A Novel Self-Sustainable Kit to Combat Rural Vitamin A Deficiency During Pregnancy and Early Childhood

Vitamin A

- A fat-soluble vitamin, vital for maintaining healthy vision, supporting immune function, and promoting cell growth.
- It plays an essential role during pregnancy, and is also crucial for the healthy growth, an development, of neonates.

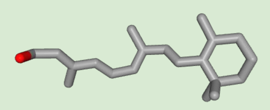
Fig.1: Structure of Retinol

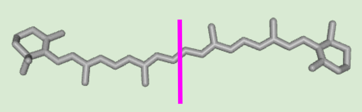
Fig.2: Structure of β-carotene

Vitamin A Source:
- **Retinoids**: Animal Sources (Liver, eggs, and dairy)
- **Carotenoids**: Carrots, sweet potatoes, and leafy greens

β-Carotene → Retinol (Active Vitamin A)

β-carotene $\xrightarrow[\text{15,15'-Monooxygenase}]{\text{Intestine}}$ 2-Retinal $\xrightarrow[\text{Retinal reductase}]{\text{Liver \& Intestine}}$ 2-Retinol

Vitamin A Deficiency

Risks:

Vision problems: Dry eyes, night blindness, and in severe cases, permanent blindness (esp. in infants, children and pregnant women).

Complications during pregnancy: Pregnancy-induced hypertension, preterm delivery, and intrauterine growth restriction, etc.


Fig. 3: Child with night-blindness.[3]


Fig. 4: Pregnant lady being examined. [4]

Fetal abnormalities: Bone and Epithelial cell growth-related.

Delayed Growth and Development in pre/neonatal stages and and low birth weight.

Weakens the immune system: especially in children, and pregnant women: Increasing the risk of infections particularly respiratory and diarrheal infections, as well as measles.

Prevalence:

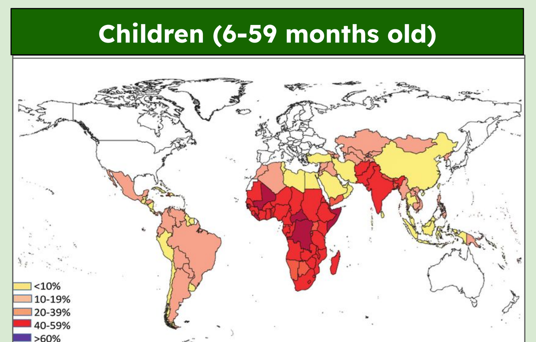

Fig. 5: Global prevalence of VAD in children. [5]

- ~19 million pregnant women suffering from VAD.
- Highest rates in Africa (14-20%) and South-East Asia (10-15%).

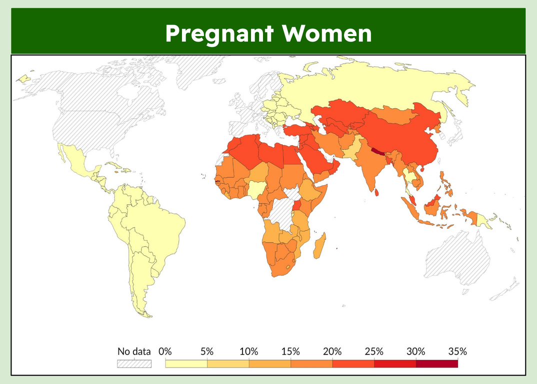

Fig. 6: Prevalence of VAD in Pregnant Women. [6]

- VAD effects one-third of children worldwide.
- Highest rates in sub-Saharan Africa (48%) and South Asia (44%).
- Vitamin-A supplementation potentially reduce all-cause mortality in children by ~12-24%.

Objectives

Long-Term Goal: To create a self-sustainable dietary kit that supplements Vitamin A

HYPOTHESIS: Selective microbial systems can be used to produce sufficient β-carotene in staple starch diets to alleviate vitamin-A deficiency

Phase 1: To establish the feasibility of growing edible microbial cultures that produce β-carotene, the precursor of Vitamin A.

The specific objectives for phase one are:

1. Identifying edible microbes that produce β-carotene.
2. Selecting appropriate starches for the fermentation process.
3. Developing methods for extracting and detecting β-carotene from biomass.
4. Evaluating the feasibility of producing β-carotene through fermentation

Methods

1) **Seed Culturing of Blakeslea trispora: a)** Potato Dextrose Agar (2%) petri dishes at room temperature for **2–5 days**. b) 1:1 ratio of water to oatmeal, at **215 RPM** at **25°C** for **3–6 days.**
2) **Tapioca Culture:** 5 % Tapioca for submerged culture and 20 % for solid state culture. Variations included the presence or absence of other supplements (**0.5% of yeast extract, 0.13% of potassium phosphate, and 0.04% of magnesium sulfate.** The cultures were incubated for **5, 7, or 9 days**.
3) **Sterilization:** All media were autoclaved at **121°C for 45 minutes**. The sterilized samples were handled under a laminar flow hood to maintain sterility.
4) **Drying of Biomass**: Post-fermentation, the the whole biomass was dried using one of the following methods: a) **Oven Drying:** Heated at **70°C for ~24 hrs, or b) Freeze Drying:** Samples were frozen at a slant and placed in a freeze dryer at **-80°C for ~48 hours**.
5) **β-Carotene Extraction:** The dried samples were crushed using a **hand grinder**. **0.2 grams** of each sample was mixed with a teaspoon of sand and had grinded for ~30 sec. B-carotene was extracted from the grinded sample with **5 mL of ethyl acetate** in a shaker for 30 min. Finally, the extracted solution was injected into **HPLC** for analysis.
6) **β-Carotene** was quantified using **Shimadzu LC-40D HPLC** with a **C18 column** and **Diode Array Detector (DAD)**. The mobile phase consisted of **Acetonitrile:Methanol (90:10)** at a **flow rate of 1.5 mL/min**. The concentration in the sample was determined by comparing chromatogram peak areas to a standard curve prepared with commercially purchased **β-carotene**.
7) **Data Analysis and Statistics**: Data analysis and the preparation of graphs was performed using Microsoft Excel. All experiments are performed at least in triplicates; repeated in 3 different days. Differences between groups were analyzed using a paired two-tailed Student's t-test, with statistical significance defined at p < 0.05

Selecting Microbes and Starches

Edible Microbes that Produce β-Carotene

1. *Mucor circinelloides (Fungi)*
2. *Rhodosporidium toruloides (Yeast)*
3. ***Blakeslea trispora(Fungi)***
4. *Sporidiobolus salmonicolor(Fungi)*
5. *Phaffia rhodozyma(Yeast)*

Blakeslea trispora produces high yields of β-carotene, thrives on a wide range of substrates, and environmental conditions.

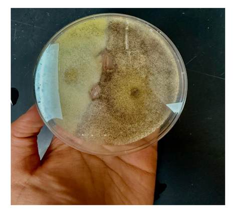
Fig. 7: Blakeslea trispora agar plate, 2024. [7]

Starches in the Affected Areas

1. Rice
2. **Tapioca (Cassava Starch)**
3. Taro
4. Yams
5. Finger Millet
6. Maize

Tapioca was selected for:
- its high starch content
- widespread availability in affected areas.
- fewer microbial growth inhibitors and complex fibers—efficient microbial metabolism.

Feasibility of Growth in Tapioca (Tp)


Fig. 8: Tapioca solid-state fermentation method, 2024.[7]

vs.


Fig. 9: Tapioca submerged-state fermentation method, 2024.[7]


Fig.9 [7]

+ KH_2PO_4 and $MgSO_4$ + Yeast extract

Solid state	Submerged-state	Submerged-state
20 % w/v Tp	5 % w/v Tp	5 % w/v Tp

The fungi grew in Tapioca but didn't produce any detectable β-carotene

B-carotene: 84.22 µg/g of dry weight.

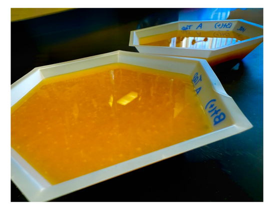
Fig. 10: Tapioca submerged-state fermentation samples, 2025.[10]

Tapioca serves as a viable energy source for *Blakeslea trispora* but requires salts and yeast extract to enable any β-carotene production.

Seeding Method & Harvesting Time

Seeding Method: The fungi was grown on an agarose petri dish or in oatmeal broth before inoculating it in Tapioca broth

Agar Plate

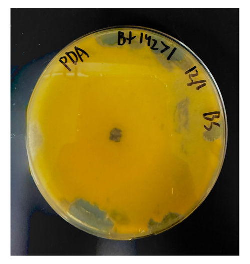
Fig. 11: Blakeslea trispora agar plate 2024. [7]

44.9 ± 8.2 µg/g On Day 9

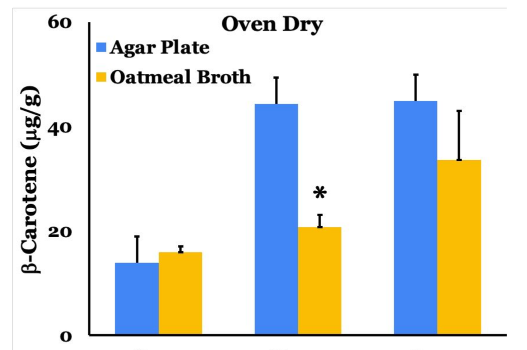

Fig. 12: Oven dry(Agar plate vs oatmeal broth) graph.[8]

*p<0.05; Student t-test, two tailed & unpaired, Agar plate vs. Oatmeal broth from the same day sample

Oatmeal Broth

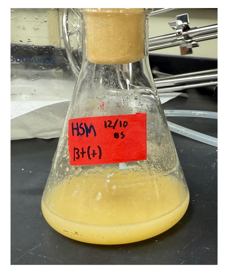
Fig. 13: Blakeslea trispora Oatmeal broth,2024. [7]

33.6 ± 9.2 µg/g On Day 9

Fungi seeds collected from Agar seed plate produced more β-carotene

Effect of Drying Method on Stability

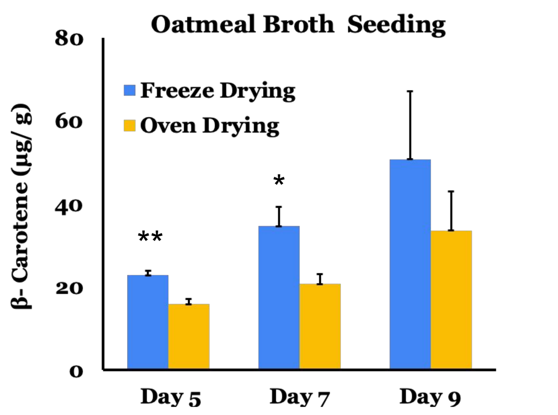

Fig. 14: Oatmeal broth(Freeze dry vs Oven dry) graph.[8]

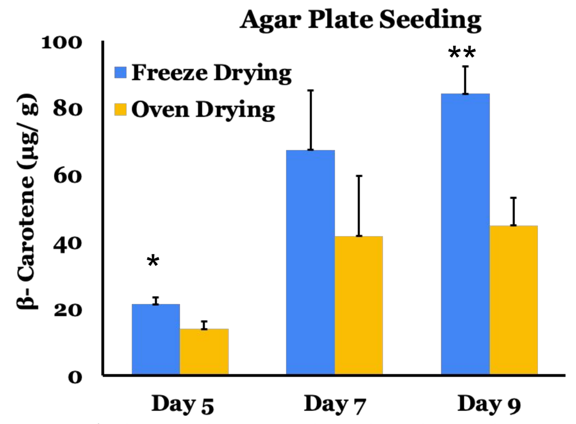

Fig. 15: Agar Plate(Freeze dry vs Oven dry) graph.[8]

*p<0.05; **p<0.005: Student t-test, two tailed & unpaired, comparison between freeze drying vs. oven drying from the same day sample

Freeze drying enhanced yield of β-carotene upto ~68 % versus oven drying

Biomass Production

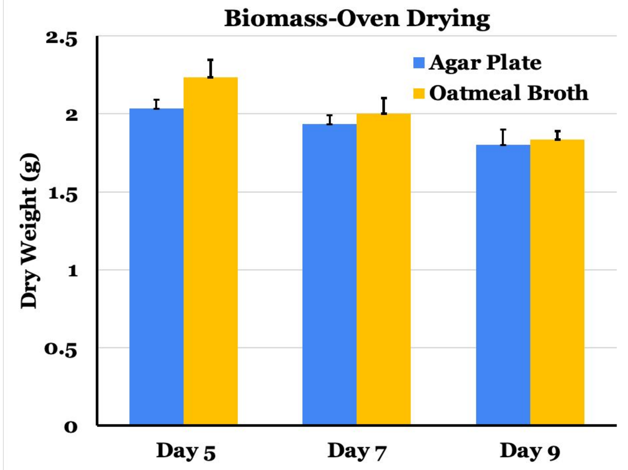

Fig. 16: Oven dried biomass(Agar plate vs Oatmeal Broth) graph.[8]

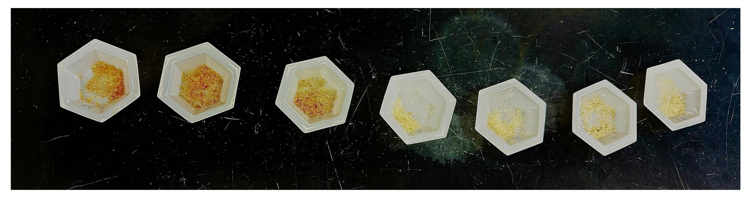
Fig. 17: Blakeslea trispora Oatmeal broth inoculation method, 2025. [7]

Biomass = Total Dry Weight of the Whole Culture

**p<0.005: Student t-test, two tailed & unpaired, comparison between Agar plate vs. Oatmeal broth seeding from the same day sample

There no significant difference in the total biomass produced

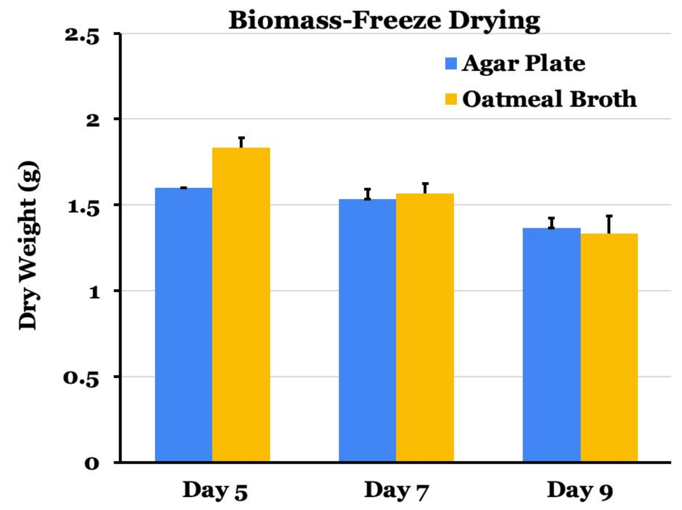

Fig. 18: Freeze dried biomass(Agar plate vs Oatmeal Broth) graph.[8]

Discussion

- Recommended daily dose of Vit-A for Pregnant women:
  - 770 RAE (Retinol Activity Equivalents). **[9]**
  - 1 RAE = 10-12 µg of dietary β-carotene.
  - 9240 µg of β-carotene needed as a diet.

Goal: 2-3 mg/g dry weight

- With **84.22 µg/g of β-carotene** achieved with our fermentation, we need to consume of dry weight of around **109.7g powder per day.**
- Other byproducts & their benefits: a) **Lycopene** (Antioxidant and Anti inflammatory) and b) **Protein source.**

Conclusions

1. Tapioca serves as a viable energy source for *Blakeslea trispora.*
2. Spores collected from Agarose plate produced more β-carotene than spores from Oatmeal broth culture.
3. Freeze drying enhanced yield of β-carotene upto ~68 % versus oven drying.
4. Addition of minerals is necessary for the production of β-carotene.
5. The source of Tapioca, Cassava could be a viable source for producing Beta-carotene using *Blakeslea trispora.*

Fermenting Blakeslea trispora in Tapioca or cassava provides a viable option for creating a at-home sustainable dietary kit for promoting the supplementation of Vitamin A

Future Studies

1. Fermentation Additives
   a. Use of edible oils in the culture *[10]*
   b. Hydrogen Peroxide (through Soybean oil) *[11]*
2. Cassava as a energy source
3. Finger Millets as energy source
4. Optimization of Carbon to Nitrogen source on β-carotene yield
5. Subculturing- Sustainability
6. Heat Stability of the fermented product
7. Nutrient Analysis

References

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7) Fig. 7-11, Fig.13 and 17: Images were taken by student, 2024-2025.
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