

New methods to determine the level of low oxygen limit

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INTRODUCTION

The use of controlled atmosphere facilities for long-term storage has long been accepted as the optimum method for preserving the quality of fruits and vegetables. Controlled atmosphere (CA) facilities use a range of sensor technologies to monitor temperature, oxygen and carbon dioxide levels within the controlled environment. Nowadays, the state of the art CA technology is provided facilities to store fruits and vegetables at ultra low oxygen level. Damage resulting from incorrect environmental storage conditions reduces the quality and market potential of the produce. As you know, when the O₂ concentration is lowered below the Fermentation Induction Point (FIP), anaerobic metabolism induces accumulation of acetaldehyde (AA) and ethanol (EtOH) in higher concentration, which can lead to the development of off-flavours and to damage of the produce.

The main objective of this work was to determine the low oxygen limit of 'Granny Smith' apples (*Malus domestica* L.) which were harvested from a local commercial orchard in Érd. Two methods were applied on this study. The first method was based on the measurement of chlorophyll fluorescence to detect the FIP. The second method was the application of solid phase microextraction (SPME) for detection and qualification the volatiles components in the gas-phase beyond the fruits. The purpose of this procedure was to recognize the effect of different storage conditions on the volatiles aroma production capacity. Furthermore to investigate how these components follow the physiological processes and to determine the anaerobic respiration metabolites under the level of low oxygen limit.

MEASUREMENT OF THE CHLOROPHYLL FLUORESCENCE

9 litres chambers containing the fruit were sealed with a range of atmospheres. The level of oxygen were decreased continuously and it was monitored by ICA41 Gas Analyser (International Controlled Atmosphere Ltd. United Kingdom) The changing of chlorophyll fluorescence was measured by MONITORING PAM - Multi-Channel Chlorophyll Fluorometer (MONI-PAM, Heinz Walz GmbH, Effeltrich, Germany) up to 48 hours. The maximum chlorophyll fluorescence intensity (F_m) and current chlorophyll fluorescence intensity (F_o) were recorded every 10 minutes. To estimate PSII photochemistry fluorometrically, PSII maximum efficiency in dark-acclimated fruits ($\Phi_{Pmax} = (F_m - F_o)/F_m$; were commonly determined (figure 1.). The fruits are mounted at a distance of 25 mm from the MONI-head's optical window so that fruit surface and longitudinal axis of the MONI-head form an angle of 120°.

APPLICATION OF THE SOLID PHASE MICROEXTRACTION (SPME)

12 L chambers containing 1kg fruit were sealed with a range of atmospheres. The atmospheres supplied to the chambers were monitored with ICA41 Gas Analyser (International Controlled Atmosphere Ltd. United Kingdom). AA and EtOH were induced in fruit by exposure to atmospheres of 0.6, 0.9, 1.2, 1.5, 2, 10 and 21% O₂ for up to 24 hours at 20°C. The volatiles were sampled by means of solid phase microextraction (SPME) using 1 cm long fibers coated with a 65µm thick layer of polydimethylsiloxane and divinylbenzene (Supelco Co., Bellefonte, USA). The volatiles were subsequently desorbed during 30 sec at 250°C into the splitless injection port of the GC (Perichrom 2100, France) and separated on a capillary column (DB-WAX length: 30 meter, inert diameter: 0.32 mm, 0.25 mm film thickness). The temperature program started at 40°C, was then raised at the rate of 5°Cmin⁻¹ to 250°C.

RESULTS

Optimal saturation time of the SPME fibers: Constant flow rate (120 ml min⁻¹) combined with different sampling time (15, 20, 25 min) were used for this measurement. **By right of the chromatograms 20 minutes for sampling is enough for the total saturation in the used system.** There was no significant difference (5%) between the chromatograms of 20 and 25 minutes sampling time. 15 minutes are too short to fully impregnate the area of the SPME fibers.

Optimal rate of flow: 3 different rate of flow (40, 80, 120 ml min⁻¹) were applied at this measurement with 20 minutes sampling time. Known amounts of nC₁₄ were added to the chambers as an internal standard. **The chromatograms showed that no relationship between the different rate of flow and the intensity of the peaks.**

Detection of the anaerobic metabolites: The results of our measurements show us that the volatile range of the anaerobic metabolics is located at the first 6 minutes retention time. Therefore during the estimation of different chromatograms this area were interested. Figure 2. shows four chromatograms which are near by the low oxygen limit. **The quantity of acetaldehyde and ethanol increased below the 1,5% O₂ concentration. Therefore the Fermentation Induction Point of 'Granny Smith' apples is estimated between 1,5% and 1,2% level of O₂.**

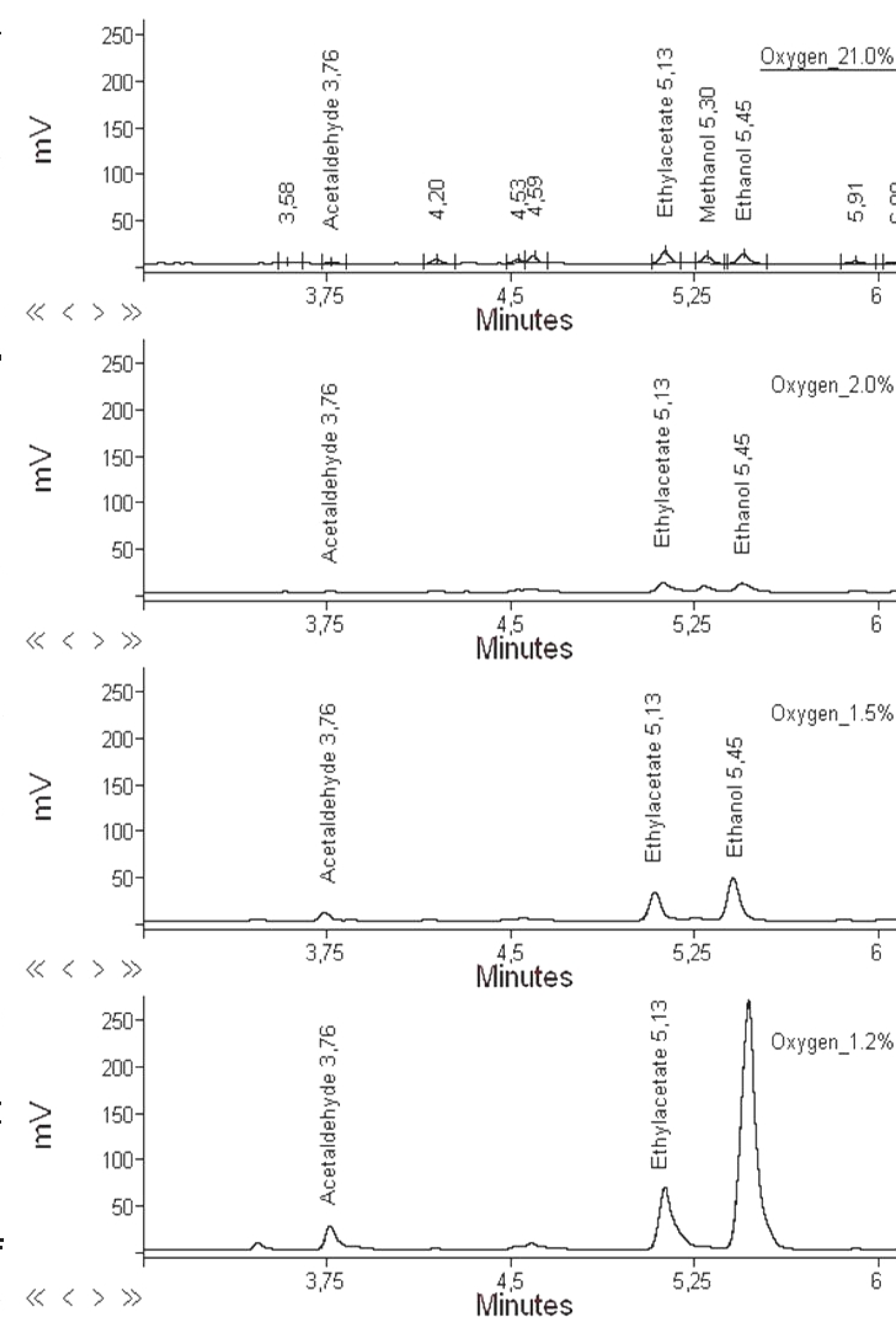


Figure 2. Chromatograms of the “Granny Smiths” apples at four different oxygen concentration (21.0% 2.0% 1.5% 1.2%)

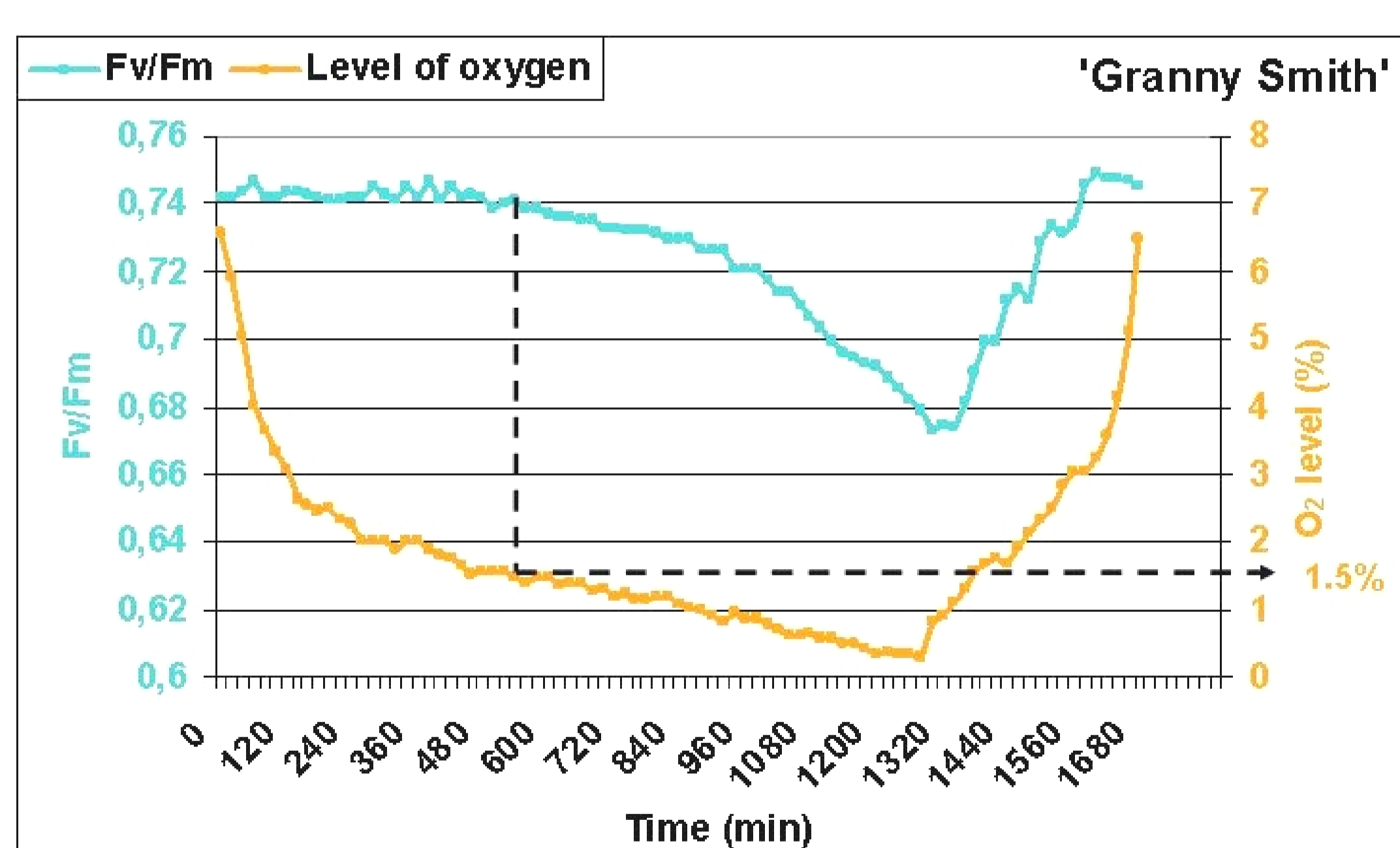
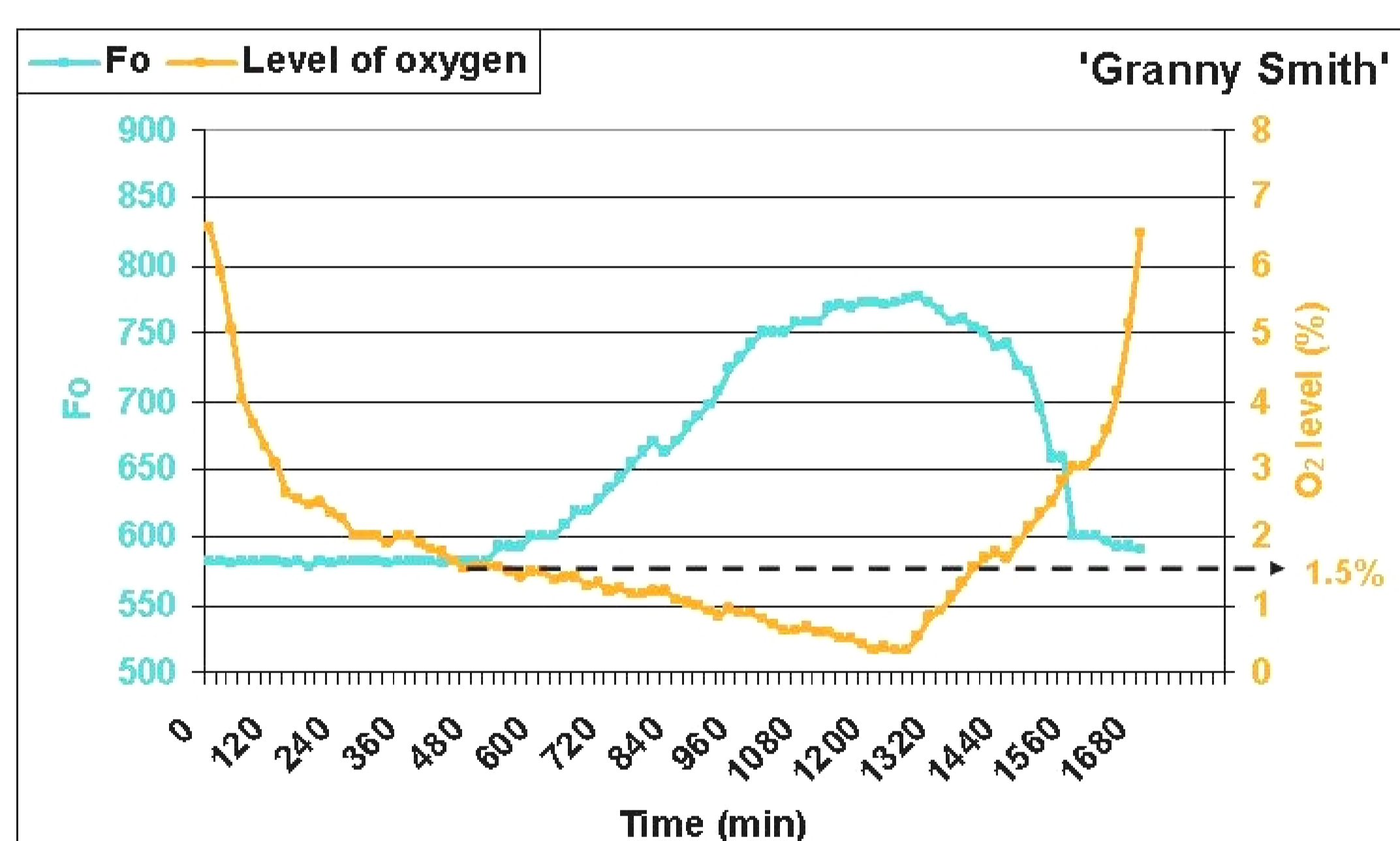


Figure 1. Changes the current chlorophyll fluorescence intensity, the PSII maximum efficiency and the oxygen concentration over time.

Below the fruit specific oxygen-threshold (1.5%) the F_v/F_m values were decrease, while the F_o values were increased.

CONCLUSION

Several book, journal article and industrial application proved it already with usage of other measurements and examination methods, that the optimal oxygen level of 'Granny Smith' apples during the controlled atmosphere storage is between 1,5 and 1,3 %. There was a challenge to confirm these research results and industrial experiences with our new measuring method. The results showed that our measurement methods are right sensitive and correct to use for investigation of the **Fermentation Induction Point** in the future.