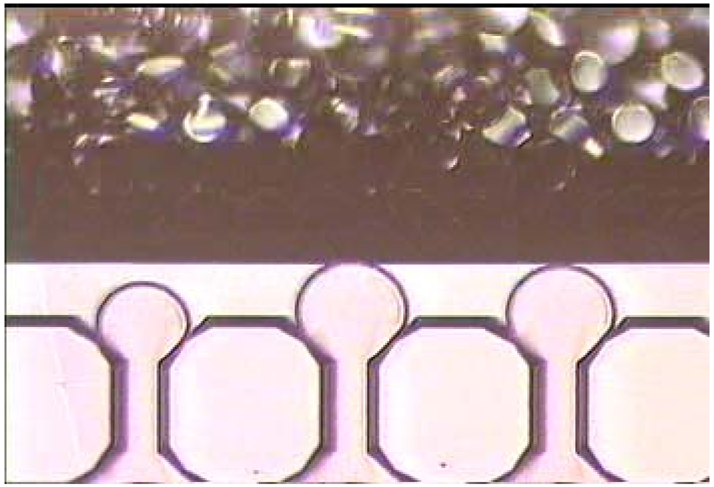


* Bovine serum albumin (1 wt%)

Microchannel emulsification

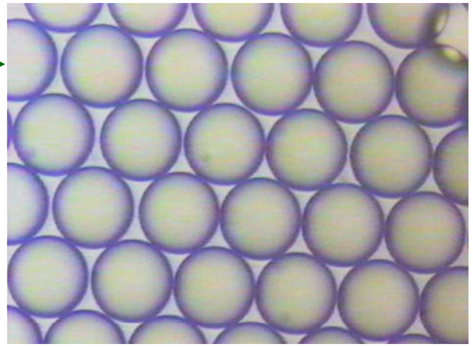
Droplet generation





Uniform droplets

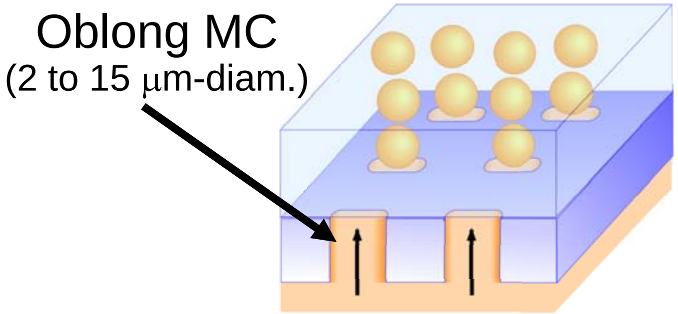
(d_{av}: 1 μm to 100 μm)



- Very mild droplet generation by spontaneous transformation of a dispersed phase that passed through MCs
- Controllable generation of uniform droplets with CV of less than 5%
- MC array devices consisting of many MCs (>100)

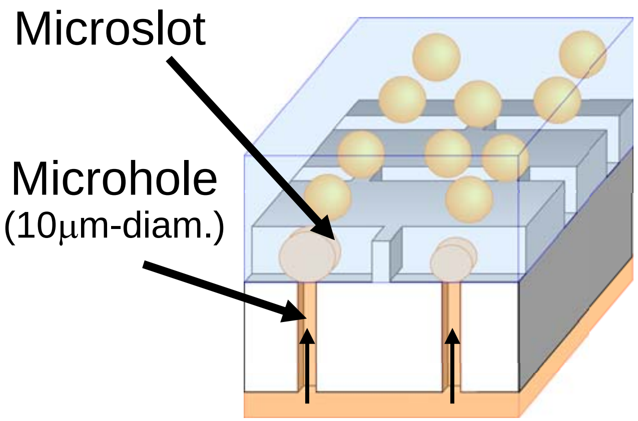
Emulsification using straight-through MC arrays

Symmetric type



Kobayashi *et al.*, *AIChE J.*, 2002

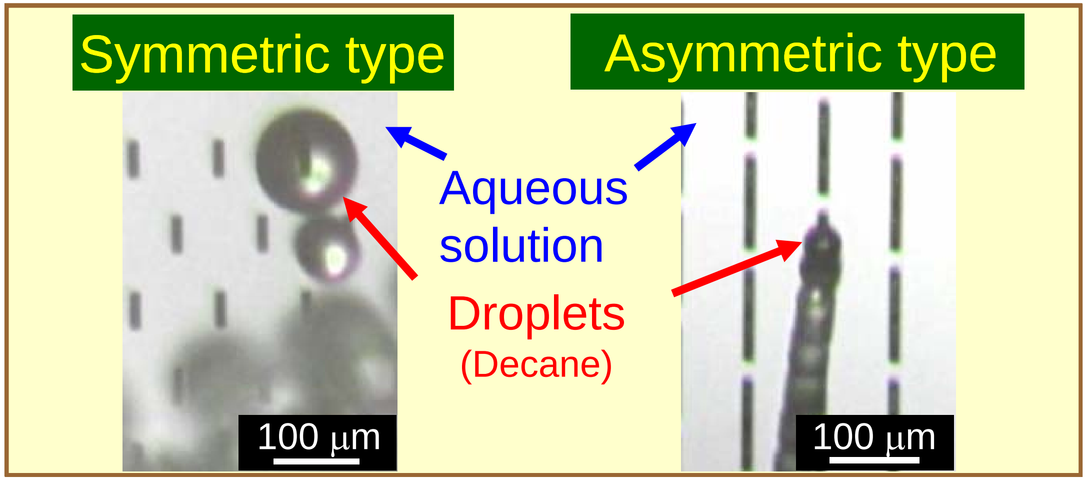
Asymmetric type



Kobayashi *et al.*, *Langmuir*, 2005

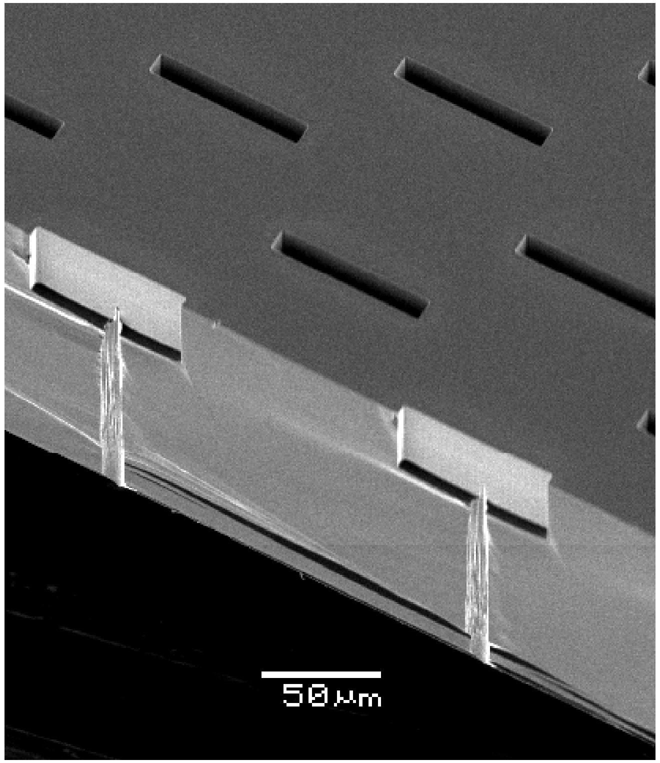
Major features

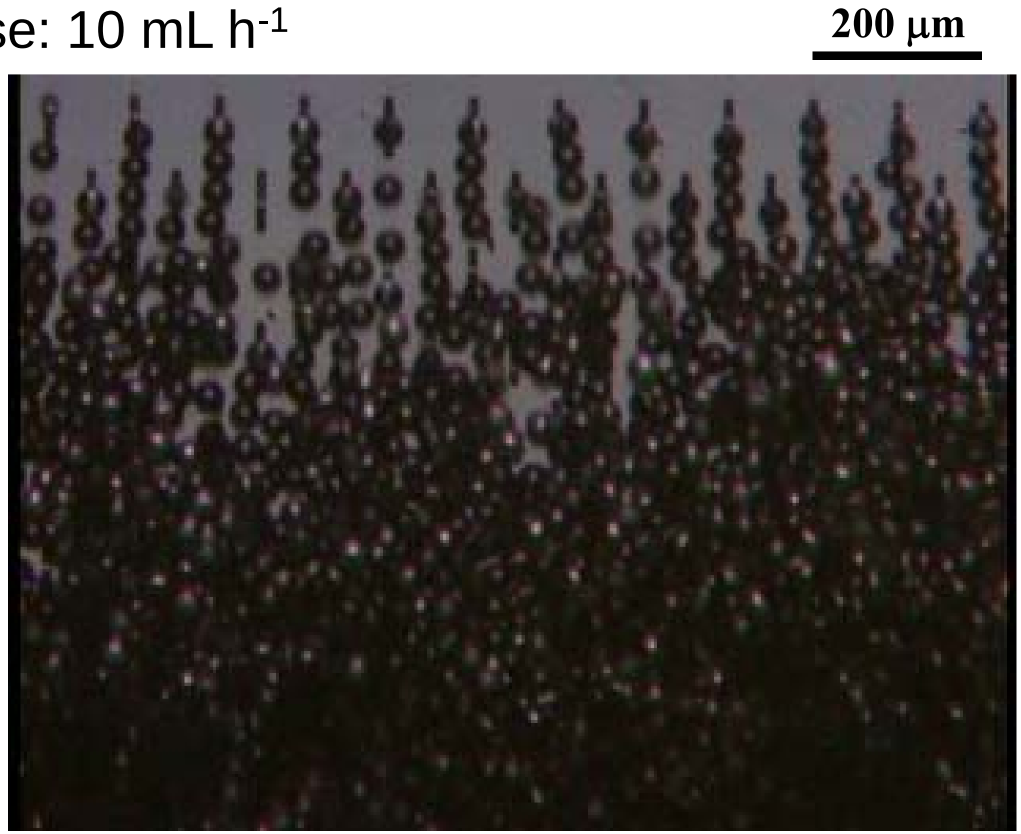
- High-performance production of monodisperse emulsions due to highly integrated MCs
  (Droplet productivity：10 to 2,000 L/(m^2 h))
- Stable generation of highly uniform droplets of low viscosity using asymmetric straight-through MCs



High-performance production of O/W emulsion using asymmetric straight-through MC array (WMS2-2)

- Continuous phase: Milli-Q water with 0.3wt% SDS
- Dispersed phase：Refined soybean oil
- Flow rate of dispersed phase: 10 mL h^{-1}





Highly uniform oil droplets with an average diameter of about 30 µm were generated at a high dispersed-phase flux (100 L m^{-2} h^{-1}).

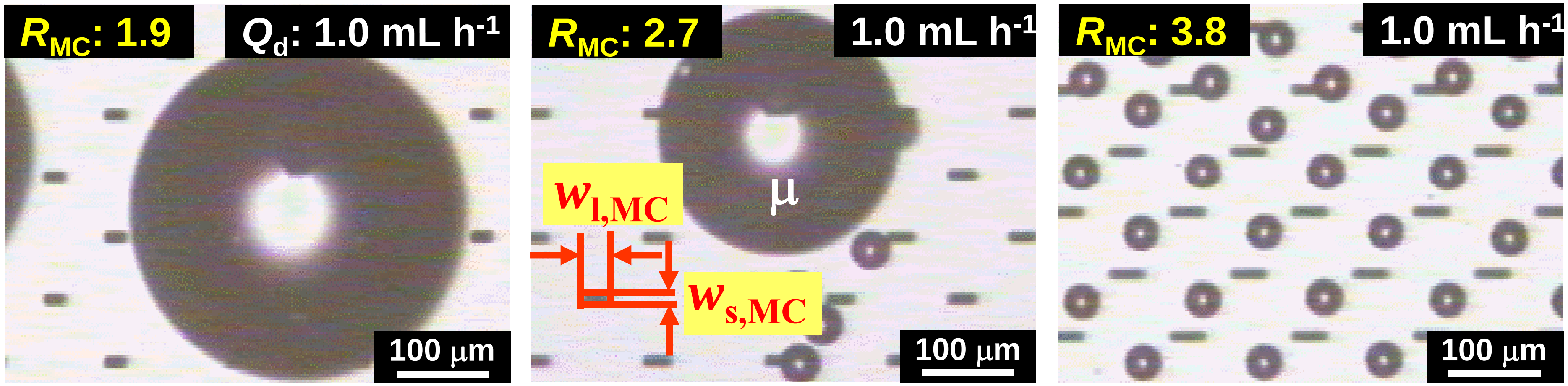
Kobayashi *et al.*, MicroTAS2007

Effect of aspect ratio of oblong MCs (R_{MC})

($w_{s,MC}$: ~10 μm, R_{MC}: $w_{l,MC}$ /$w_{s,MC}$)

- Refined soybean oil-in-Milli-Q water with 1.0 wt% SDS system

Continuous expansion of dispersed phase | **Generation of polydisperse droplets** | **Stable generation of monodisperse droplets**



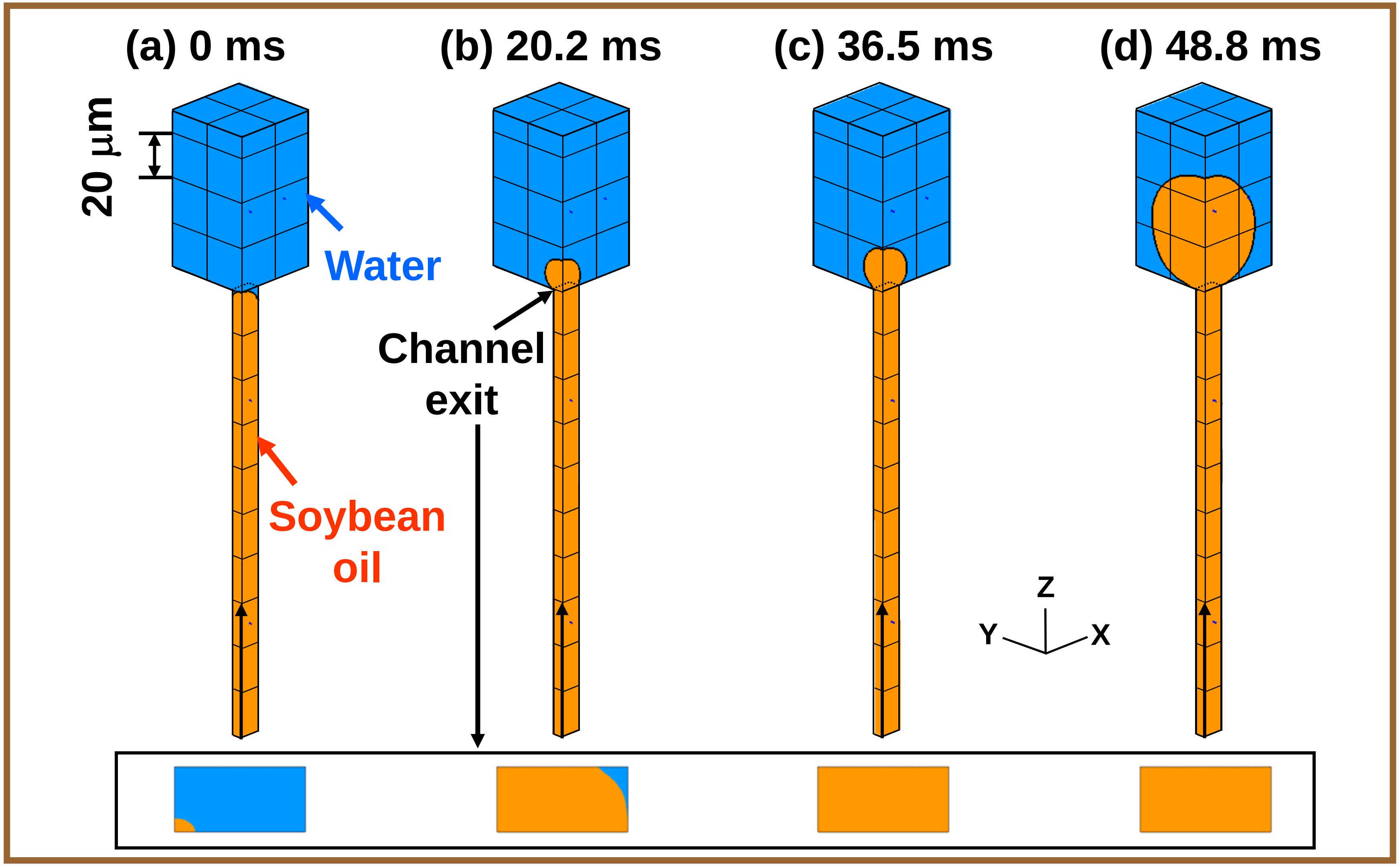
d_{av}: 41.9 μm
CV: 1.9%

Oblong straight-through MCs with R_{MC} over a threshold value of about 3 are needed for stably generating uniform droplets.

Kobayashi *et al.*, J. Colloid Interface Sci. (2004)

Droplet generation process calculated using CFD

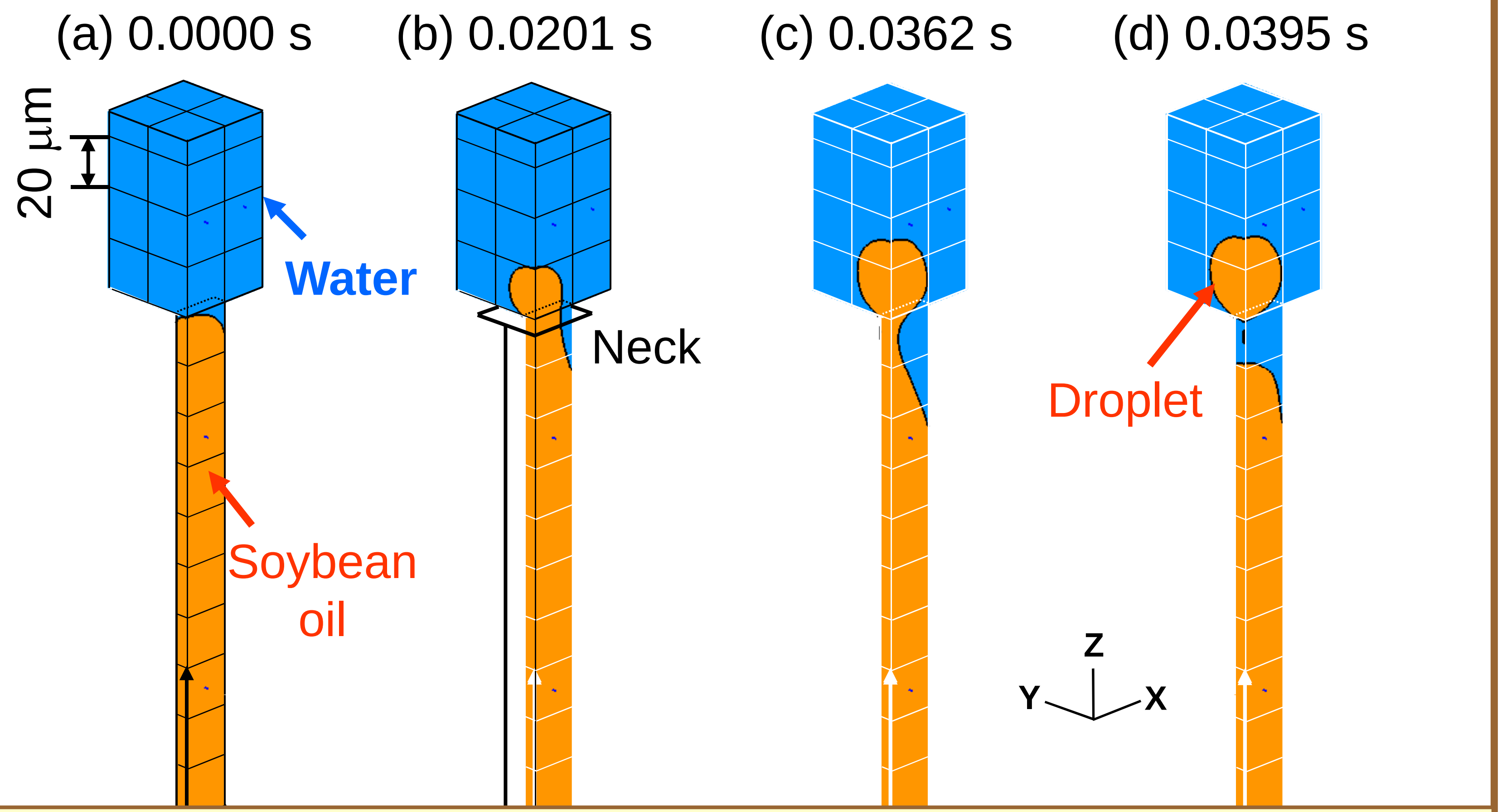
(Oblong straight-through MC, R_{MC}: 2)



Droplet generation process calculated using CFD

(Oblong straight-through MC, R_{MC}: 4)

- Flow velocity of the dispersed phase at the MC inlet ($U_{d,MC}$): 1.0 mm/s

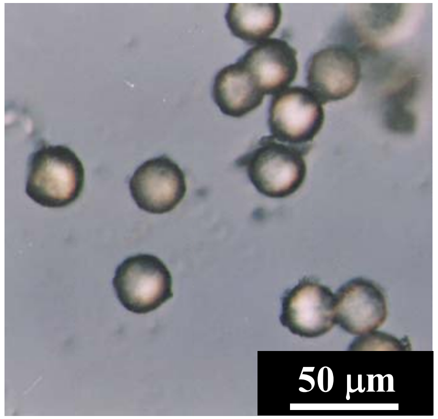


Sufficient space for the continuous phase at the MC outlet must be kept to achieve successful droplet generation.

Applications of MC emulsification

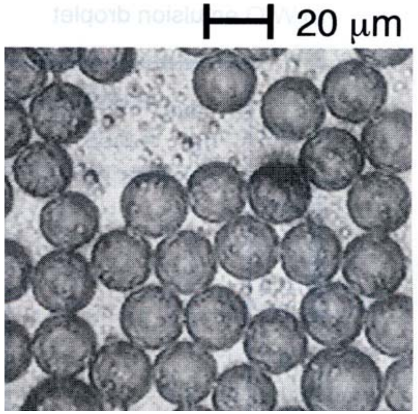
Solid microparticles

(Sugiura *et al*., 2000)



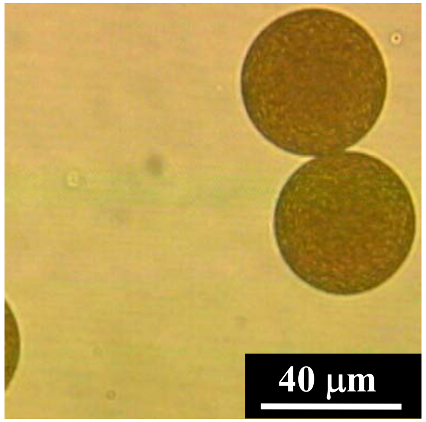
Gel microparticles

(Kawakatsu *et al*., 1999)



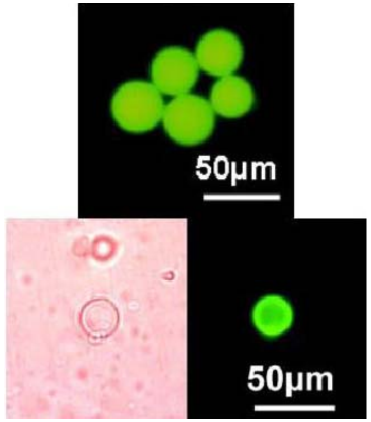
W/O/W emulsions

(Kobayashi *et al*., 2005)



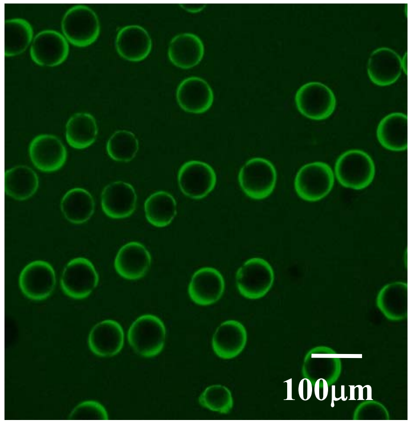
Giant vescicles

(kuroiwa *et al*., 2008)



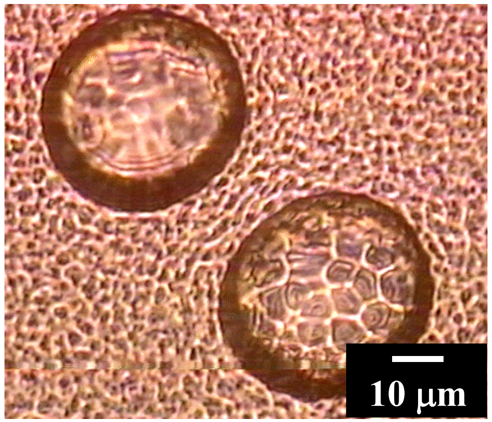
Coaservate microcapsules

(Nakagawa *et al*., 2004)



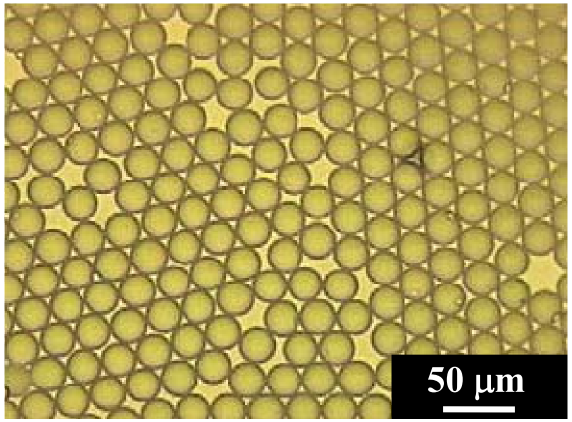
Nanoparticle stabilized O/W emulsions

(Xu *et al*., 2005)



O/W emulsions stabilized by modified lecithin and chitosan

(Chuah *et al*., 2009)



Formulation and characterization of oil-in-water emulsions containing bioactive compounds

- Approach: **To develop a method efficient to produce monodisperse emulsions with antioxidant food materials, and evaluate their stability**

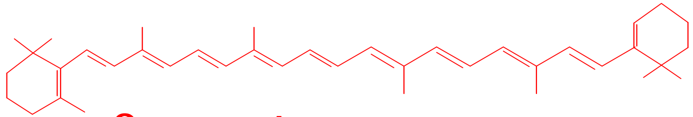
Bioactive food compounds

➢ Palm oil

Oil palm fruit

Palm

Functional compound



β-carotene

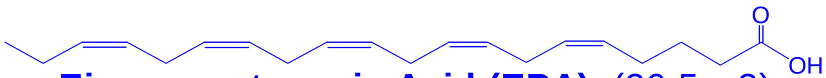
➢ Fish oil

Atlantic Menhaden
(*Brevoortia tyrannus*)

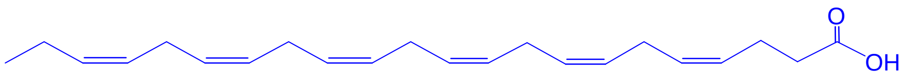
Fish oil

Functional compounds

α-Linolenic Acid (ALA) (18:3ω-3)



Eicosapentaenoic Acid (EPA) (20:5ω-3)

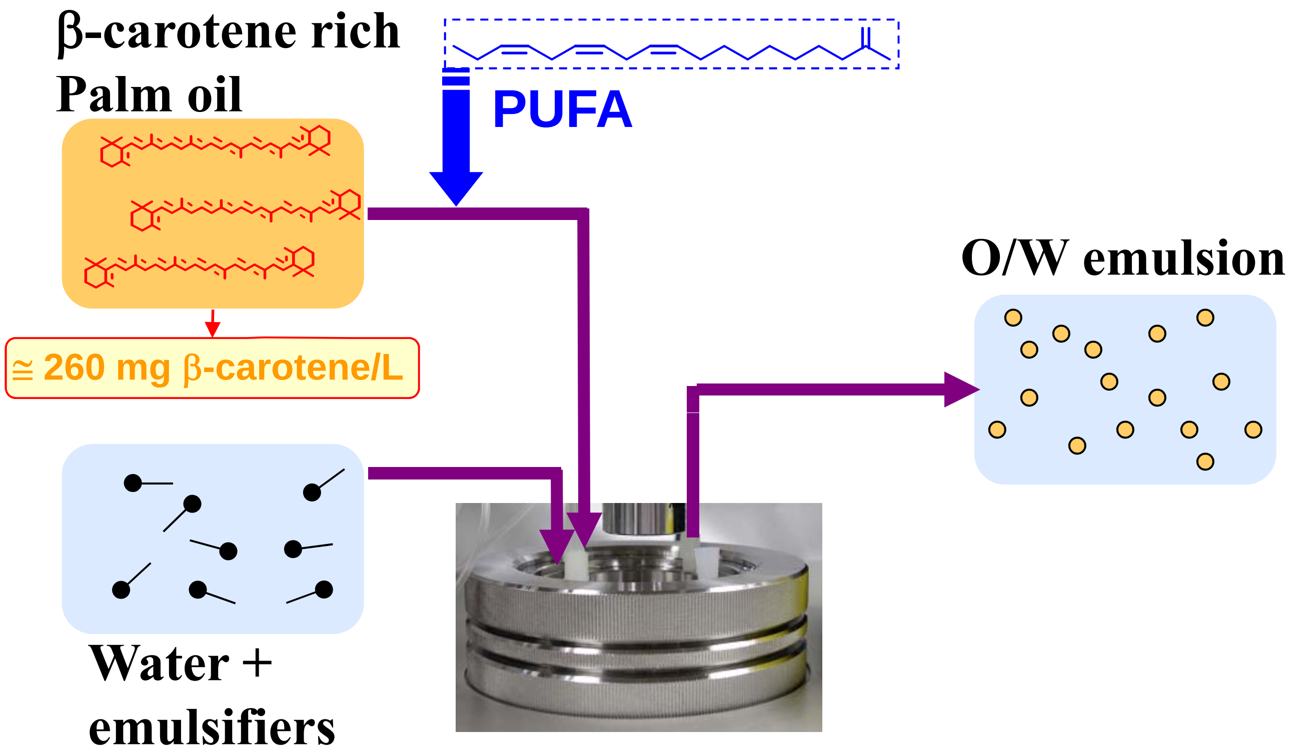


Docosahexaenoic Acid (DHA) (22:6ω-3)

Omega-3 polyunsaturated fatty acids (ω-3 PUFAs)

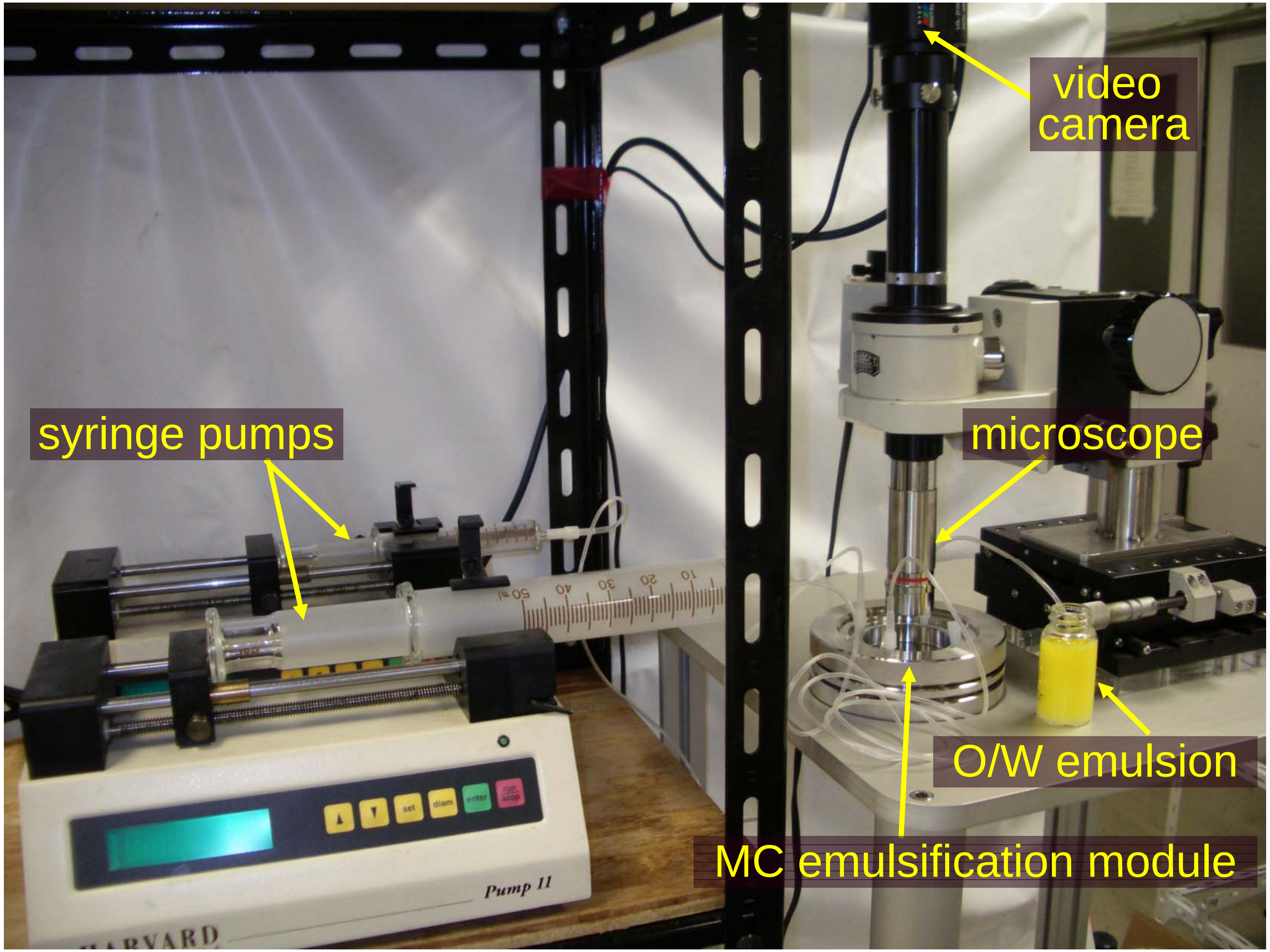
Methodology

- **Disperse phase: Red palm Superolein PUFA (45 g/L)**
- **Continuous phase: Water + β-Lactoglobulin (1wt%) + Sucrose laurate (L-1695) (1wt%)**



Scheme of microchannel (MC) emulsification process

Experimental setup for MC emulsification



Microchannel plate

➢ Asymmetric Straight Through (AST)

- Alternate vertical-horizontal slits
- Silicon fabricated
- Hydrophilic (surface oxidized)

Specification: WMS 1-4

Dimensions:

Diameter: 10 μm

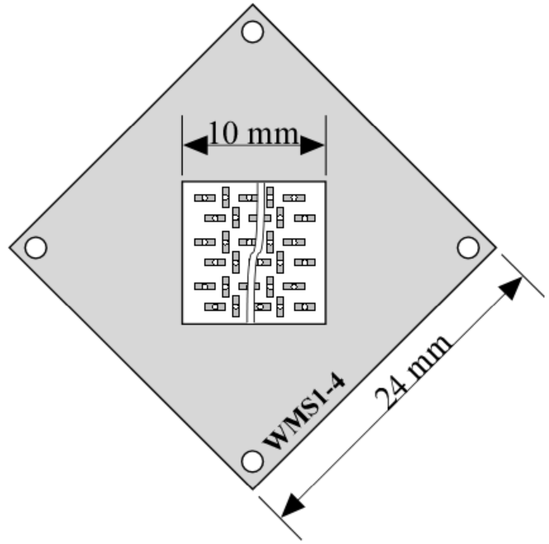
Slit: - longer line: 50 μm

- shorter line:10 μm

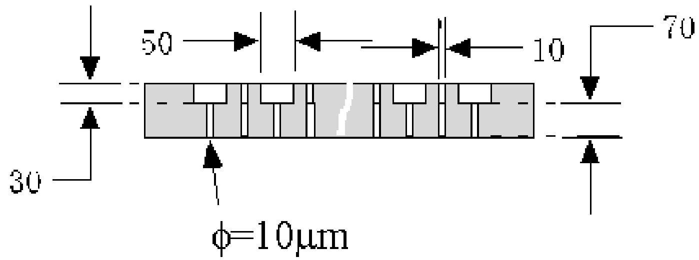
Number of channels: ≅ 23, 400

Active area: 1 x 10^{-4} m^2

Top view



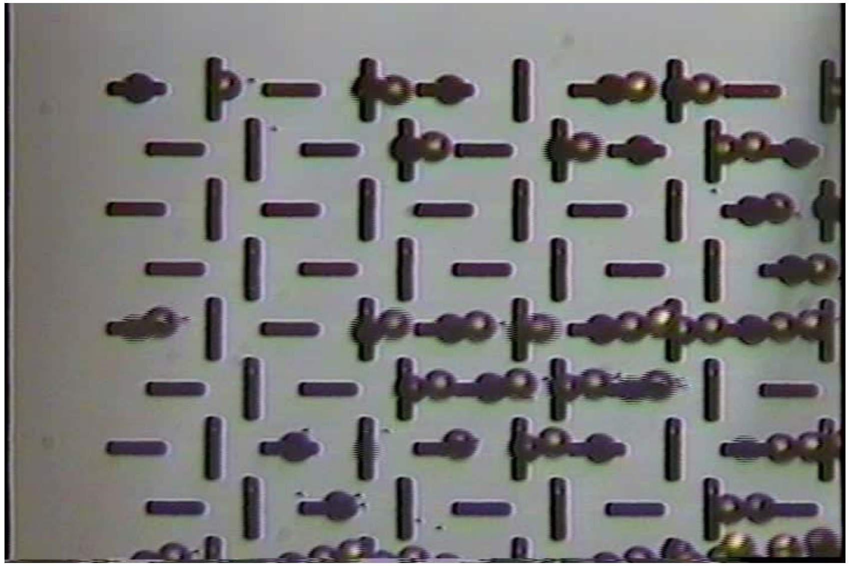
Cross-sectional view



Results

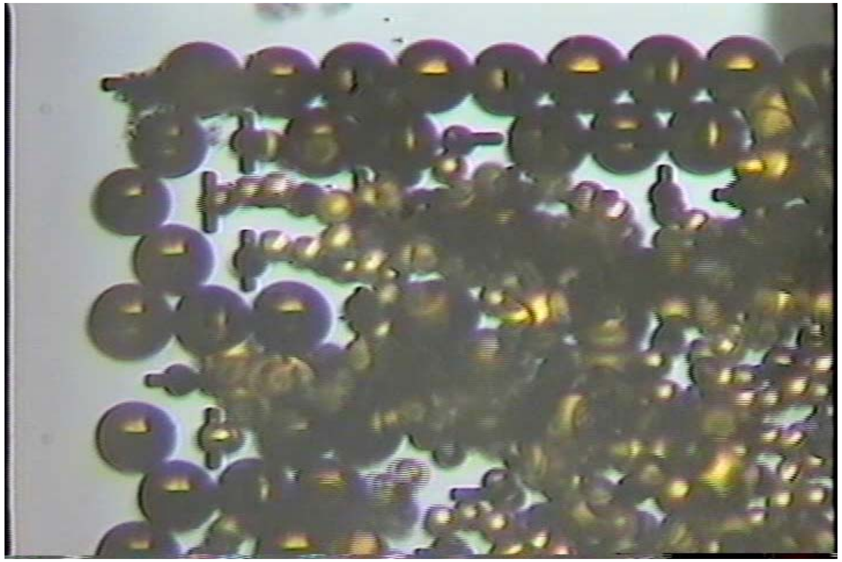
- **Emulsification at various levels of oil flux using β-carotene rich palm oil loaded with PUFAs**

Oil flux: 10 L/(m²·h) 50 μm



d_{av} = 27.6 μm
CV = 3.3 %

Oil flux: 80 L/(m²·h) 50 μm



d_{av} = 33.7 μm
CV = 15.1 %

Images of PUFA-loaded droplets formed using the AST MC plate at various oil fluxes

Neves *et al.*, Food Biophysics 2008

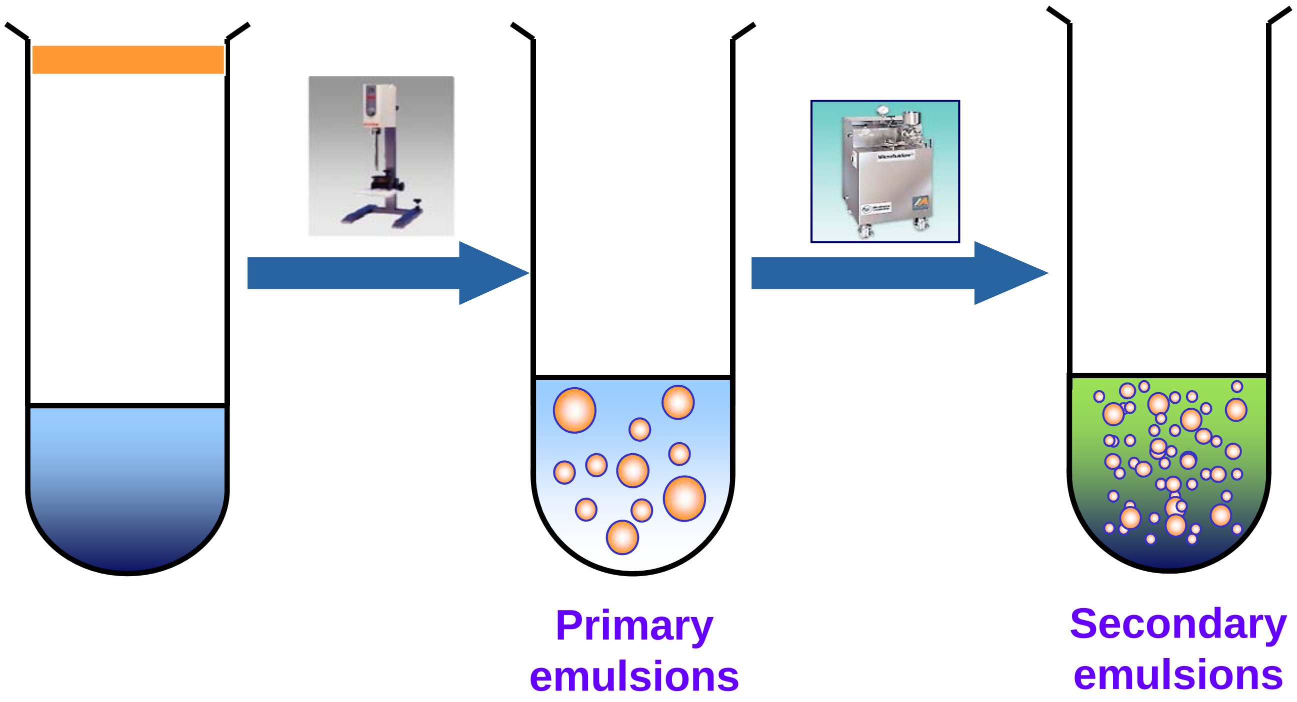
Conclusions (1)

- **Monodisperse PUFA-loaded emulsions containing β-carotene were obtained successfully by Microchannel emulsification.**
- **Increasing the oil flux above 40 $Lm^{-2}h^{-1}$, polydisperse droplets with high coefficient of variation were produced.**
- **The emulsions formed were nearly stable for 3 weeks without coalescence or phase separation.**
- **Monodispersed droplets and droplet size are of essential importance because of its great influence on physical stability.**

β-carotene nanoemulsions passing through an *in vitro digestion model*

- Approach: - To investigate the digestibility of β-carotene nanoemulsions passing through an *in vitro* digestion model.
  - To investigate the effect of different emulsifiers

Methodology



Secondary emulsions:

<u>A: Fine emulsion</u>

Hemogenization at 100MPa for 3 cycles

<u>B: Coarse emulsion</u>

Hemogenization at 10MPa for 1 cycle

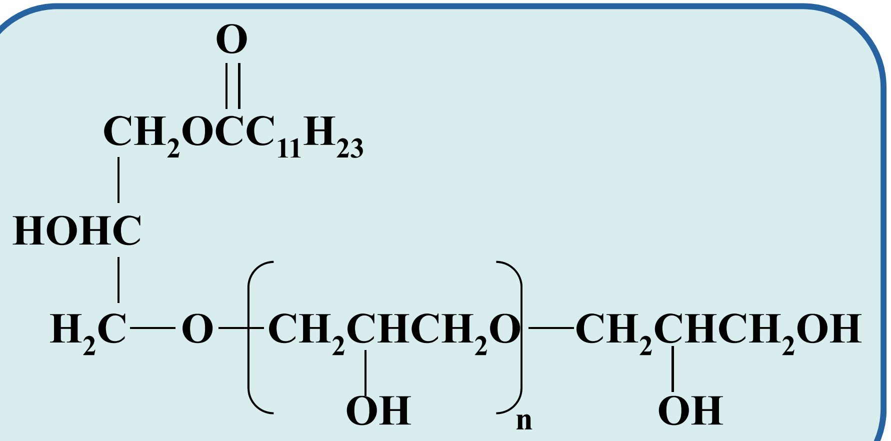
Analysis

- **Particle size analysis**
- **TEM analysis**

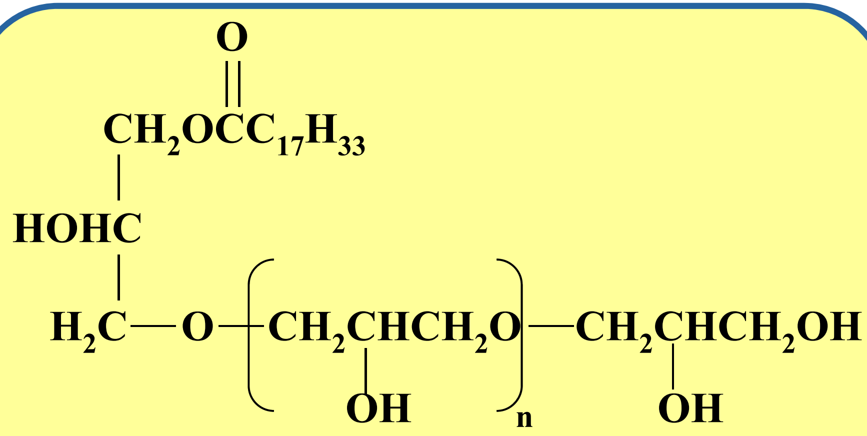
Materials

Polyglycerol Fatty Acid Esters

Supplier: Sakamoto Yakuhin Kogyo Co., Ltd. (Osaka, Japan)



Polyglycerol monolaurate, ML



Polyglycerol monooleate, MO

PGFE type	Chemical name	Polymerization degree, n
ML310	Tetraglycerol monolaurate	2
ML500	Hexaglycerol monolaurate	4
ML750	Decaglycerol monolaurate	8
MO310	Tetraglycerol monooleate	2
MO500	Hexaglycerol monooleate	4
MO750	Decaglycerol monooleate	8

Digestion model

Procedure

(particle digestibility by gastric-intestinal digestion)

Stomach

①pH 7.
- stored for 1 h at room temperature.

②pH 2
- 95 rpm
- 37℃ for 1h.

Stomach digestate

Intestinal

③pH 5.3
- mix with Bile extract/pancreatin solution,
- 95 rpm,
- 37℃ for 2 h.

④pH 7.5
- 2 h at 37℃, 95 rpm

Intestinal digestate

Modified from literatures: Miller et al. (1981)
Beysseriat et al. (2006)

Conclusions (2)

- **Both gastric digestion and intestinal digestion caused the increase of particle size.**
- **Bile extract and pancreatic lipase could absorb to the surface of emulsion and made them more negatively charged.**
- **Bile extract played different role on the release of fatty acid from emulsions when various emulsifiers were used to prepare emulsions.**

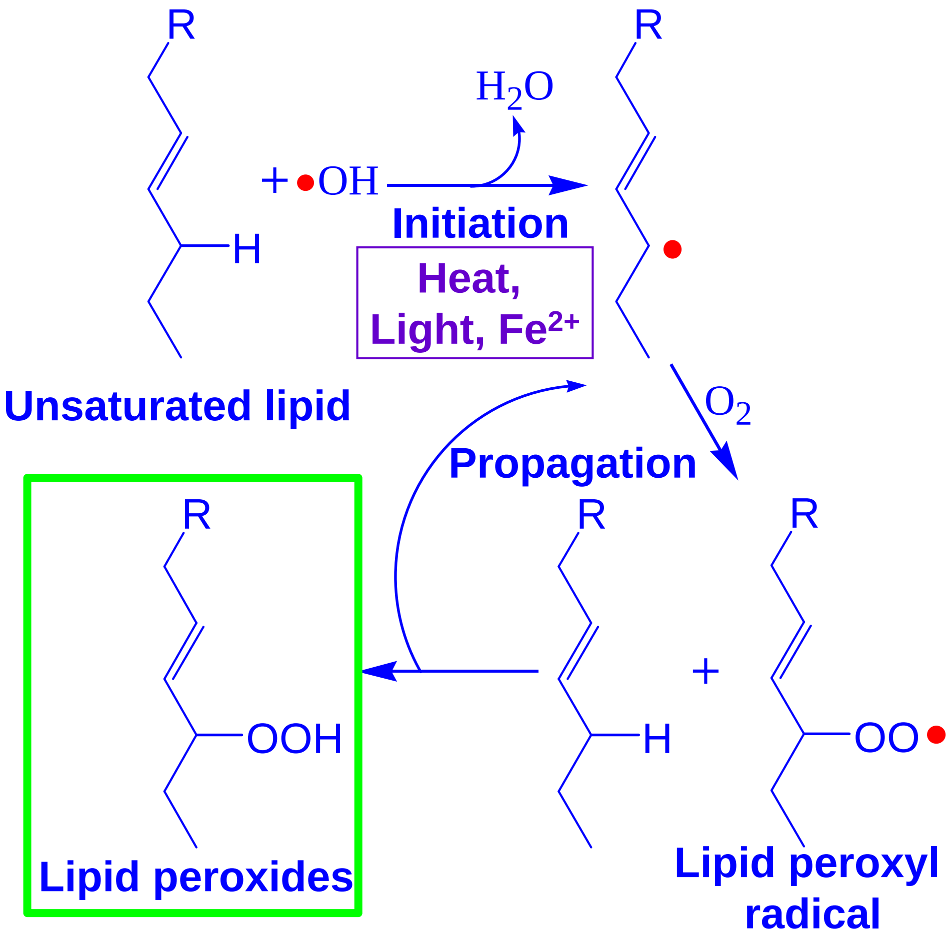
Formulation of food nanoemulsions containing bioactive compounds

- Approach: **To develop a method efficient to produce food nanoemulsions with increased oxidative stability**

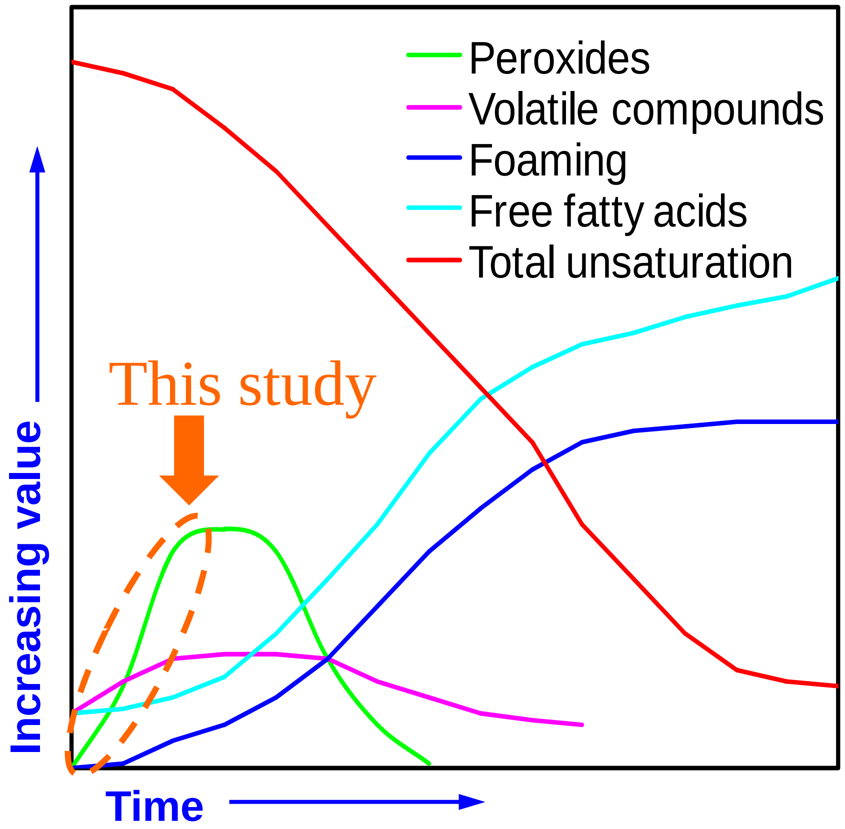
Lipid oxidation in food emulsions

- Great importance to food technologists (undesirable flavors)
- Environmental and processing factors (O_2, heat, light)

Reactions involved in lipid oxidation



Indicators of lipid autoxidation



Akoh & Min, Food Lipids, 840 p. (1998)

Objectives

- To determine the relationship between droplet size and the oxidation rate of fish oil-in-water (O/W) emulsions with different droplet sizes.

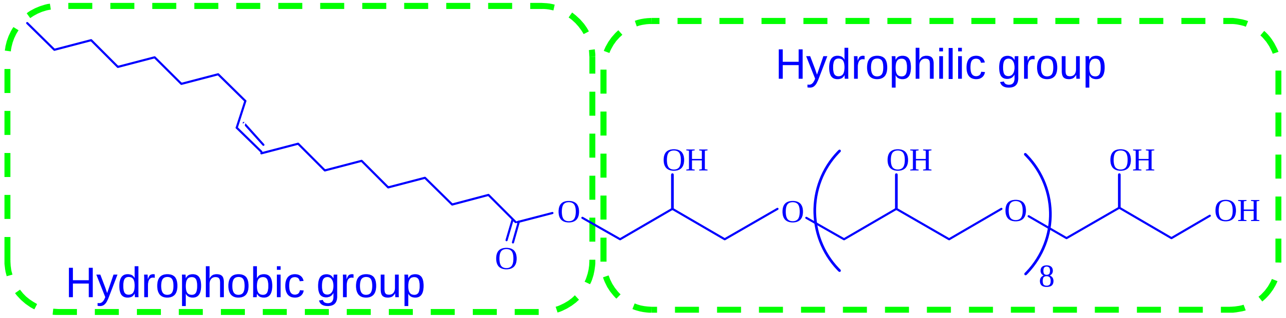
- To develop a method for preventing, or at least retarding lipid oxidation in food emulsions.

- To elucidate the mechanisms by which lipid oxidation occurs, in order to design an oxidatively stable emulsion containing bioactive PUFA.

Materials

➢ Continuous phase: 1 wt% MO750

- ◆ Sakamoto Yakuhin Kogyo Co.
- ◆ Viscosity = 1.05 mPa s; Density = 998 kg/m^3
- ◆ Dissolved in phosphate buffer (50 mM; pH 7); NaN_3 (0.02 wt%)

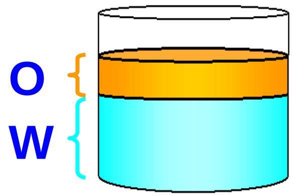


Decaglycerol monooleate (MO750)

- ◆ Nonionic food-grade surfactant (HLB: 12.9)

Pre-mixture:

1:9 w/w (O:W)

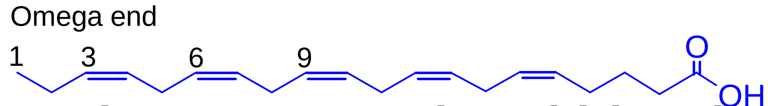


Used in all experiments, except for MC emulsification

➢ Disperse phase: Fish oil

Atlantic Menhaden

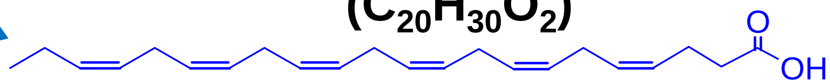
～14 %



Eicosapentaenoic Acid (EPA)

($C_{20}H_{30}O_2$)

～10 %



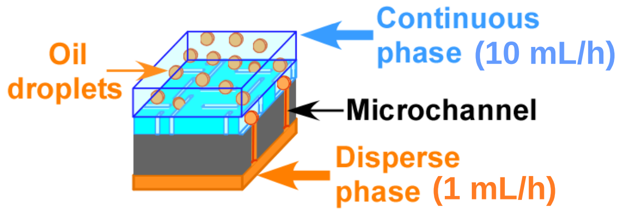
Docosahexanoic Acid (DHA)

($C_{22}H_{32}O_2$)

- ◆ Sigma Aldrich Co.
- ◆ Viscosity = 44.3 mPa s; Density = 924 Kg/m^3
- ◆ Interfacial tension between the phases = 10.5 mN/m

Emulsification Processes

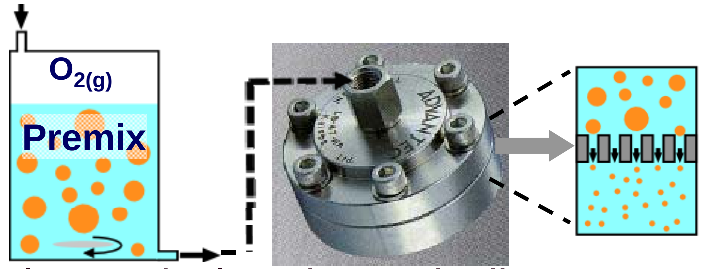
Microchannel (MC) Emulsification



- Asymmetric Straight-Through MC
- Silicon plate: WMS 1-4
- d_{MC}= 10 μm

(Neves *et al*., 2008)

Premix Membrane Emulsification



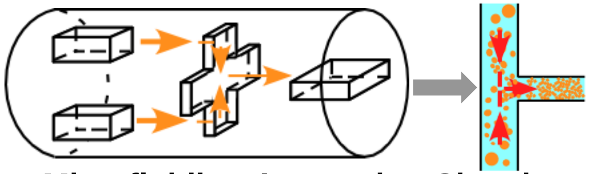
- Nuclepore (polycarbonate); disc: 47 mm
- Pore: 100 nm (0.5 MPa)~800 nm (0.06 MPa)
- Stirrer (500 rpm); 1 cycle

(Kukizaki, 2005)

Vacuum Homogenizer

- 10,000 rpm / 5 min
- 25 °C
- Mizuho Co.
- Coarse emulsion

High Pressure Homogenizer



Microfluidizer Interaction Chamber

- Pressure: 20, 40, 80, 100, 160 MPa
- 1 cycle ; 10 °C
- Fine emulsion

(Toda *et al*., 1997)

All emulsions prepared were stored either at 5 or 30 ºC, in absence of light.

Analyses

➢ <u>Droplet Size and Size Distribution</u>:

✓ **Light Scattering** (Beckman Coulter, LS 13320): size range: 40 nm~2 μm

Sauter Mean Diameter ($d_{3,2}$)

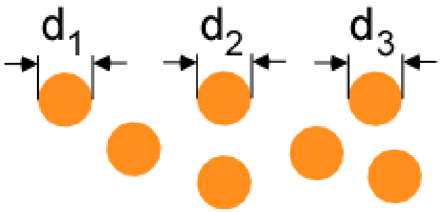
$$d_{3,2} = \frac{Volume}{Surface\ Area} = \frac{\sum n_i\, d_i^{3}}{\sum n_i\, d_i^{2}}$$

n_i= Nº. of droplets

d_i= Diameter

✓ **Image analysis** (WinRoof 5.6, Mitani Co.)

Mean Diameter ($\bar{d}$)



$$\bar{d} = \frac{d_1 + d_2 + \mathrm{K} + d_n}{n}$$

n = 200 droplets

➢ <u>Lipid Hydroperoxides</u> (LOOH):

✓ **Ferric Thiocyanate:** **Major reaction:** **$LOOH + Fe^{2+} \rightarrow LO\cdot + OH^- + Fe^{3+}$**

Analysis: **- Lipid extraction with isooctane/2-propanol;**

- Reaction of the organic phase with methanol/1-butanol;

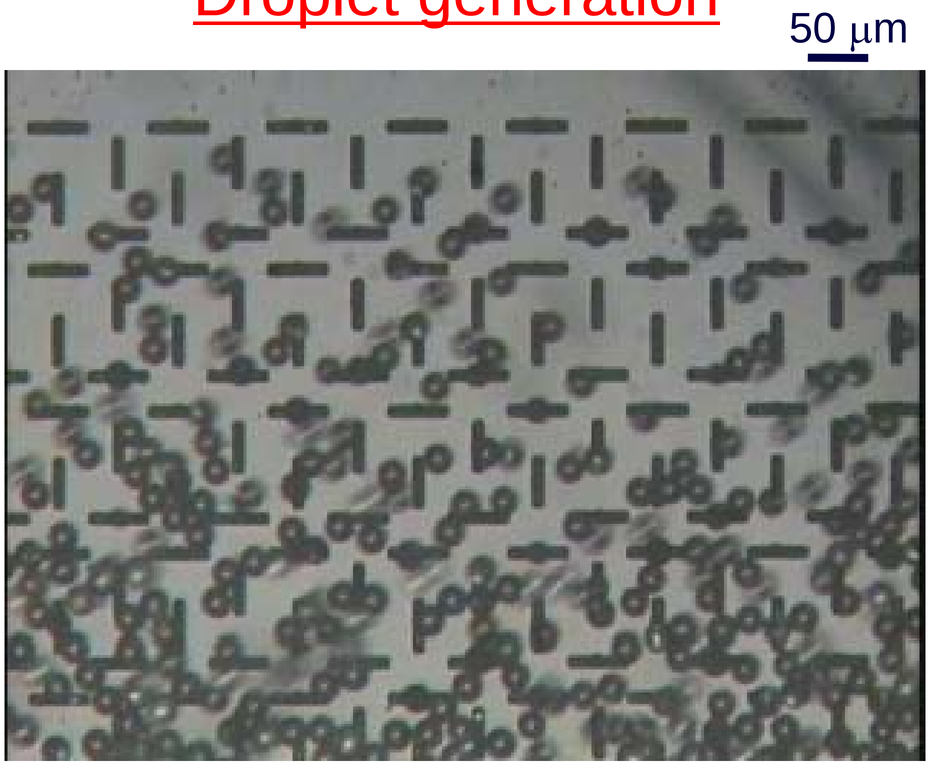
- React for 20 minutes with ammonium thiocyanate and ferrous ions solution;

- Read Absorbance at 510 nm using a spectrophotometer (Jasco V530, Japan).

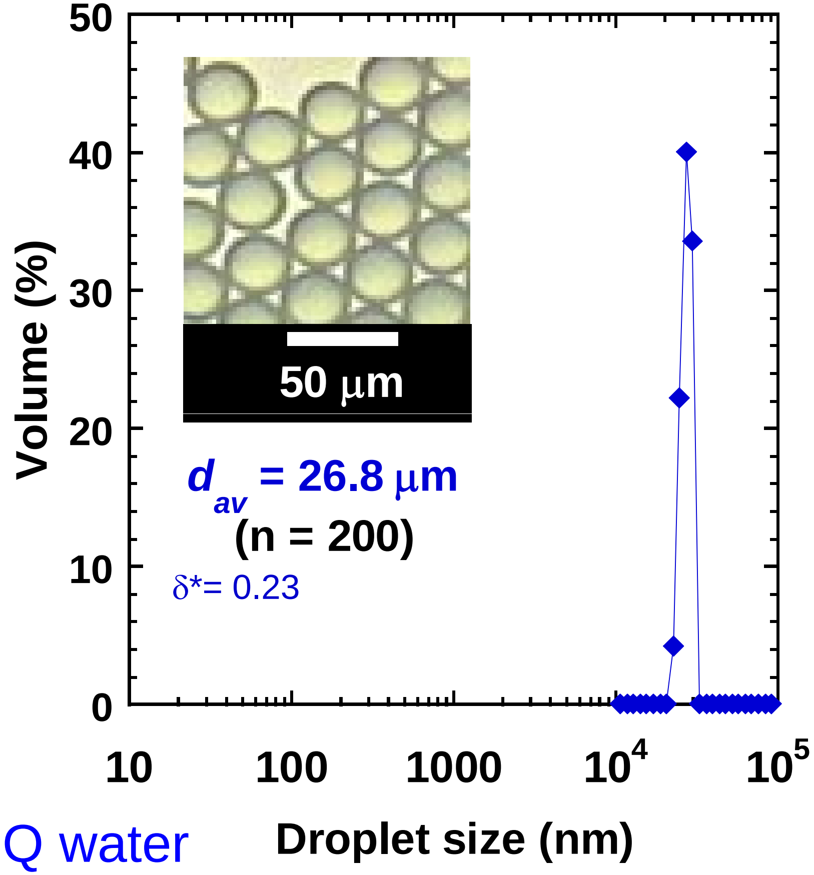
McClements & Decker, J. Food Sci., 65, 1270 (2000)

Generation of uniform droplets using straight-through microchannels (WMS1-4)

Droplet generation



Size distribution



- Dispersed phase: Menhaden fish oil
- Continuous phase: 1.0wt% MO750 in Milli-Q water

Highly monodisperse fish oil droplets containing PUFA with an average droplet diameter of around 30 μm were obtained stably using the microchannel device.

Conclusions (3)

- Fish O/W emulsions were formulated successfully using various processes, and their chemical stability was evaluated.
- The oxidative stability of O/W emulsions containing PUFA was found to be prone to various factors, in the following order:

Emulsification Process

Plays a major role on lipid oxidation in food emulsions. For instance, the spontaneous droplet formation in case of MC emulsification resulted in emulsions with the highest oxidative stability, compared to conventional homogenizers which generally employ high energy input (10^5 to 10^9 J/m^3).

Storage Temperature

Lipid autoxidation in food emulsions is strongly temperature-dependent. In general, all emulsions stored at 30 ºC had higher oxidation activity compared to 5 ºC.

Droplet Size

Within the micrometer size range, decreasing droplet size promoted lipid oxidation. Further reduction to sub-micron size did not have significant effect on oxidative activity. Most likely, this was caused by surfactant molecules packing around the oil-in-water interface so that suppressing oxygen diffusion.

Melting point and solubility for nanoparticles

Gibbs-Thomson Equation

$T(r) / T_\infty = \exp(-(2\gamma V_D)/(r\Delta H_{fus}))$

Kelvin Equation

$S(r) / S_\infty = \exp(2\gamma V_D/rRT)$

particle (radius, r)

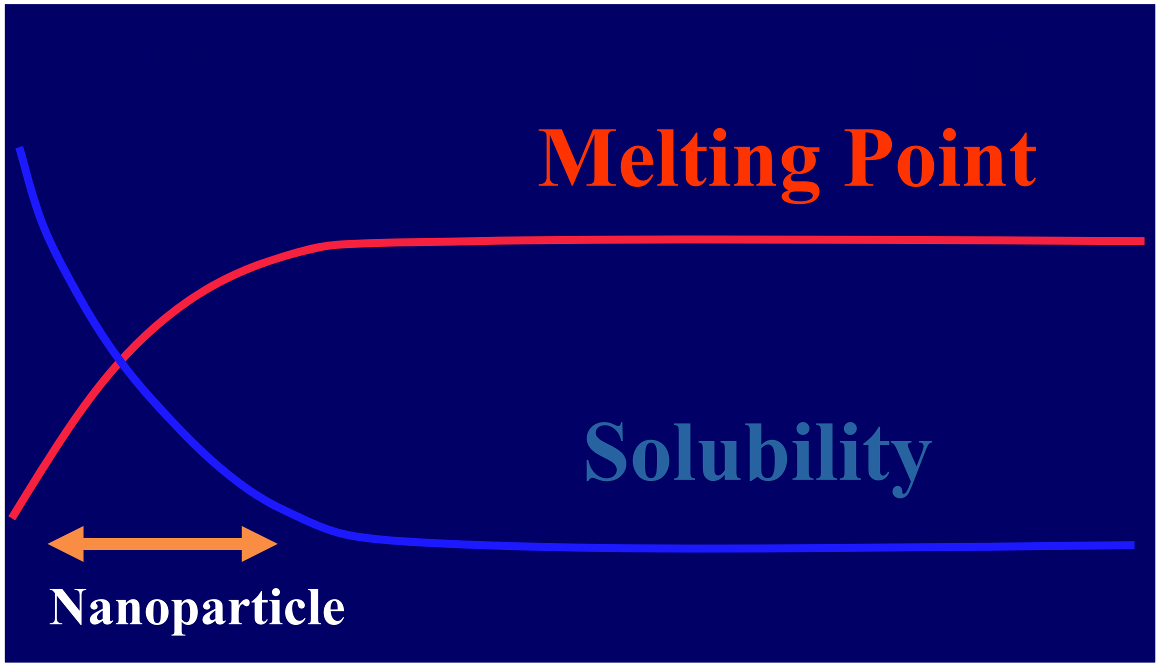
$T(r)$: Solubility of particle

T_∞ : Bulk melting point

V_D : molar volume

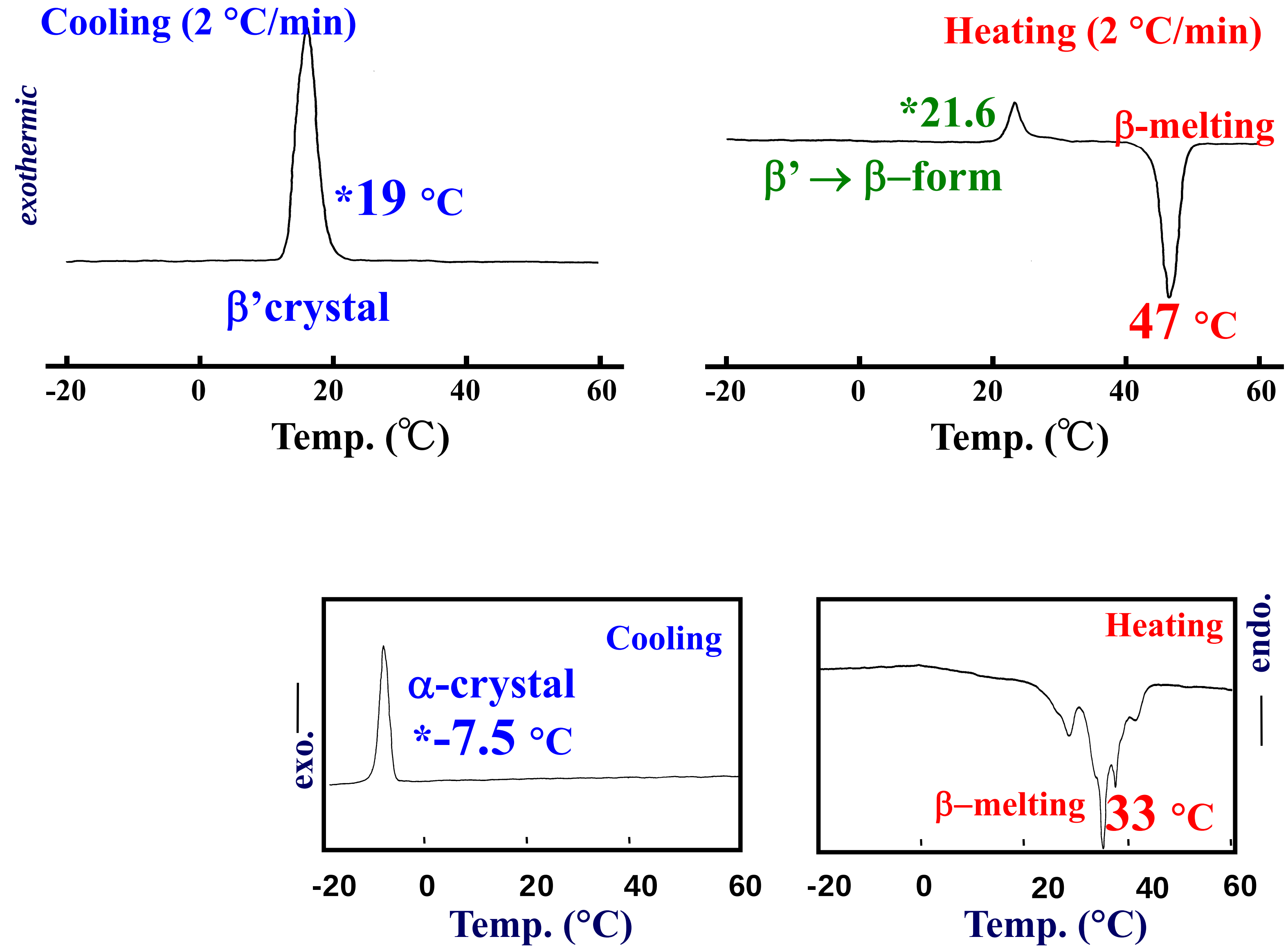
$S(r)$: Solubility of particle

S_∞: Bulk solubility



Particle size

Crystalization and melting phenomena of trilauryn



Sato

Nanotechnology and microengineering for food industry

- Utilization of micro/nano-fabrication technology
- Micro/nano-scale designed processes
- Micro/nanotechnology: Integrated, multi-disciplinary

  Acquisition of nanoscience knowledge,

  Development of microengineering processes,

  Establishment of useful technology, and

  Formulation of premium products

Food Processes and Nanotechnology

- Emulsification, Dispersion, Mixing：　Microfabrication technology and Microchannel emulsification, Membrane emulsification, Micro-mixer, Food rheology control
- Pulverization, Formation：Stainless steal mortar for flour milling, Extruder, Powders/Particles
- Separation, Classification, Extraction：Chromatography particle, Nanofiltration, Removal of impurities, Size classification, Microchannel extraction
- Micro-nozzle: Micro-capsule, Spray-dry
- Sterilization, Heating：Rapid temp increase, Micro-heat exchanger, Micro/nano-bubble, Micro-mist
- Application of CFD（Computational fluid dynamics） to Food Process

Food quality and safety control by nanotechnology

- Food freshness control: Food packaging, Micro-mist
- Food packaging・container： Development of nano-structured film, Antimicrobial film, gas transfer-controlled film, long-term preserved film, high-durable film, high-resistant film, light-weight container, temp measurement during food preservation, Traceability, RFID (Radio Frequency identification) certification system, Antimicrobial surface treatment of refrigerator and food containers
- Taste sensor, Smell sensor： Artificial tongue, Visualization
- Food safety：Rapid detection of microbial contamination, Sensor of Food poisoning bacteria, Antigen detection sensor, Alien substances detection, Poisonous substance detection, Agricultural chemicals detection

Formulation and Evaluation Food functionality by nanotechnology

- Food for Specified Health Uses (FOSHU), Health foods, Food supplements： Emulsions and microcapsules with functional components, Increase of health foods/supplements market：Establish of evaluation of food functionality and safety
- Stomach, intestine models (Digestion and absorption)：Analysis of digestion and absorption for carbohydrates, protein and lipids by intestinal epithelial cell device (absorption, immunity, stress etc), Comparison between animal and human tests
- Absorption control by food structure design, Feedback from digestion/absorption analysis to food processings
- Lung, skin and blood capillary model：safety of nanoparticles

Nanotechnology for Food Industry

1. Just beginning state
   More research from seed to need oriented one
2. More analytical study for food micro/nano-structure and functionality; Characterization of size reduction of food; decrease of melting point, increase of solubility, etc.;
3. More systematic study for micro/nanoscale engineering; Design of micro/nano processings; Nanoscale sensing for food safety;
4. Collaboration of researchers majoring food and nanotechnology; Collaboration of industry, academia and government, including International collaboration
5. Nanotechnology-oriented food processing system; Formulation of functional food and materials

Thank you for your attention!

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