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CelloFuel
Portable Biomass Refinery

History of Making Omega-3 Lipids with Torula Yeast

- Waldhof is near Mannheim, which was in the American sector after the war, and American engineers interviewed Waldhof engineers and made copies of all the drawings for this fermenter.
- Waldhof fermenters were built at sulfite mills in Great Lakes area (Rhinelander, Wisconsin), most wood was hardwood
- Wasn't very profitable, but cleaned up water from sulfite mills
- Eventually most sulfite mills closed, Kraft pulp mills became dominant, only one Waldhof fermenter remained (Rhinelander).
- In 1947, the long-time director of Waldhof, E. Schmidt, published a complete technical description of the experiences at Waldhof, doubtful that anyone in USA ever read it.
- Schmidt, E. "Eiweiß-und Fettgewinnung über Hefe aus Sulfitablauge." *Angewandte Chemie* 59.1 (1947): 16-20.
- "the sum of fat and protein in Torula, around 50%, i.e. if you have 10% fat, there are also 40% protein, but you have 25% fat, so only 25% protein, etc."

Making Torula Yeast with Omega-3 Lipids

- Growing Candida utilis in high concentrations of glucose accumulates fatty acids
- About 5% glucose accumulates about 7% fatty acids
- Waldhof fermenter can produce 150 g/L yeast dry mass
- This is probably how Schmidt produced 25% lipids in Candida utilis
- Babij, T., F. J. Moss, and B. J. Ralph. "Effects of oxygen and glucose levels on lipid composition of yeast Candida utilis grown in continuous culture." *Biotechnology and bioengineering* 11.4 (1969): 593-603.

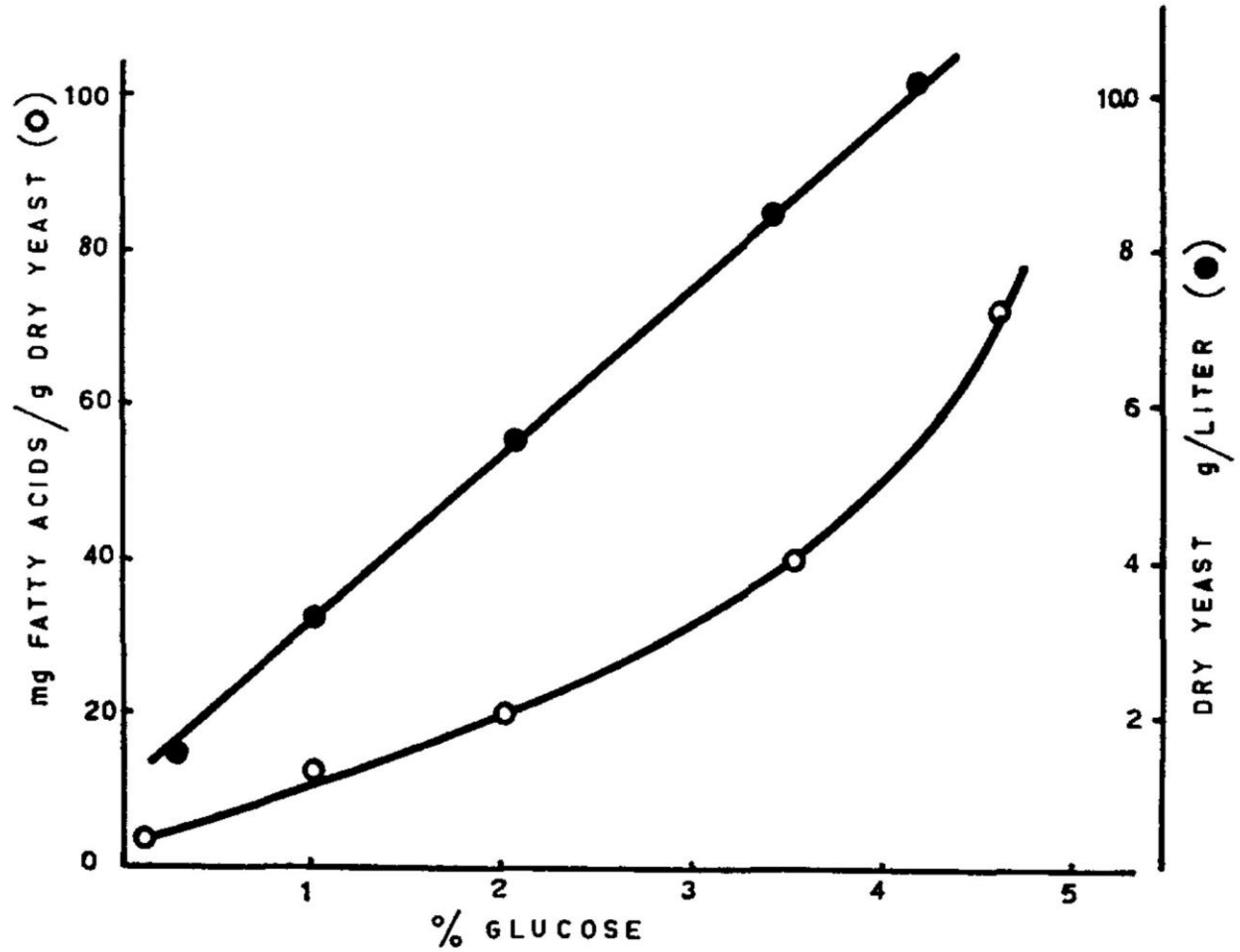


Fig. 3. Changes in lipid content of yeast cells with increasing glucose concentration.

Making Torula Yeast with Omega-3 Lipids

- Higher oxygen concentration leads to higher linonenic acid (C18:3, Omega-3) concentration
- Can produce up to 240 uM oxygen concentration in foam fermenter
- This shows that a foam fermenter with Candida utilis can produce lipids with 35% Omega-6 and 30% Omega-3
- Babij results confirmed in Johnson, B., Sheila J. Nelson, and C. M. Brown. "Influence of glucose concentration on the physiology and lipid composition of some yeasts." *Antonie van Leeuwenhoek* 38 (1972): 129-136.

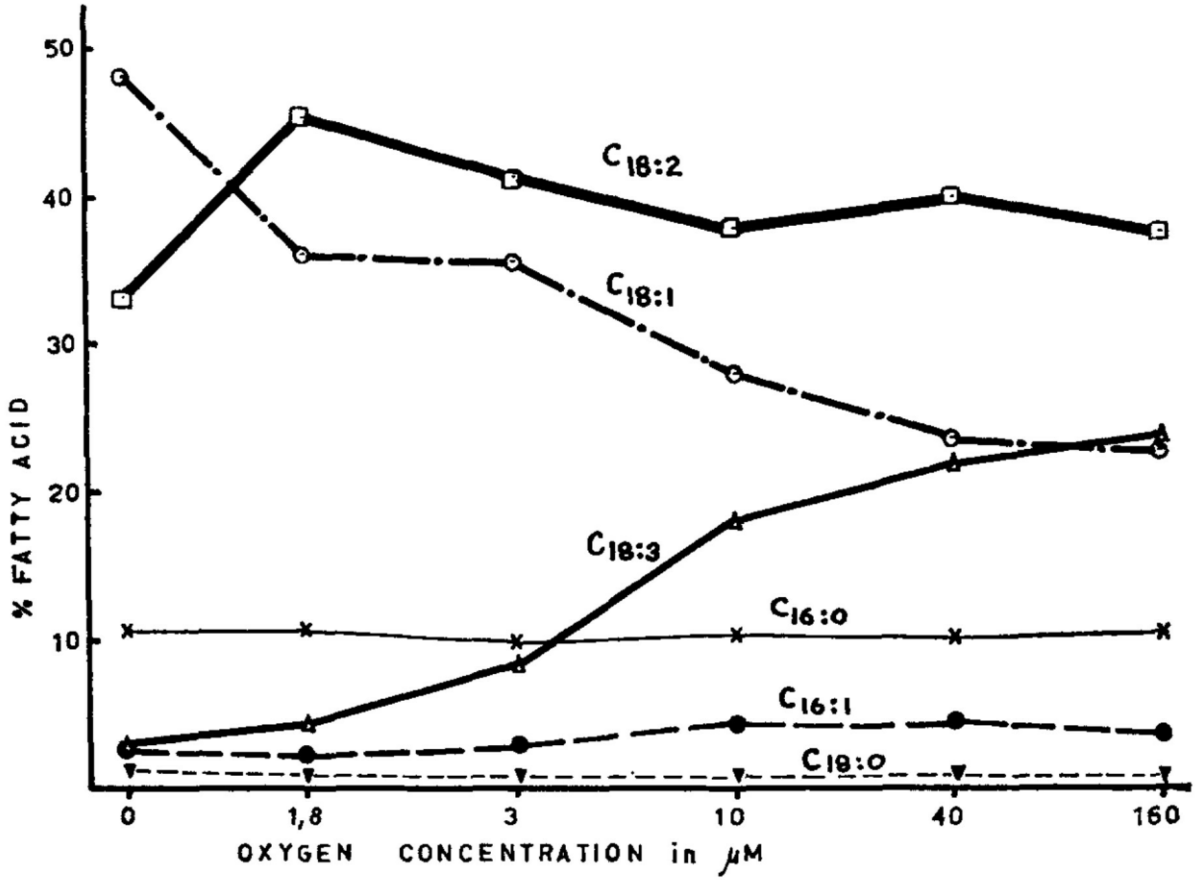
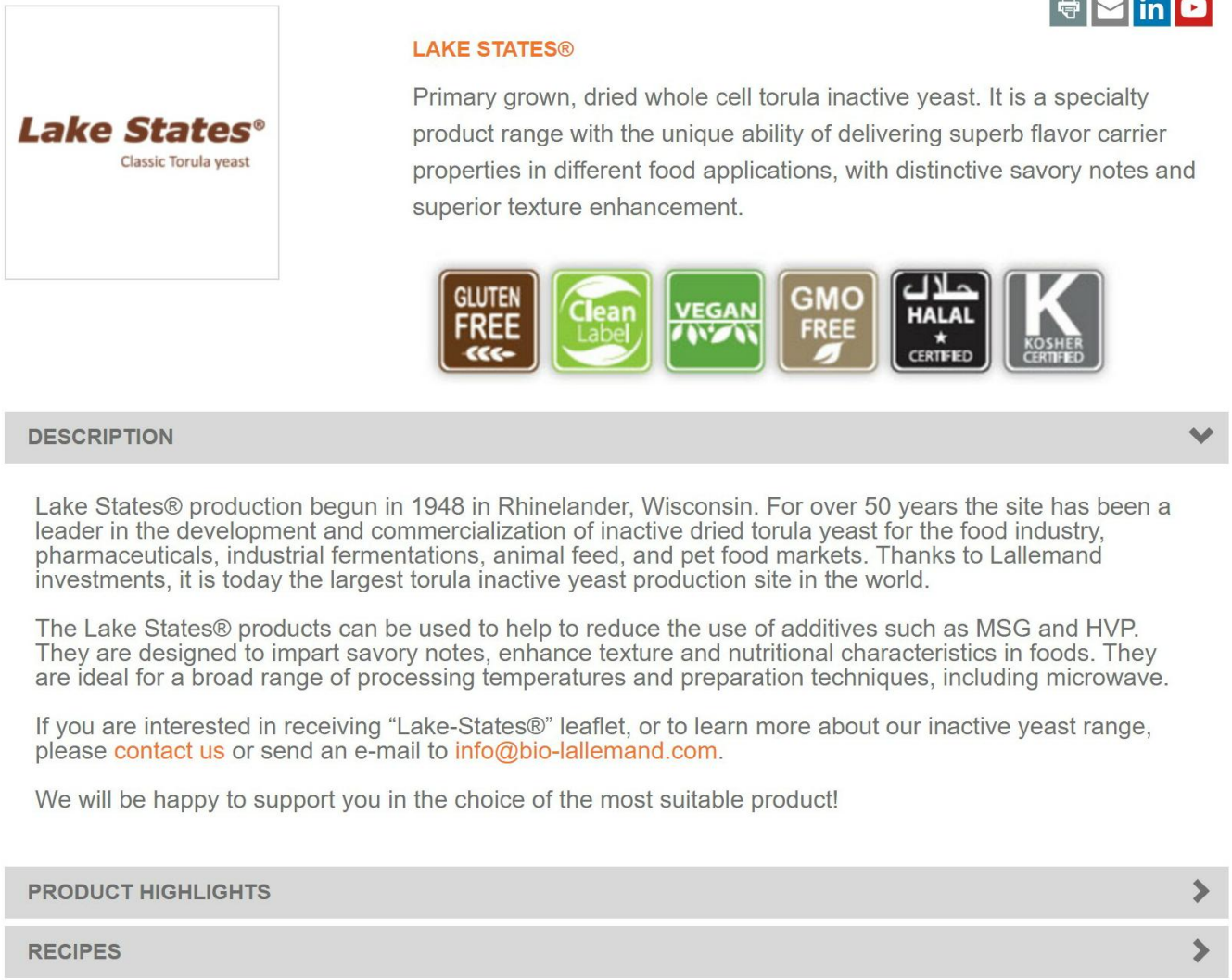


Fig. 2. Effects of oxygen tensions on fatty acid composition expressed as % of total fatty acids at high glucose concentration.

Key: $C_{16:0}$ Palmitic; $C_{16:1}$ Palmitoleic; $C_{18:0}$ Stearic; $C_{18:1}$ Oleic; $C_{18:2}$ Linoleic; $C_{18:3}$ Linolenic.

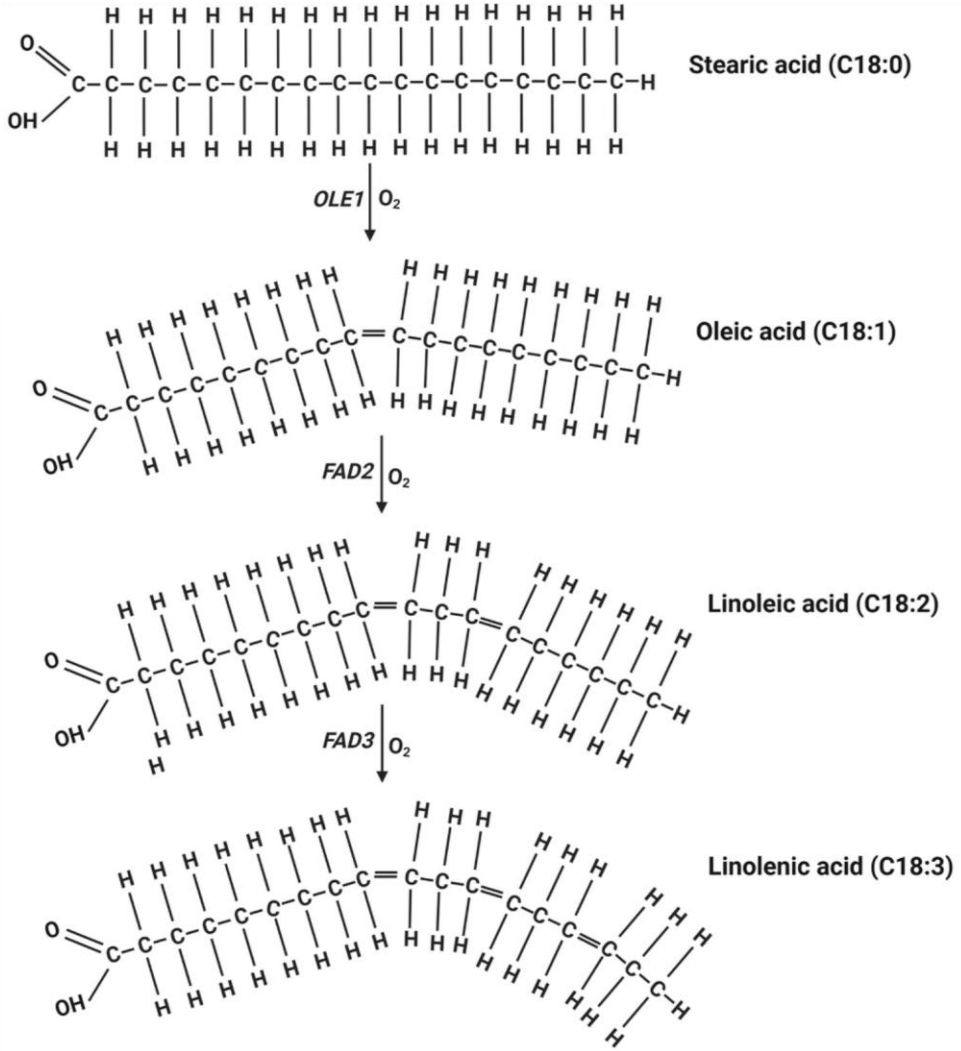
Current market for Torula yeast (Lallemand)

- Lallemand is now the world leader in producing Candida utilis (Torula) for the food industry, in Rhinelander, Wisconsin
- Purchased Waldhof fermenter and assets of Provesta (Phillips Petroleum fermenter)
- Sulfite pulp mill is closed, Lallemand uses glucose and molasses, standard batch aseptic aerobic fermenter, doesn't use Waldhof fermenter any more.
- Lallemand Torula has no Omega-3 lipids
- There's something magic about a Waldhof fermenter that produces Torula yeast with Omega-3 lipids



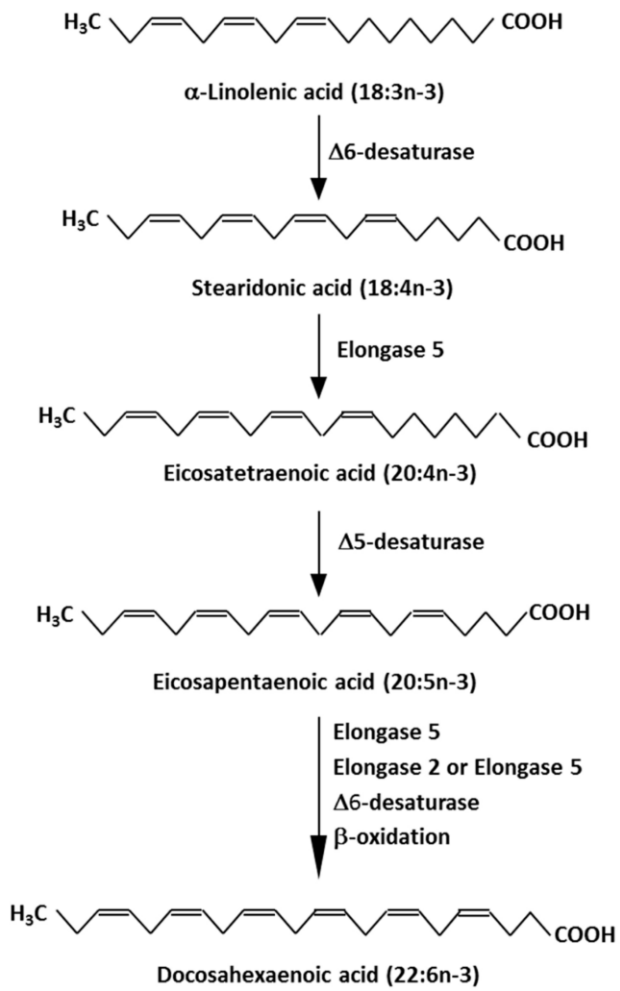
Importance of Omega-3 Lipids

- Omega-3 and Omega-6 fatty acids are essential to human life
- The human body does not have FAD2 and FAD3 enzymes so we need linoleic acid (Omega-6) and linolenic acid (Omega-3) in our diet
- Ratio of Omega-6 to Omega-3 must be between 2:1 and 1:2 for optimal health
- More oxygen = more Omega-3
- The Waldhof fermenter has higher dissolved oxygen at high cell concentrations than any other type of fermenter



Importance of Omega-3 Lipids

- Omega-3 is precursor to EPA and DHA, the major fatty acid in the brain is DHA
- The human body can produce EPA and DHA, but the more Omega-6, the less EPA and DHA (competition for the same enzymes)
- Chickens and salmon (and most animals) can produce EPA and DHA from Omega-3
- Epidemiological studies suggest that consumption of DHA is associated with a reduced incidence of Alzheimer disease. Animal studies demonstrate that oral intake of DHA reduces Alzheimer-like brain pathology.



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Why are Americans Fat?

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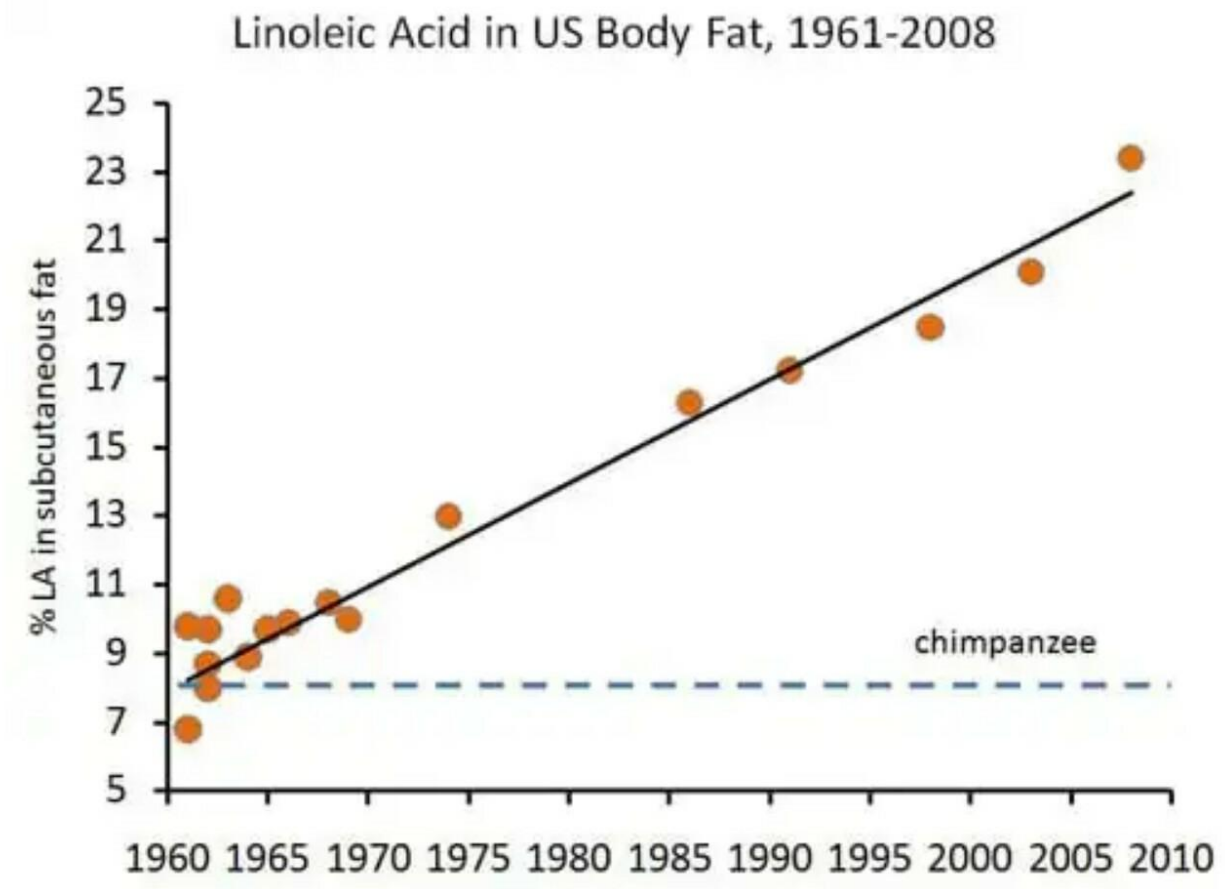
- America's most widely consumed oil causes genetic changes in the brain. New UC Riverside research shows soybean oil not only leads to obesity and diabetes, but could also affect neurological conditions like autism, Alzheimer's disease, anxiety, and depression.

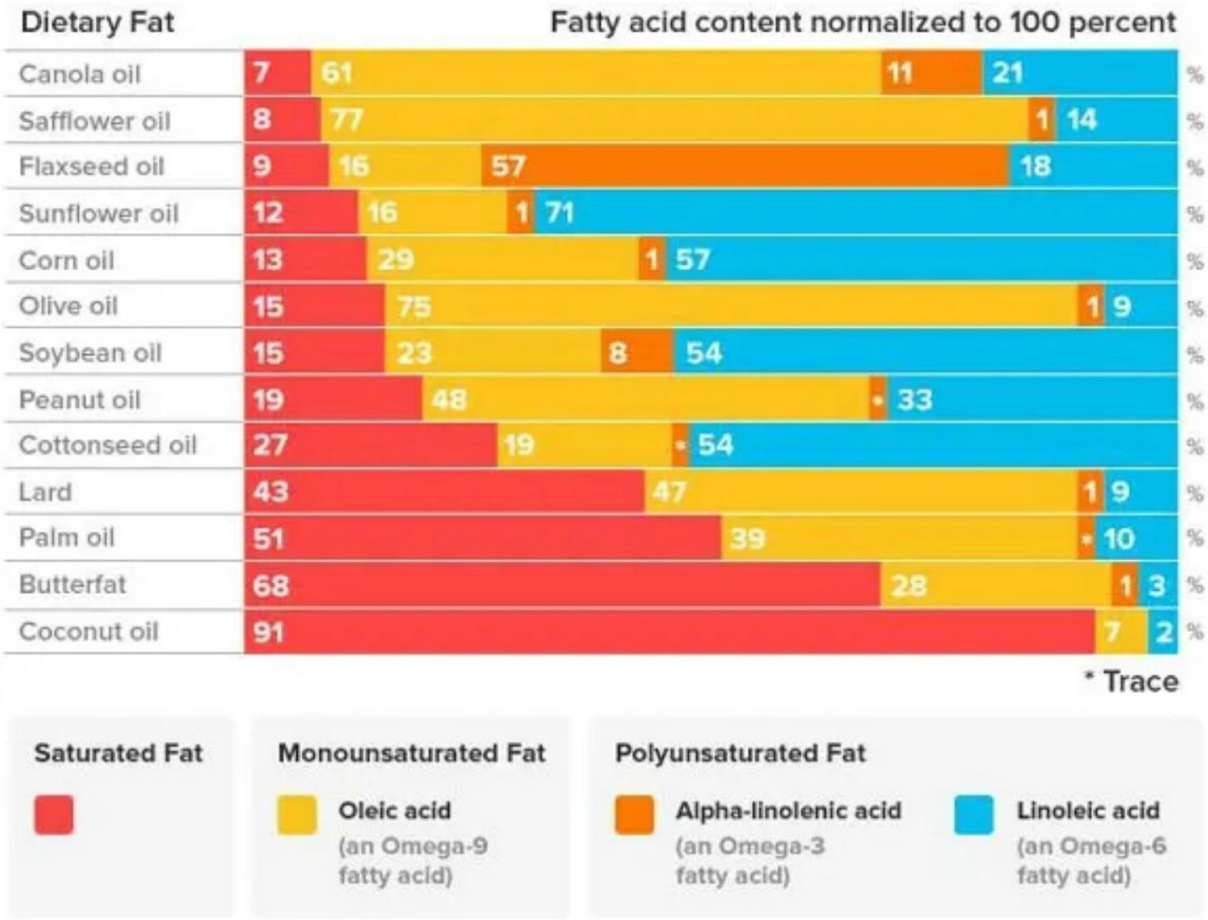
  University of California, Riverside, Jan 17, 2020

- Soybean oil has a 7:1 ratio of Omega-6 linoleic acid to Omega-3 linolenic acid.
- A ratio of more than 2:1 Omega-6 to Omega-3 leads to reduced levels of DHA in the body. DHA is the main fatty acid in the brain.
- Animals are usually fed grain-based feeds containing soy and corn. Fats in the meat are mostly Omega-6 while grass fed animals are 1:1.



Healthy and Unhealthy Fats

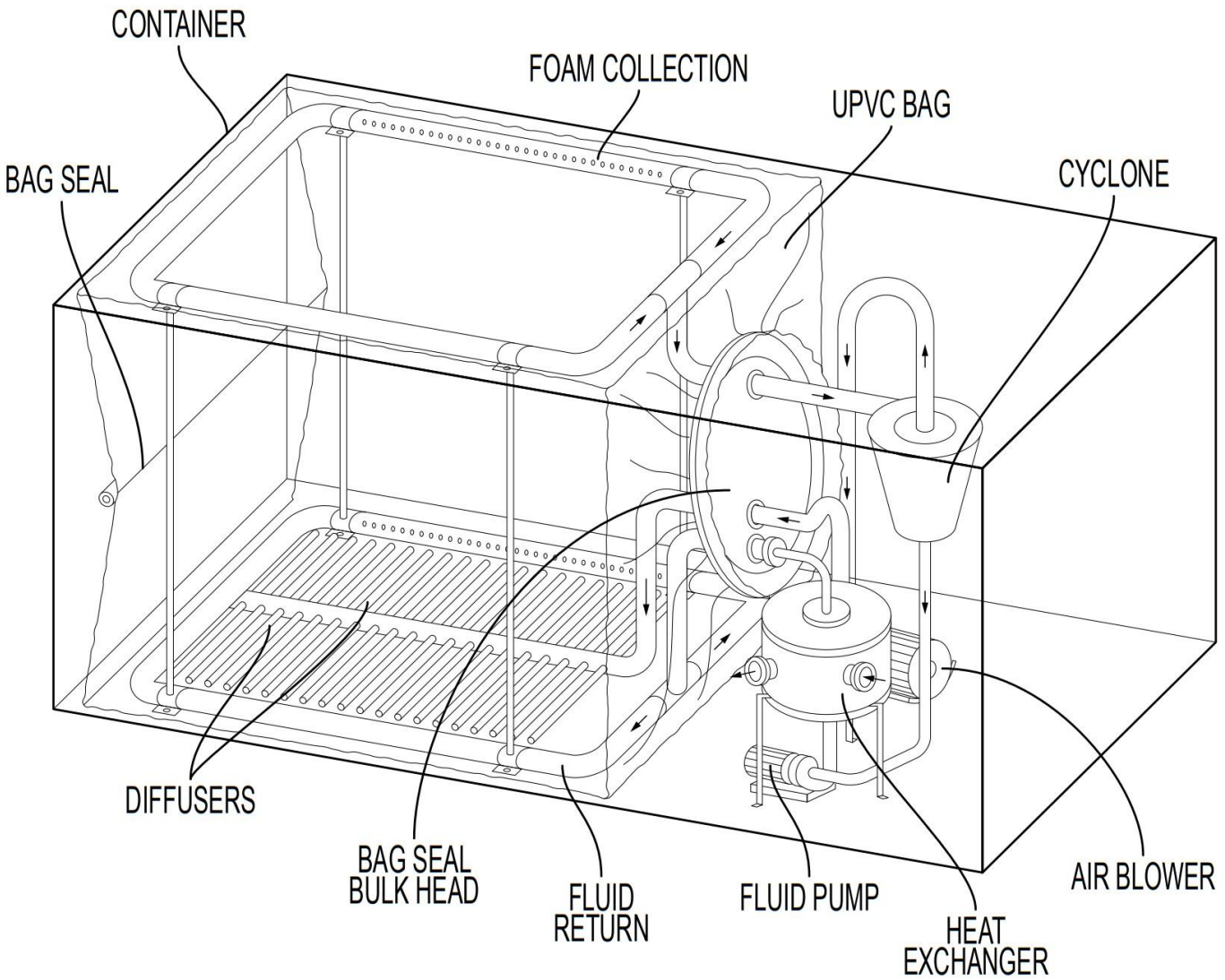
- Unhealthy fats have a ratio of linoleic acid to linolenic acid greater than 2:1. This leads to low production of DHA, which is needed for the brain.
- Soybean (7:1), corn (57:1), sunflower (71:1) and cottonseed oil (54:1) are very unhealthy
- Canola (rapeseed) oil (2:1) is reasonably healthy, most margerine in the world today uses this
- Flaxseed (linseed) oil (1:3) is the healthiest, but oxidizes easily so not useful for cooking oil and margarine
- Can feed flaxseed to chickens without grinding, commonly used to make Omega-3 eggs



What's New in CelloFuel Fermenters?

- Waldhof fermenter has a 1.5 m diameter spinning disc to make a 10% emulsion, Cellofuel fermenter uses 75mm bubbler tubes to make foam with 10% liquid, using much less energy with no moving parts.
- Waldhof fermenter removes heat by cooling emulsion with heat exchanger, but emulsion is an insulator and can't easily be cooled. Cellofuel fermenter cools fermentation liquid after breaking foam with cyclone.
- Waldhof fermenter uses spinning disk to break foam and reinject air. CelloFuel fermenter uses cyclone to break foam and reinject air, which uses much less energy with no moving parts.
- Waldhof fermenter uses a stainless steel fermenter, Cellofuel fermenter uses a UPVC fermenter in a shipping container, reducing cost.
- Waldhof fermenter uses stainless steel heat exchanger, Cellofuel fermenter uses either titanium or 316L heat exchanger to reduce nickel leaching, which works with contamination control (discussed later).

20 ft. CelloFuel Container



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40 ft. CelloFuel Container