Effect of Oxygen Level and Temperature on Stability of Lipids

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BACKGROUND

- Lipids are common excipients used to deliver active pharmaceutical ingredients (APIs). Two factors that affect the quality of lipids are oxygen level and temperature.
- In the presence of oxygen, lipids undergo autoxidation to form oxidative degradation products (Fig. 1).
- Degradation products lower the quality of oils and cause rancidity.

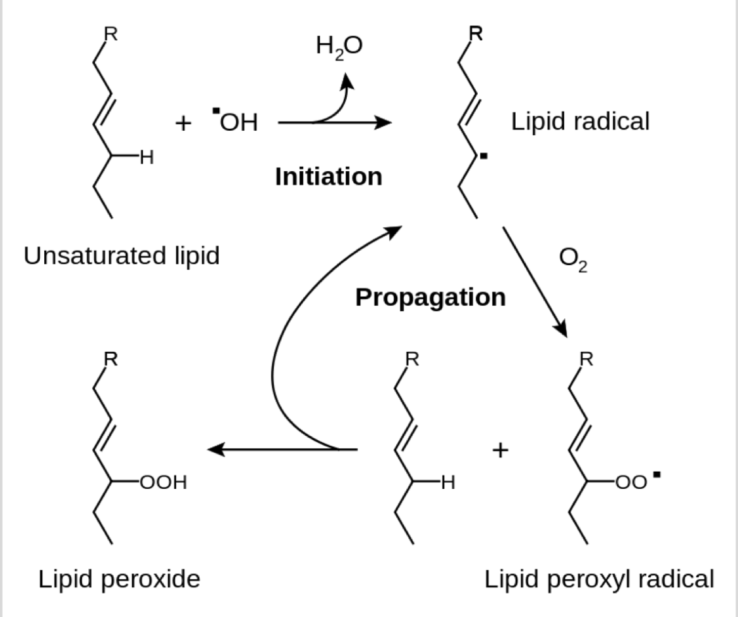


Fig. 1

- In this experiment, a predictive model of lipid stability was developed based on the growth of primary and secondary oxidation products in a model oil under a variety of accelerated stress conditions.
- FreeThink Technologies has previously studied oil oxidation, but samples were tested only for primary oxidation products (peroxide value).

OBJECTIVES

- Conduct an Accelerated Stability Assessment Program (ASAP) case study with sesame oil to model oil oxidation.
- Stress sesame oil samples at different times, temperatures, and oxygen levels.
- Measure primary and secondary oil oxidation products using peroxide value (PV) and anisidine value (AV) tests, respectively, using methods described in USP 41 <401> (titration for PV and spectroscopic measurements for AV).
- Use PV and AV to calculate total oxidation value (TOTOX) to show overall oxidation.
- Use ASAP*prime*® software to model oxidative degradation.
- Set up samples for real-time measurements for comparison to the model.

Accelerated Stability Assessment Program (ASAP) Approach

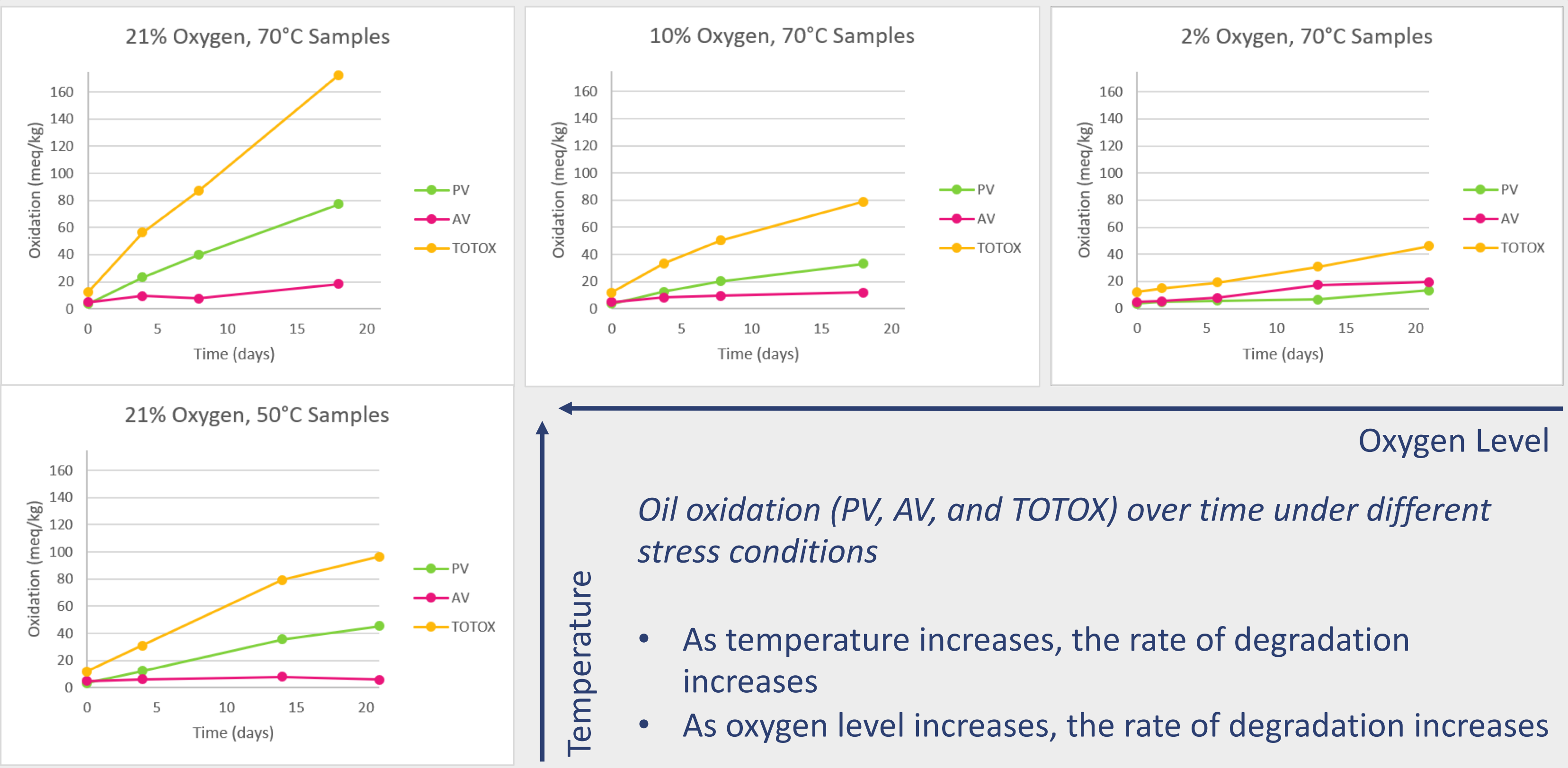
- Time to the edge of failure (i.e. when the product reaches the specification limit) was experimentally measured for several stress conditions.
- A predictive model of product stability was obtained using a standard or modified Arrhenius equation.
- The model can be used to predict time to the edge of failure under different storage conditions.

EXPERIMENTAL DESIGN

T (°C)	$\%O_2$	Time in days (repeats)
		0 (4)
50	4%	2, 6, 14, 21
50	10%	4, 21
50	21%	4, 14, 21
60	0%	7, 14, 21
60	2%	5, 12, 21
60	21%	2, 7, 12, 21
70	2%	2, 6, 13, 21
70	10%	4, 8, 18
70	21%	4, 8, 18
80	0%	6, 12, 21
80	4%	2, 6, 12, 21
80	21%	1, 2, 6

1) Samples of sesame oil (NF grade) between 15 and 16 g were placed in uncapped 20 mL vials.
2) Samples were degassed and prepared at a variety of oxygen levels inside a glove box.
3) Each sample was stored in Mason jars of sufficient volume such that oxygen was in excess.
4) Sealed Ball® jars were placed in ovens until sampled.
5) Samples were tested for PV by titration, AV with an Agilent 8453 UV-Vis spectrophotometer, and TOTOX (calculated).
6) Shelf life based on PV, AV, and TOTOX was modeled using ASAP*prime*® software using the specification limits: 10 milliequivalent oxygen atoms (meq)/kg oil for PV, 15 meq/kg for AV, and 35 meq/kg for TOTOX.
7) Oxygen in the sesame oil was measured optically with a FireStingO$_2$ meter and an Erlenmeyer flask equipped with two oxygen sensing dots (one for the oil and one for the headspace). Measurements were used to calculate the oxygen solubility in the oil.

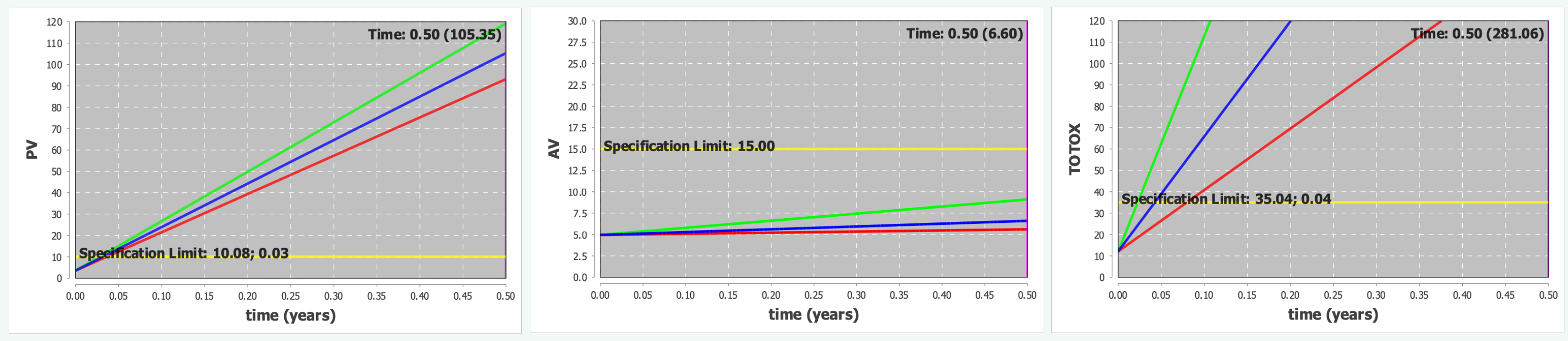
RESULTS



Oil oxidation (PV, AV, and TOTOX) over time under different stress conditions

- As temperature increases, the rate of degradation increases
- As oxygen level increases, the rate of degradation increases

ASAP model predictions for PV, AV, and TOTOX at 21% oxygen (ambient oxygen)

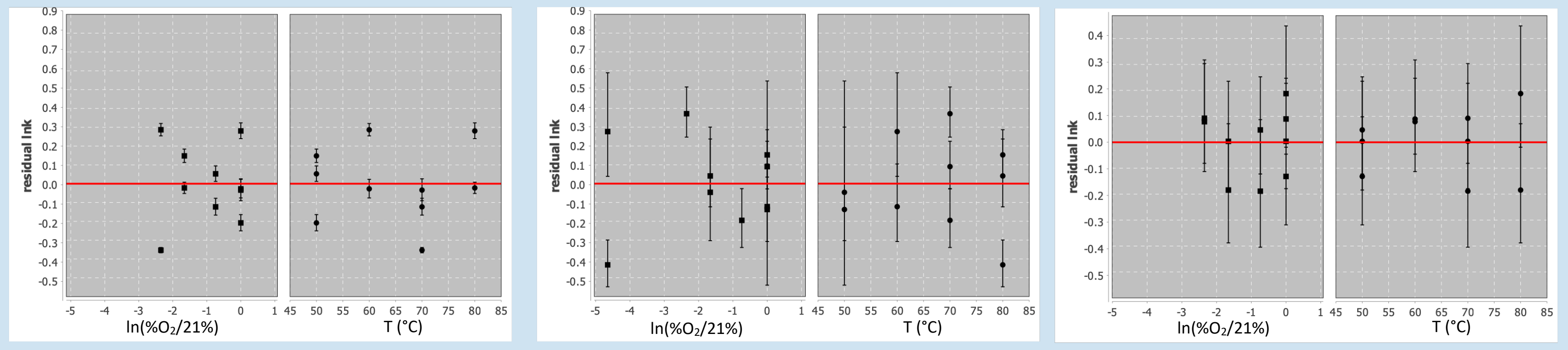


PV, Default fit *AV, Avromi-Erofeyev fit* *TOTOX, Default fit*

Arrhenius Equation: $lnk = lnA - \frac{E_a}{RT}$

	Spec Limit	lnA	E_a (kcal/mol)	R^2	Q^2
PV	10	17.1 ± 1.5	10.5 ± 1.0	0.985	0.917
AV	15	27.8 ± 6.0	19.2 ± 4.1	0.998	0.993
TOTOX	35	16.3 ± 5.1	9.4 ± 3.4	0.952	0.817

ASAP model residual plots for PV, AV, and TOTOX at all oxygen levels



PV, Default fit *AV, Avromi-Erofeyev fit* *TOTOX, Default fit*

Modified Arrhenius Equation: $lnk = lnA - \frac{E_a}{RT} + Cln\frac{\%O_2}{21}$

	Spec Limit	lnA	E_a (kcal/mol)	C	R^2	Q^2
PV	10	12.0 ± 2.2	7.0 ± 1.5	0.97 ± 0.08	0.961	0.906
AV	15	24.1 ± 4.2	16.8 ± 2.9	0.13 ± 0.06	0.928	0.800
TOTOX	35	12.4 ± 3.0	6.8 ± 2.0	0.98 ± 0.07	0.978	0.932

RESULTS (cont.)

Oxygen solubility in sesame oil

- Oil oxidation occurs when oxygen is dissolved in oil, even if the headspace is purged of oxygen.
- ~200 g of oil put in 250 mL Erlenmeyer flask (total volume 285 mL) equipped with oxygen sensing dots.
- Two methods:
  - a) Oil is saturated with oxygen by bubbling ambient air; headspace is purged with nitrogen. Flask sealed and oil stirred until equilibrium.
  - b) Oil is purged with nitrogen; headspace is filled with ambient air. Flask sealed and oil stirred until equilibrium.

Dissolved oxygen results
O_2 solubility in sesame oil at room temperature (µg/gm)

Starting with air saturated oil	43.1
Starting with purged oil	45.1
Average	44.1

CONCLUSIONS

- PV, AV, and TOTOX were well modeled by a modified Arrhenius equation assuming a log dependence on the oxygen concentration. No cross-term involving both temperature and oxygen was needed.
- PV and TOTOX were modeled with a default fit. AV was modeled with an Avromi-Erofeyev fit (a kinetic fit with lag), which is characteristic of secondary degradation.
- The PV and TOTOX values showed a low E_a (~ 7 kcal/mole) and strong oxygen dependence. The AV values had a higher E_a (~20 kcal/mole) and weak oxygen dependence.
- The oxygen solubility in sesame oil at room temperature was 44.1 µg/gm, which compared well with literature values.
- Data from samples stressed for three weeks under accelerated conditions were used to develop a model using ASAP*prime*® to quantify the temperature and oxygen level dependence of the growth of oxidative degradation products in sesame oil.

FUTURE WORK

- Test for oxygen solubility in sesame oil at higher temperatures.
- A real time study with sesame oil is in progress for comparison with the model.

REFERENCES

Figure 1 from Tim Vickers, after Young IS,McEneny J (2001). "Lipoprotein oxidation and atherosclerosis".Biochem Soc Trans 29 (Pt 2): 358–62

ACKNOWLEDGEMENTS

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