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# Evaluation of a Nitrogen Flush System to Prevent Oxidation of Fish Oil Encapsulated in Licaps® Capsules Using CFS1200 Equipment

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# Evaluation of a Nitrogen Flush System to Prevent Oxidation of Fish Oil Encapsulated in Licaps® Capsules Using CFS1200 Equipment

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**Key words**: encapsulation, oxidation prevention, nitrogen, capsules, Fish oil, stability

## PURPOSE

The work presented hereafter demonstrates the efficiency of the Nitrogen Flush system installed on CFS1200 machine (Photo 1) to reduce oxidation of oil encapsulated in Licaps® capsules.


**Photo 1**: CFS1200 machine

Unsatured oils are prompt to oxidation in contact with oxygen from the air (Fig. 1). During encapsulation in hard capsules, theses oils are in contact with the air at filling and after the closing steps (Photo 2, orange arrows at filling and after closing).

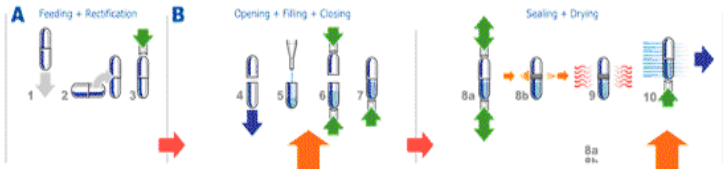


**Photo 2**: Process of encapsulation on CFS1200 machine

This air can be replaced by nitrogen or another inert gas before closing thanks to Nitrogen Flush accessories. The stability of the oil encapsulated in these conditions increases to a large extent. The purpose of this study is to evaluate how long the capsules will remain below the limit of oxidation when stored under I.C.H. conditions.

## METHODS

### Product description

The capsules considered are Licaps® capsules size #0, natural transparent (Capsugel). The fish oil encapsulated is EPAX6000 TG (Polaris). The nitrogen used is technical grade with less than 5ppm traces of oxygen (Gaz Liquide). The sealing fluid is a mixture of pure ethanol and water 50/50 w/w.

## Encapsulation conditions

- A design of experiments was performed using two factors at two levels: nitrogen flush (Yes/No) and product degassing in the CFS1200 container prior to encapsulation (Yes/No). The four runs were repeated the day after to confirm repeatability of results.
- Each Licaps® capsule was filled with 500mg fish oil, closed and sealed in the presence of 20µL sealing fluid on CFS1200. Nitrogen flow was 11L/min at closing, and 2L/min during 1 hour for degassing the oil in the CFS1200 container.
- Samples of oil were taken before and after encapsulation of each run; samples of filled capsules were stored in HDPE bottles in climatic cabinets under I.C.H. conditions (25°C/60%RH, 30°C/65%RH and 40°C/75%RH) for 6 months to study their stability over time in standard and accelerated conditions.

## Measurement of oil oxidation

The oxidation of oil is measured using two parameters:

- Peroxide index (IP): milliequivalents of peroxide per kg of fat are measured by titration with iodide ion.
- Anisidine value (AV): this method measures the level of non-volatile carbonyl components (aldehydes and ketones), which are formed during deterioration of oils. They react with p-anisidine determining an absorption that can be measured at 350 nm.

According to European Pharmacopeia, maximum limits for oxidation products are IP<10 and AV<30. IP points out the first phase of oxidation, whereas AV stands for the second phase of oxidation that ends up with total degradation of oil (brownish color and release of rancid odor) (Fig 1).

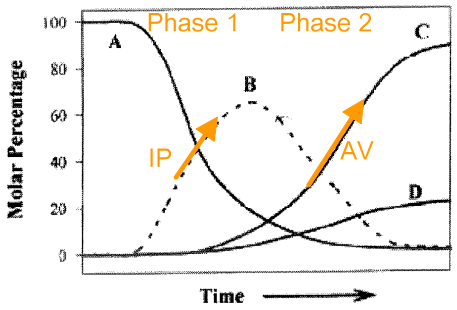


**Fig. 1**: Oxidation of unsatured oils: *(A) unsaturations, (B) peroxides, (C) products of degradation, (D) volatile compounds (odor)*

## RESULTS

The overall results are consistent between repeats (Fig 2: day factor).
Nitrogen flush is the main significant factor that affects both IP and AV: the oxidation values are lower with nitrogen flush.
The fact of degassing the oil in the container prior to encapsulation is not significant in our processing conditions (4 hours running per day).

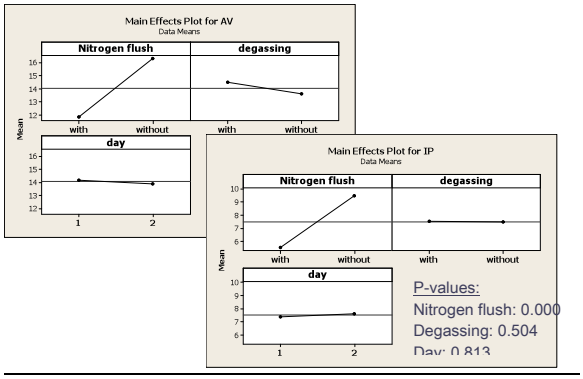


**Fig. 2**: Main effects of factors "nitrogen flush", "degassing" and "day"

The detailed results show that peroxide index (IP) increases significantly after encapsulation when nitrogen flush is not used (Fig. 3). The IP value is larger in standard conditions (25°C/60%RH) than in accelerated ones (40°C/75%RH) because peroxides destroy each other beyond a threshold of concentration, as described in literature.

Anisidine value (AV) consistently increases over the 6 months whatever the conditions. As expected, the oxidation is much more rapid under accelerated conditions: AV limit is reached after 6 months stability in oil encapsulated without nitrogen flush.

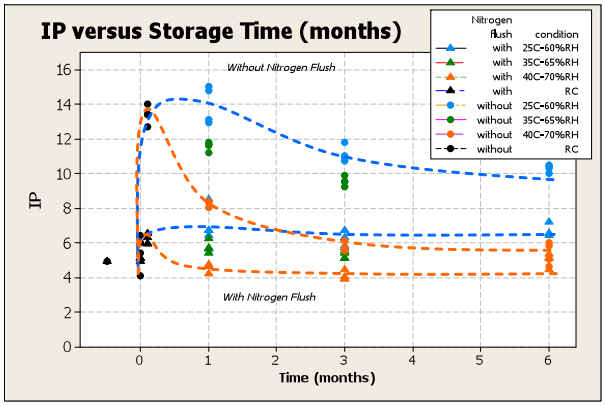


**Fig. 3:** Evolution of peroxide index oil encapsulated over time

The results are conforming to the ones expected (Table 1): fish oil oxidation is significantly slowed down thanks to nitrogen flush during closing.

Under accelerated conditions, Fish oil oxidation is prevented for 6 months with nitrogen flush: this corresponds to 2 years shelf-life extension under standard conditions.

Degassing of oil in CFS1200 container has minor effect on oxidation because encapsulation time was short; it would have been significant at larger scale.

| Runs | Nitrogen flush | Oil degassing | T=0 | 1 Month | 3 Months | 6 Months |
|---|---|---|---|---|---|---|
| 4 & 8 | No | No | IP>10 | IP>10 | IP>10 | AV>30 @40°C/75%RH |
| 2 & 6 | No | Yes | IP>10 | IP>10 | IP>10 | AV>30 @40°C/75%RH |
| 3 & 7 | Yes | No | OK | OK 3C | OK 3C | OK 3C |
| 1 & 5 | Yes | Yes | OK | OK 3C | OK 3C | OK 3C |

**Table 1:** Stability of oil encapsulated. "OK 3C":IP<10 and AV<30 at the 3 ICH conditions

## CONCLUSIONS

Fish oil encapsulated in Licaps® capsules under nitrogen is stable after 6 months storage under standard and accelerated conditions thanks to nitrogen flush. This means the product has 2 years shelf life instead of 1 year without nitrogen flush.

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