



Effect of gases on biochemical stabilization

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Chapter Outline: Philippe Cayot

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Oxidation, lipid, protein, aroma, phenol, inert gas, carbon dioxide, nitrogen

Abstract:

Lipid or protein are sensitive to peroxidation and inert gas limit or stop adulterations. Unsaturated lipids are peroxidized by ground state dioxygen in presence of catalysts (iron, enzymes) or after thermal dissociation of C=C bond, and by excited state dioxygen without any assistance (photo-oxidation). Inerting fragile oils by bubbling with dinitrogen or carbon dioxide that are soluble gas in oil slows oxidation process during storage or frying. In a same way, limit the oxidation of the tryptophan residues of protein is possible by reducing oxygen content. Oxidation leads also to oxidation of thiol in disulfide, which induced protein cross-linked and decay of functional properties, but also to change of aroma in case of volatile thiols. Bubbling with N₂ is preferable in aqueous solution because of buffering effect of CO₂. Dioxygen enters in the phenol polymerization of fresh-cut fruits and vegetable but dioxygen cannot be completely eliminated in order to preserve the life of cells.

Gases used in food industry can potentially react with substrate as any molecule whatever the state (gas, liquid, solid). Among gases, dioxygen (O_2 , commonly named oxygen) or ozone (O_3) are very reactive but the carbon dioxide (CO_2) is not really inert. Dinitrogen (N_2 , commonly named nitrogen) or dihydrogen (H_2) are in other side neutral when dissolved in aqueous solvent. Substrates that can react with gases are mainly unsaturated lipids, proteins, aromas and vitamins. Saccharides and polysaccharides are less reactive, except with dihydrogen in presence of Raney nickel, a catalyzer allowing the production of sugar alcohol (xylitol, sorbitol, mannitol for ex.) by reduction of saccharides.

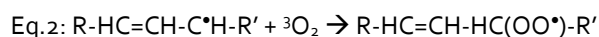
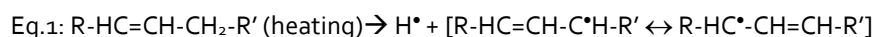
Lipids & gas reaction

Lipids & O_2 - oil oxidation

The molecular dioxygen possesses a bond dipole moment equal to zero and is naturally more soluble in oil (molar fraction oxygen/oil of $5 \cdot 10^{-3}$ at $20^\circ C$) than in water (molar fraction oxygen/water of $2.5 \cdot 10^{-5}$ at $20^\circ C$; Battino et al. 1983), *i.e.* 200 times more soluble in oil than in water. The carbon-carbon double bonds are susceptible to react with molecular oxygen. Unsaturated lipids (that contain $C=C$ double bonds) belong logically to the family that are very sensitive to Reactive Oxygen Species (ROS), such as dioxygen in different states (O_2^1 , $^{\circ}O_2$, $^{\circ}O_2^{2-}$, $HO-OH$, HO°).

An oxygen-rich atmosphere can be used for example for fresh bovine meat to give a bright red color, but presents clearly the risk of early peroxidation of meat lipids. Molecular dioxygen at electronic ground state (3O_2 or triplet oxygen, symbolized also by 1O_2) does not react without any assistance such as enzyme (lipoxygenase for ex.), metal catalysis (iron or copper ions), or photo-initiation. The activation energy of a direct reaction between acyl chain of triglyceride and triplet oxygen is too high ($E_a = 145-270$ kJ/mole). The only possibility of the triplet ground-state dioxygen to react with lipids (without any catalysis) is observed with frying oil: a thermal homolytic dissociation of double carbon-carbon bond is possible at temperature generally over $170^\circ C$ (Rudnik et al., 2001). The allyl group loses easily a radical hydrogen (H^{\bullet}) (eq.1) because the energy of an allylic C-H bond is only 364 kJ/mol (compared to

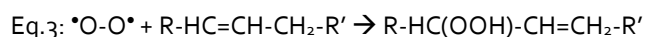
397 kJ/mol for alkane; Blanksby & Ellison, 2003), due to an allylic resonance stabilizations (eq. 1). The acyl-radical reacts with ground-state oxygen to form peroxide (eq. 2).



Oxygen transfer depends on the geometry of the frying-bath and greatly influences the kinetics of oxidation of oil (Patsioura et al., 2017). Without oxygen, no oxidation!

The photo-oxidation is due to the excited-state of molecular dioxygen. Triplet oxygen is transformed in singlet oxygen in presence of a sensitizer (for ex., coloring agent) and light. The myoglobin in the meat is for example a sensitizer and can promote singlet oxygen with a modified atmosphere rich in oxygen. In the electronic structure of the molecular orbital of the ${}^3\text{O}_2$, $(\sigma_{1s})^2(\sigma^*_{1s})^2(\sigma_{2p})^2(\pi_{2p})^4(\pi^*_{2p})^2$, where the two degenerated antibonding orbitals $\pi_{g,x}$ and $\pi_{g,y}$ are not occupied with a pair of electrons on the opposite of the other orbitals but by single electrons. Due of this, different spin orientations of electrons are possible (Schweitzer & Schmidt, 2003) and offer the possibility of higher level of energy. The characteristics of singlet oxygen, ${}^1\text{O}_2\ ^1\Delta_g$, explains its reactivity: 94.3 kJ/mol above triplet state, ${}^3\Sigma_g^-$ that is the ground state (that means no assistance-catalysis is necessary); the singlet-triplet transition is spin forbidden; consequently the lifetime of singlet oxygen is important (74 minutes) in gas phase; and the solution lifetime is also quite important (but solvent dependent; 30 s in benzene and maybe in apolar solvent such as lipid, 3.5 s in water). In reality the dioxygen can have two physical excited states, one $\text{O}_2\ (^1\Delta_g)$ and the second, $\text{O}_2\ (^3\Sigma_g^+)$ that presents 155 kJ / mol above the ground state (${}^3\text{O}_2$) (Schweitzer & Schmidt, 2003). To simplify, the two states are combined in a simple excited state, ${}^*\text{O}_2$ (symbolized also by ${}^5\text{O}_2$ for singlet), and the ${}^*\text{O}_2$ is considered as a diradical, $\cdot\text{O-O}\cdot\ (^*\ddot{\text{O}}-\ddot{\text{O}}\cdot)$, when the ground state is written usually $\text{O}=\text{O}$ (or $:\ddot{\text{O}}=\ddot{\text{O}}:$ with two non-bonding electron pair). The atmosphere contains naturally a small content of singlet oxygen: the mean peak concentration of ${}^1\text{O}_2$ is currently estimated at about 10^8 molecules·cm⁻³ of air (Wayne, 1994) but the concentration depends on the pollution and the sunshine (Ogawa et al., 1991). For comparison, one cm³ of air contains $5.64\cdot 10^{18}$ molecules of dioxygen.

Because of a permanent presence of excited oxygen in air (even at low content), peroxidation of oil occurs without any other prooxidant (eq.3).



Lipids & N₂

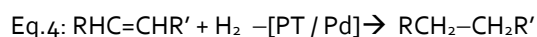
Dinitrogen is inert for unsaturated carbon-carbon bonds. The N₂ gas is used to protect lipids against oxidation by bubbling or with a liquid nitrogen drop in an oil container. The N₂ gas allows anoxic conditions that prevent the oxidation initiation. A modification of atmosphere with nitrogen gas surrounding the frying-bath can limit the oxidation of oil: with a residual of 2 or 4% of oxygen in atmosphere, the oxidation stability of the frying oil is maintained (Fujimoto, 2003). The modified atmosphere with 100% N₂ of a packaged ground beef patties avoids lipid oxidation (detected by carbonyl content; Lund et al., 2007a).

Lipids & CO₂

The oxidation of oil at deep-frying temperature was remarkably inhibited by carbon dioxide blanketing, with a minimum carbon dioxide flow rate necessary to blanket 4 L of frying oil in an electric fryer (Totani, et al. 2016). In this case, the effect is the same that was obtain with nitrogen, meaning the reduction of oxygen content in the headspace and so dissolved in oil by convection during the frying. The advantage of carbon dioxide is the high density of the gas compared to oxygen & dinitrogen, respectively a volume mass of 1,98 kg/m³ (gas at 1 atm and 0 °C) , 1.42 and . 1.25. In the headspace of a bottle or a fryer, the carbon dioxide avoid oxygen contact without a continuous flow of gas but not the dinitrogen gas that is lighter than oxygen. In labs, chemists use argon gas to make anoxic synthesis because of its density (1.78 kg/m³) but also mainly because it is inert! The carbon dioxide is a weak electrophile (it reacts with strong nucleophile such as HO⁻ or carbanions, provided by Grignard reagents for ex.) but can be considered as quite inert with oil. In addition, because it has a bond dipole moment equal to zero value (μ = 0 Debye), it is strongly soluble in oil.

Lipids & H₂

Dihydrogen gas is not used with lipids except to saturate the fatty acid residues of oil and produce hydrogenated oil. Hydrogenation of vegetable oil is used in oil industry in order to obtain margarine, frying oils, etc. Hydrogenation of oil leads to increased melting point¹ (margarine) and reduces the number of unsaturation that lowers the susceptibility to oxidation. Hydrogenation is obtained by bubbling H₂ gas in oil with catalysts, Pt, Pd, (eq.4) or Pt–Ni bimetallic at 200°C during several hours (McArdle et al., 2014)..

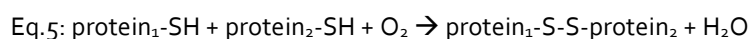


An alternative technique of hydrogenation has been tested using the catalytic transfer hydrogenation in the presence of 5 wt.% Pd/C – palladium metal supported on activated carbon - (Sancheti & Gogate, 2017). This technique does not require the use of hydrogen gas, H₂ being potentially dangerous. The hydrogen gas is replaced by hydrogen donors such as cyclohexadiene, hydrazine, formic acid or sodium formate ... phosphinic or acid or sodium hypophosphite. The use of hydrogenated oil in food industry is one of the reliable strategies to avoid oxidation in intermediate water activity products such as biscuits, very susceptible to oxidation.

Protein & gas reaction

Protein & O₂

Dioxygen can react with protein, essentially with thiol residues (Cys) and tryptophan moiety. Disulfide bond can be formed during storage with oxygen, by oxidation of two cysteinyl residues (eq.5). During storage under high-oxygen atmosphere at 4 °C, the protein thiol groups in ground beef have been decreased and irreversible disulfide bonds have been formed, resulting in a cross-linked myosin heavy chain (Rysman et al., 2014).



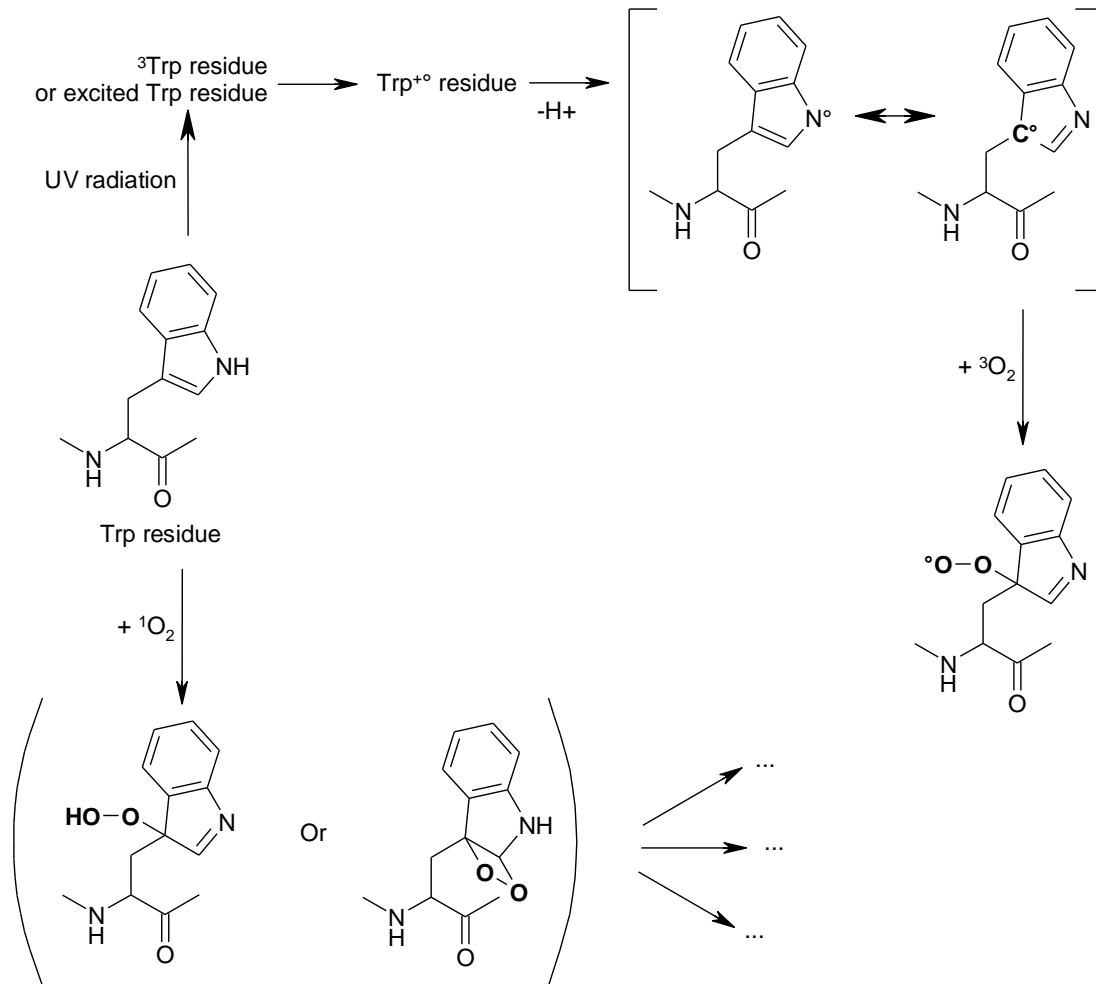
¹ The higher the number of C=C double bond, the lower the fusion point of triacylglycerol.

Because of these cross-linking of proteins under high-oxygen packaging atmosphere, the tenderness of meat can be reduced (Lund et al., 2007b). Cooking meat by heating also induces oxidation of thiol groups (Promeyrat et al., 2013), due naturally to atmospheric dioxygen, accelerated by iron presence (prooxidant). The evolution of baking quality of flour during the storage can also be explained by oxidation of air in flour (Prange et al., 2001), modifying the ratio SS/SH functions in gluten. The number of thiol groups in *Longissimus thoracis et lumborum* steaks are stable in modified atmosphere without O₂, but decrease in a storage at 5°C with an atmosphere with 20%, 50% or 80% of O₂ (Spanos et al., 2016). In lab, when it is necessary to store protein powder for a long period of study (3 years for ex.), the powder is package under nitrogen, in order to avoid disulfide bond formation during the long-term storage.

Oxygen can also favor protein oxidation, and especially the oxidation (peroxidation) of tryptophan residues. For example, oxidation damage within cells targets mainly proteins, due to oxidation of tryptophan by reaction with excited oxygen molecule (singlet dioxygen; Gracanin et al., 2009). It is possible to follow oxidation of Trp residues by fluorescence (Estévez et al., 2008). Oxidation products of tryptophan are well described and give for ex. kynureine residue, which doesn't fluoresce as tryptophan (Taylor et al., 2003), but also dityrosine residues (Dyer et al., 2006), which induces protein reticulation. As lipids, photooxidation of protein occurs with ^{*}O₂ (¹O₂) that react with Trp, but also Tyr, His and Cys residues or by excitation of Trp by UV radiation and reaction with ground-state dioxygen, ³O₂ as shown in eq. 6 (Pattison et al., 2012). The oxidation of oil in an O-in-W emulsion stabilized by protein operates at the same time with protein oxidation and tryptophan loss (Estévez et al., 2008). Milk supplemented with iron and ascorbate for infant formulas are damaged by heating at 60°C principally by oxidation of Trp residues (Leclère et al., 2002) because of iron and ascorbate addition but may be also by the action of dioxygen dissolved in milk!

The greater is the concentration of oxygen in the package, the higher is the oxidation of pork protein (Bao & Ertbjerg, P, 2015). The best manner to avoid oxidation of protein is anoxia, using enriched dinitrogen atmosphere for packaging or during the process... in a same way of lipids.

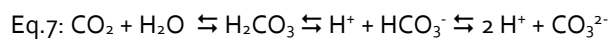
Eq.6:



Proteins & CO₂

Because carbon dioxide is soluble in water (1.45 g/L at 25 °C), bubbling CO₂ gas in water produces carbonic acid, a weak acid, with a first pK_a at 3.6 and a second at 10.3 (eq.7) and in equilibrium with bicarbonate and carbonate bases.

Bubbling with CO₂ acidifies the water solution, for example a protein solution;



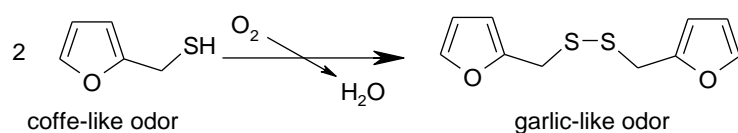
Carbon dioxide bubbling in milk is generally used for preservation against bacteria (Singh, 2012) but not only. Proteins, and especially caseins, are strong buffers (Tomasula et al., 1999) but bubbling milk for example can precipitate protein because of acidification of milk. It can be used to prepare milk protein concentrate (Marella et al.

2015), to better moisture retention and salt uptake in Cheddar curds by milk preacidification (Nelson & Barbano, 2005) or to decrease the amount of rennet required to coagulate milk (St-Gelais et al., 1997), as a yogurt manufacture assistance (Calvo et al., 1999).

Modified atmosphere and aroma molecules

The coffee beans packaged in hermetic big-bags with a CO₂ injection maintained a specialty coffee classification based on quality attributes that include the fragrance (Borem et al., 2013). As protein thiol, other thiols, and especially aroma thiols, are sensitive to oxidation. For example, the 2-furfurylthiol (CAS Registry Number 98-02-2) is one of the key coffee brew odorants and its concentration decrease modifies the sensory perception of the beverage (Hofmann & Schieberle, 2002). The change of odor of coffee during heating and storage after percolation of coffee is due to oxidation of aroma molecules, and in particular of the 2-furfuryl thiol (Kumazawa et al., 1998). The oxidation of 2-furfuryl thiol gives a disulfide with a garlic odor (cold coffee or reheated coffee is generally not appreciated), the Bisfurfuryl disulfide (CAS Registry Number: 4437-20-1) (see eq. 8). A nitrogen purge of water or headspace could preserve the characteristic coffee odor. Nevertheless, aroma thiol compounds can also react with phenol by nucleophilic addition even in absence of oxygen (Charles-Bernard et al., 2005). But anoxic conditions can slow the degradation of thiol aroma of coffee. Boiling water and percolate immediately is a manner to deoxygenate water... but coffee must be drunk immediately, in other words piping hot!

Eq.8:



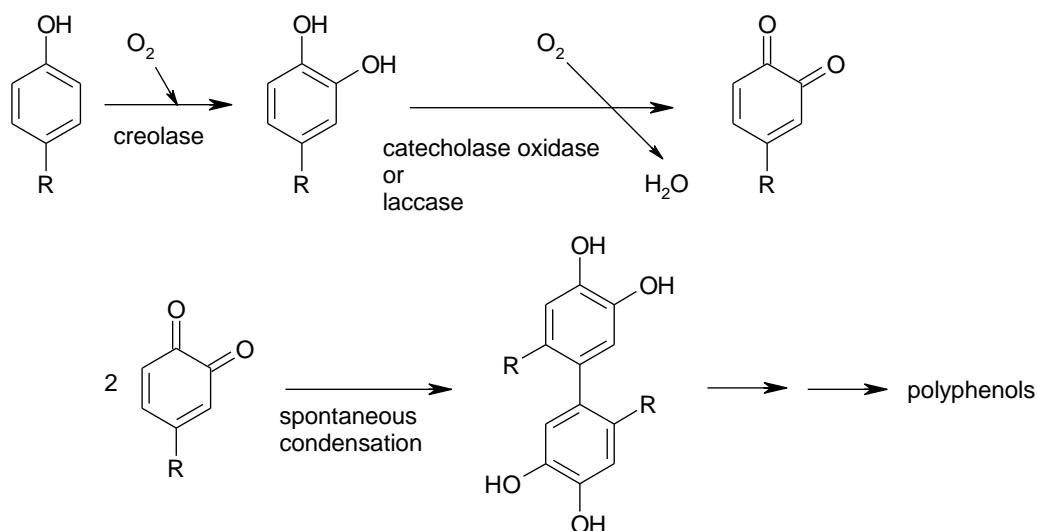
Argon seems to be more efficient to preserve aroma of food product than dinitrogen, especially against oxidation. Argon packaging and processing preserves and enhances flavor, freshness, and shelf life of different foods such as cheese, potato chips, orange juice etc. (Spencer & Humphreys, 2003).

The use of modified atmosphere to preserve a good aromatic quality of fruits and vegetables is not so simple because cells need oxygen to live, even with cut fruits & vegetables. The use of modified atmosphere has a controversial effect. The modified atmosphere packaging with very low O₂ eases the off-odor formation of iceberg lettuce for ex. (Deza-Durand & Petersen, 2014).

Modified atmosphere and phenol of fruits & vegetables

The browning of fresh cut fruits and vegetables is due to the polymerization of phenols. In presence of oxygen, monophenols are converted in diphenol then in O-quinone, owing to the catalysis of PPO, polyphenol oxidase enzymes (eq. 9). The actions to avoid browning are to eliminate oxygen, to reduce the pH in order to move off from the optimum pH value of enzymes (4 to 5), to reduce the produced O-quinones into diphenols (by adding ascorbic acid), or to modify phenol by adding sulfite (SO₂) and block the catalysis action. The fresh pre-cut vegetables are generally packaged in a modified atmosphere in order to limit the polymerization of phenols and, for fresh pre-cut fruits, citric and ascorbic acids are added. To maintain the orange color of dry apricot and avoid using sulfites, a modified atmosphere package very poor in oxygen gas (rich in dinitrogen) constitutes a very efficient alternative and is already used by food industry (see also Elmaci et al., 2008).

Eq.9:



Browning of mushrooms during senescence is due to polyphenol oxidation. The modified atmosphere with 80% O₂ + 20% CO₂ seems to inhibit the polyphenol-oxidases (Li et al., 2017), enzymes that catalyze the polymerization of phenol responsible of the browning in presence of oxygen. The CO₂ gas can play the role of added citric acid with fresh pre-cut fruits, that is, an acid that decreases the pH to move off the optimum pH of enzymes. On intact fruits, a modified atmosphere package preserves quality with a high content of oxygen: Myrtle berries stored at 2°C for 30 days in a 80% O₂ atmosphere have a higher content of anthocyanins (polyphenols) than control (fruits in air) (Fadda et al., 2016).

Conclusion

Combined to antioxidants and opaque package that control gas transfer (with oxygen absorber), the modified atmosphere very poor in oxygen can readily increase the shelf of life of product sensitive to oxidation. Unsaturated lipids (especially omega-3 rich oils), proteins rich in tryptophanyl residues (α -lactalbumin enriched infant formula), and food with fragile fragrance can be stored a long time with nitrogen, argon or carbon dioxide gas. For living fresh products or fresh pre-cut fruits or vegetables, the strategy of aroma preservation is more difficult because the cell life has to be maintained. For dry fruits, anoxic atmosphere is however recommended and avoids uses of sulfites.

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