Chlorophyllfluoreszenz als sensorischer Parameter in der Apfellagerung

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Zusammenfassung

Die Lagerung von Früchten unter kontrollierter Atmosphäre ermöglicht es die Fruchtqualität und Haltbarkeit zu bewahren. Insbesondere die dynamisch kontrollierte Lagerung mit Messung der Chlorophyllfluoreszenz (Dynamic Controlled Atmosphere - Chlorophyll Fluorescence = DCA-CF) von Äpfeln (*Malus* x *domestica*, BORKH.) bietet großes Potential. Bei diesem Typ der DCA-Lagerung wird die Chlorophyllfluoreszenz gemessen, um das Stressverhalten von Äpfeln als Folge von zu niedrigen O_2-Konzentrationen in der Lagerraumatmosphäre zu bewerten. Gegenwärtig werden in der DCA-CF-Lagerung nur nicht-bildgebende Fluoreszenzmessverfahren verwendet. Jedoch kann das Potential der DCA-CF-Lagerung nur teilweise genutzt werden, weil Äpfel gleicher Sorte und Herkunft bei völlig identischen Lagerungsbedingungen ein unterschiedliches Stressverhalten hinsichtlich niedriger O_2-Konzentrationen zeigen können. Daher ist die Auswahl einer repräsentativen Probe für die Fluoreszenzmessung beeinträchtigt.

Ziel dieser Dissertation war es, die Ursachen für das unterschiedliche Stressverhalten von einzelnen Äpfeln hinsichtlich niedriger O_2-Konzentrationen zu finden, um die Auswahl einer repräsentativen Probe für die Fluoreszenzmessung zu erreichen. Darüber hinaus wurde mit einem bildgebenden Messverfahren die Chlorophyllfluoreszenz auf der Apfelschale gemessen, um die Fluoreszenzheterogenität sowie die Reaktion verschiedener Fluoreszenzparamter bezüglich O_2-Mangel zu untersuchen.

Unter Verwendung eines nicht-bildgebenden Fluoreszenzmessverfahrens wurde die kritische O_2-Konzentration (Lower Oxygen Limit = LOL) einzelfruchtweise identifiziert. Der Reifegrad der Apfelfrüchte, insbesondere kurz nach der Ernte, wenn der Stärkeabbau noch nicht abgeschlossen war, beeinflusste den LOL signifikant. An einem Teil der Apfelfrüchte, die zuvor vier Monate gelagert wurden, konnte der LOL nicht identifiziert werden (variierend von 12,5 % bis 41,7 % der untersuchten Äpfel; n = 96). Zudem zeigte sich, dass der Chlorophyllgehalt in der Apfelschale die Fluoreszenzmessmethode massiv beeinflusste. Des Weiteren konnte mit einem bildgebenden Fluoreszenzmessverfahren die räumliche Verteilung der Chlorophyllfluoreszenz auf der Apfelschale dargestellt werden. Der Fluoreszenzanstieg infolge von niedrigen O_2-Konzentrationen in der Lagerraumatmosphäre wurde visualisiert. Ferner wurde eine neue Methode für die Identifizierung des LOL mit einem bildgebenden Fluoreszenzmessverfahren entwickelt, welches die Heterogenität der Fluoreszenz berücksichtigt und die gemessenen Daten als Histogramm bündelt. Darüber hinaus zeigte die Fluoreszenzkinetik spezifische Änderungen, wenn Äpfel anaeroben Bedingungen ausgesetzt wurden. Insbesondere die Parameter *Fv/Fm, ϕPSII_D1, ϕPSII_D2* und *ϕPSII_D3* waren für die frühzeitige Detektierung von Stress aufgrund von O_2-Mangel geeignet.

Schlüsselwörter: Fluoreszenzkinetik; Histogramm-Teilung; Lower Oxygen Limit; Nachernte

Abstract

Storing fruits under controlled atmosphere makes preserving fruit quality and shelf life possible. In particular, the Dynamic Controlled Atmosphere storage with Chlorophyll Fluorescence (DCA-CF) measurement of apples (*Malus* x *domestica*, BORKH.) offers great potential. In this type of DCA storage, chlorophyll fluorescence is measured to evaluate the stress behavior of apples due to low-O_2 concentrations in the storage room atmosphere. Currently, only non-imaging fluorescence measurement methods are used in DCA-CF storage. However, the potential of DCA-CF storage can only be partially used because apples of the same variety and origin can show different stress behavior with regard to low-O_2 concentrations under completely identical storage conditions. Therefore, the selection of a representative sample for fluorescence measurement is hindered.

The aim of this dissertation was to find the causes of the different stress behavior of individual apples with regard to low-O_2 concentrations in order to select a representative sample for fluorescence measurement. In addition, the chlorophyll fluorescence on the apple skin was measured using an imaging measurement system in order to investigate the fluorescence heterogeneity and the reaction of various fluorescence parameters to low-O_2 conditions.

The Lower Oxygen Limit (LOL) was identified on individual fruit using a non-imaging fluorescence measurement method. The ripeness of the apples influenced the LOL significantly, especially after harvest when starch degradation had not yet been completed. The LOL could not be identified in some of the apples stored for four months (varying from 12.5 % to 41.7 % of the examined apples; n = 96). It was also shown that the chlorophyll content in the apple skin had a massive influence on the fluorescence measurement method. Furthermore, the spatial distribution of chlorophyll fluorescence on the apple skin could be shown using an imaging fluorescence system. The increase in fluorescence due to low-O_2 concentrations in the storage room was visualized. Additionally, a new method for identifying the LOL using an imaging fluorescence system was developed, which considers the heterogeneity of the fluorescence and bundles the measured fluorescence data as a histogram. Also, the fluorescence kinetics showed specific changes when apples were exposed to anaerobic conditions. In particular, the parameters *Fv/Fm*, *ϕPSII_D1*, *ϕPSII_D2*, and *ϕPSII_D3* were suitable for early stress detection due to low-O_2 conditions.

Keywords: fluorescence kinetics; histogram division; Lower Oxygen Limit; post-harvest

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Abkürzungsverzeichnis

1-MCP	1-Methylcyclopropene
AL	Actinic light
ATP	Adenosintriphosphat
CA	Controlled Atmosphere
CCD	Charged Coupled Device
CTIFL	Center Technique Interprofessionnel des Fruits et Légumes
D	Dip
DCA	Dynamic Controlled Atmosphere
DCA-CF	Dynamic Controlled Atmosphere - Chlorophyll Fluorescence storage
Fm	Maximale Fluoreszenz
Fm'	Maximale Fluoreszenz (lichtadaptiert)
Fo	Grundfluoreszenz
Fp	Peak-Fluoreszenz
Ft	Terminaler Fluoreszenzwert
Fv	Variable Fluoreszenz
h^*	Intervals of reference histogram
h_t	Intervals of nth histogram at time *t*
k	Histogram intervals
I	Inflection
LED	Light-emitting diodes
LOL	Lower Oxygen Limit
M	Maxima
ML	measuring light
Mt	Megatonne
NAD^+	Nicotinamidadenindinukleotid
NDVI	Normalized Difference Vegetation Index
NPQ	Non-photochemical quenching

P	Peak
PAM	Pulse Amplitude Modulation
PFM	Pulse Frequency Modulation
PQ	Plastochinon
PSII	Photosystem II
Q_a	Primärer Chinon-Elektronenakzeptor (PSII)
qP	Photochemical quenching
RQ	Respiration quotient
r_s	Spearman-Rho's rank correlation
S	Steady-state
S_n	Singulett
SD	Standard deviation
SP	Saturation pulse
T	Terminal
TA	Titratable Acidity
TSS	Total Soluble Solids
ULO	Ultra Low Oxygen
Δd_t	Histogram division quotient at time *t*
$\phi PSII$	Photosystem II quantum yield
_D	Dark
_L	Light
_Lss	Light steady-state

1 Einleitung

1.1 Grundlagen der Chlorophyllfluoreszenzanalyse

Allgemein betrachtet ist Fluoreszenz ein physikalisches Phänomen von bestimmten Stoffen Photonen zu absorbieren und wieder zu emittieren. Wenn ein Elektron (eines fluoreszierenden Stoffes) durch die Absorption eines Photons angeregt wird, erreicht das Elektron ein höheres Energieniveau. Das Elektron verlässt seinen Grundzustand (S_0; S_n = Singulett) und gelangt auf ein höheres Energieniveau (S_n). Der erreichte höhere Energiezustand ist für das Elektron instabil und es kehrt in Nanosekunden zurück in den Grundzustand. Hierbei wird ein Photon emittiert, was energieärmer ist als das zuvor absorbierte Photon (Stokes-Verschiebung) (Lichtman and Conchello 2005; Matyssek und Herpprich 2019).

Photosynthetisch aktive Pflanzen nutzen die Lichtenergie, um energiereiche Biomoleküle aufzubauen. Die Lichtenergie wird über die Chlorophylle a und b sowie weitere akzessorische Pigmente, wie Carotinoide absorbiert. Das in Pflanzen enthaltende Chlorophyll ist eine fluoreszierende Substanz. Die Absorption von Photonen aus dem roten Spektralbereich bewirkt den Übergang von S_0 in den S_1-Zustand. Die Absorption von Photonen aus dem blauen Spektralbereich bewirkt den Übergang in den energetisch höheren S_2-Zustand. Der Rückfall von S_2 zu S_1 ist begleitet durch Wärmeabgabe an die Umgebung. Beim Übergang von S_1 zu S_0 kommt es zum Phänomen der Chlorophyllfluoreszenz (Murchie und Lawson 2013; Matyssek und Herpprich 2019).

Die Messung der Chlorophyllfluoreszenz lässt sich in drei wesentliche Messprinzipien einteilen. Mit der *Fluoreszenzspektroskopie* kann das Fluoreszenzemissionsspektrum aufgenommen werden. Mit der *Fluoreszenzkinetik* werden Änderung der Fluoreszenzsignale im zeitlichen Kontext bewertet. Des Weiteren kann die *Fluoreszenzintensität*, also die Amplitude des Fluoreszenzsignals, gemessen werden (Lichtman and Conchello 2005; Baker 2008; Murchie und Lawson 2013).

Kautsky und Hirsch (1931) beschrieben zum ersten Mal ein Phänomen, welches als Kautsky-Effekt bekannt wurde. Sie konnten die Änderung der messbaren Fluoreszenz von zuvor dunkeladaptierten Pflanzenblättern beobachten. Wird ein zuvor dunkeladaptiertes Pflanzenblatt mit aktinischem Licht bestrahlt, ist eine Änderung der Fluoreszenz messbar. Der Fluoreszenzinduktionskurve wurden nach Lavorel und Etienne (1977) Fixpunkte (O, I, D, P, S, M, T) zugeordnet. Der Fluoreszenzinduktion ist in Abb. 1.1 dargestellt und gliedert sich in die schnelle und langsame Kinetik. Mit dem Einschalten des Messlichtes wird der Grundwert der Fluoreszenz erreicht (O). Mit der aktinischen Belichtung erhöht sich die Fluoreszenz (I = inflection; D = dip) bis zu einem maximalen Wert (P = peak). Mit Erreichen des maximalen Wertes nimmt die Fluoreszenz ab und gelangt zunächst zu einem stationären Zustand (S = steady-state) und anschließend zum sekundären Maximum (M = maxima). Nach einigen Minuten wird ein mehr oder weniger konstanter Endwert (T = terminal) erreicht. Aus den Fixpunkten der Fluoreszenzinduktionskurve lassen sich wesentliche Fluoreszenzparameter ableiten (Baker 2008; Matyssek und Herpprich 2019).

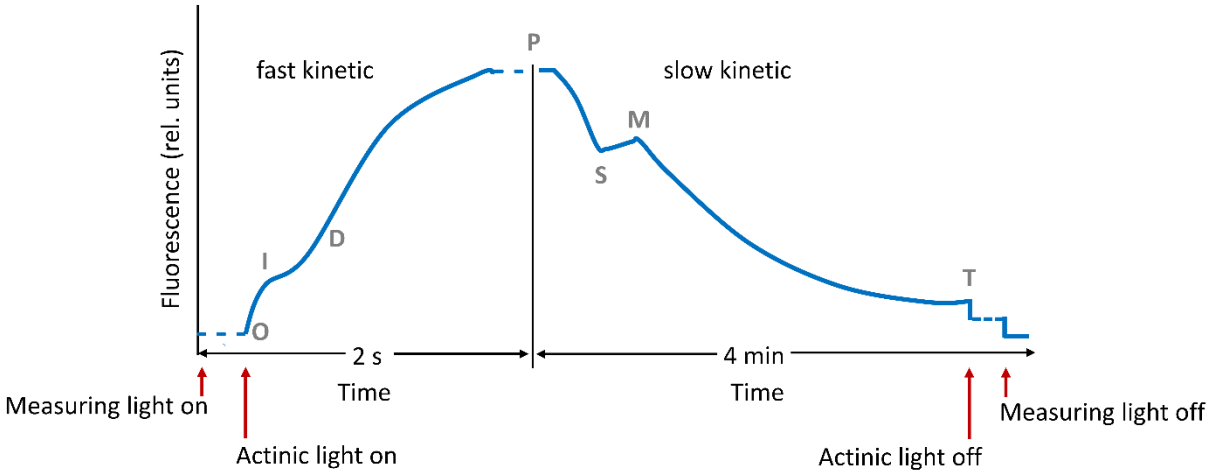

Abb. 1.1: Die schnelle und langsame Kinetik der Chlorophyllfluoreszenzinduktion (in vivo), als Messlicht wurde gepulstes Schwachlicht verwendet (basierend auf Matyssek und Herpprich 2019, verändert).

Im Plastochinon-Pool (PQ), welcher an der Thylakoidmembran der Chloroplasten lokalisiert ist, wird der primäre Chinon-Elektronenakzeptor Q_A des Photosystems II (PSII) verortet. Die Dunkeladaption einer Pflanze führt zur Inaktivierung der Photosysteme. Nun besteht keine Möglichkeit zur photochemischen Energienutzung. Somit liegt Q_A im oxidieren Zustand vor. Die Reaktionszentren des PSII werden als *offen* bezeichnet (Maxwell und Johnson 2000; Baker 2008). Die Belichtung der Pflanze mit einem schwachen Messlicht (0,1 µmol m^{-2} s^{-1} bis 1 µmol m^{-2} s^{-1}) ermöglicht, dass Q_A weiterhin oxidiert vorliegt und gleichzeitig Fluoreszenz emittiert wird. Diese Fluoreszenz (bei Fixpunkt O) wird als minimale Fluoreszenz oder Grundfluoreszenz (*Fo*) bezeichnet. Die Belichtung mit einem kurzen und starken Lichtpuls (je nach Verfahren mit 1 000 µmol m^{-2} s^{-1} bis 10 000 µmol m^{-2} s^{-1}) führt zu der vorübergehenden *Schließung* aller PSII Reaktionszentren. Der Elektronenakzeptor Q_A liegt vollständig reduziert vor und kann kein weiteres Elektron akzeptieren. Zu diesem Zeitpunkt gilt das PSII als *geschlossen*. Die Fluoreszenzausbeute erreicht einen maximalen Wert und wird als maximale Fluoreszenz (*Fm*) bezeichnet (Baker 2008; Matyssek und Herpprich 2019). Die variable Fluoreszenz ($Fv = Fm - Fo$) beschreibt den Übergang vom dunkeladaptierten Zustand mit vollständig *offenen* Reaktionszentren zu vollständig *geschlossenen* Reaktionszentren. Neben den primären dunkeladaptierten Parametern (*Fo* und *Fm*) können weitere Parameter mit Kombinationen aus Dunkelheit, Sättigungspulsen und aktinischem Licht gemessen bzw. ermittelt werden. Der Fluoreszenzparameter *Fv/Fm* ist ein robuster Indikator für die maximale Quantenausbeute von PSII. Ein unbelastetes Pflanzenblatt hat einen *Fv/Fm*-Wert von 0,75 bis 0,85 (Bjorkman und Demmig 1987; Bolhar-Nordenkampf et al. 1989; Murchie und Lawson 2013). Abb. 1.2 zeigt den schematischen Verlauf der Fluoreszenzkinetik und gibt wesentliche Fluoreszenzparameter an.

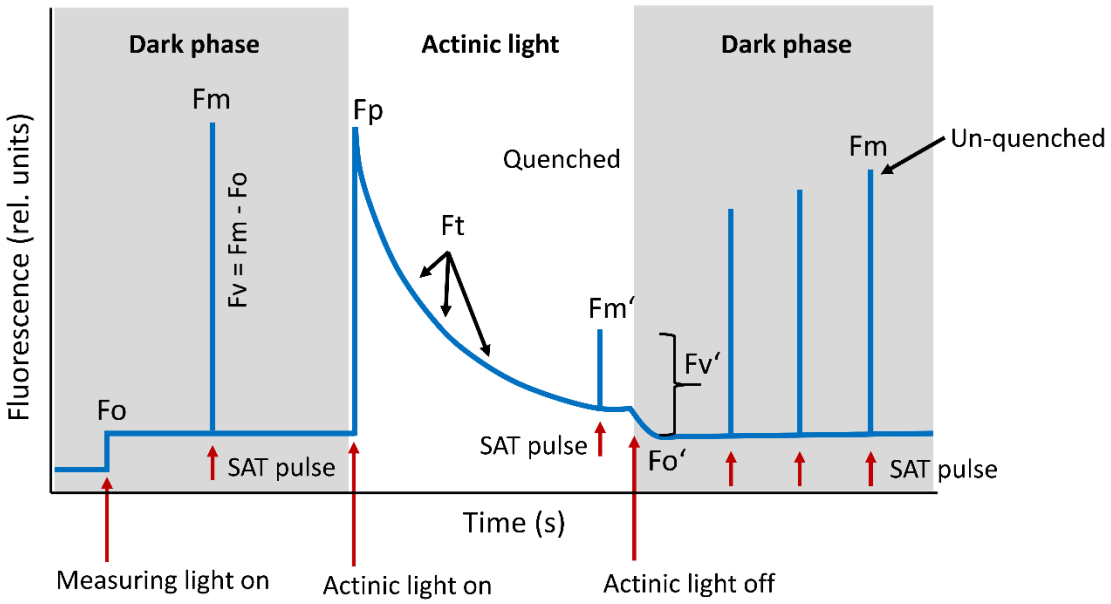

Abb. 1.2: Schematische Darstellung eines Chlorophyllfluoreszenzmessprotokoll (basierend auf Murchie und Lawson 2013, verändert).

Mit dem Einschalten des aktinischen Lichtes steigt die Fluoreszenz kurzzeitig an (abhängig von der Lichtstärke) und wird als Peak-Fluoreszenz (*Fp*) bezeichnet. Dieser Anstieg resultiert aus der zügigen Reduktion des PQ-Pools und der langsamen Aktivierung von Oxidationsmechanismen (Matyssek und Herpprich 2019). Während der Lichtanpassung sinkt die Fluoreszenz auf einen terminalen Fluoreszenzwert (*Ft*). Auch in dieser Phase können Sättigungspulse gegeben werden, die zu einem Maximum in der lichtadaptierten Phase (*Fm´*) führt. Mit dem Ausschalten des aktinischen Lichtes beginnt die Dunkelrelaxationsphase nach der Kautsky-Induktion (Baker und Rosenqvist 2004; Murchie und Lawson 2013).

Die Chlorophyllfluoreszenz kann durch photochemische oder nicht-photochemische Mittel gelöscht (*quenching*) werden. Mit der Fluoreszenzlöschung können Informationen über Änderungen der Effizienz der Photosynthese und der Wärmeabgabe gewonnen werden. Die drei Bereiche Photochemie, Chlorophyllfluoreszenz und Wärmeabgabe stehen in einem *Wettbewerb* zueinander. Die Steigerung der Effizienz einer dieser drei Bereiche führt zu einer Verringerung der Ausbeute der anderen beiden (Maxwell und Johnson 2000; Baker 2008).

Das photochemische Löschen (photochemical quenching = qP) bezieht sich auf die Abnahme der Fluoreszenz aufgrund einer erhöhten Effizienz der Photochemie, insbesondere durch die Erhöhung der Geschwindigkeit, mit der Elektronen vom PSII weitergeleitet werden. Wenn der Wirkungsgrad der Wärmeabgabe ansteigt und sich die Fluoreszenzausbeute vermindert, wird dies als nicht-photochemische Löschung (non-photochemical quenching = NPQ) bezeichnet (Schreiber 2004). Das NPQ fungiert als Mechanismus zur Ableitung übermäßiger Mengen an Anregungsenergie. Überschüssige Anregungsenergie wird als Wärmeenergie abgeführt, bevor sich reaktive Sauerstoffspezies bilden können. Hierdurch werden Schäden an den Antennenkomplexen des PSII verringert (Pfündel und Dilley 1993; Demmig-Adams und Adams 1996; Matyssek und Herpprich 2019).

1.2 Grundlagen der Apfellagerung

Mit einer weltweiten Erntemenge von 86 Mt (Mt = Megatonne) im Jahr 2018 gehört der Kulturapfel (*Malus* x *domestica* BORKH.) zu den bedeutendsten Früchten, neben Bananen (116 Mt) und Wassermelonen (104 Mt) (FAO und CIRAD 2021). Um die Verbraucher das ganze Jahr mit qualitativ hochwertigen Äpfeln versorgen zu können, ist die Lagerung ein essentielles Instrument. Hierbei hat die Lagerung das Ziel, die Fruchtreife und -qualität bestmöglich zu erhalten und die Haltbarkeit (Lebensdauer, Verwendbarkeit) zu verlängern (Abbott, 1999; Harker et al. 2002; Dilley 2010). Optimale Lagerungsbedingungen sind entscheidend für ein gutes Auslagerungsergebnis (Saltveit 2003; Dilley 2010). In der Apfellagerung werden insbesondere die Faktoren Temperatur, O_2- und CO_2-Gehalt geregelt. Weitere Faktoren sind die Luftbewegung und -verteilung sowie die Luftfeuchtigkeit (Geyer und Praeger 2012). Welche Temperaturen, O_2- und CO_2-Werte in der Lagerung eingestellt werden, ist sortenspezifisch und variiert hinsichtlich der geplanten Lagerungszeit und der Anbauregion (Prange und Wright 2023). Äpfel, die unter nicht optimalen Bedingungen gelagert werden, verlieren Qualität und können physiologische Lagerungskrankheiten entwickeln (Knee 1973; Hribar et al. 1993; Dilley 2010; Geyer und Praeger 2012).

Für die Lagerung von Äpfeln werden im Wesentlichen die Strategien Kühlhauslagerung unter Normalatmosphäre oder Lagerung unter kontrollierter Atmosphäre (Controlled Atmosphere = CA) eingesetzt. Die Temperatur wird je nach Lagerungsstrategie auf -0,5 °C bis +5 °C gesenkt (Streif 2012). Die Senkung der Temperatur verkörpert den effektivsten Faktor für die Verlängerung der Haltbarkeit. Niedrige Lagerungstemperaturen verlangsamen die Fruchtatmung sowie die enzymatische Aktivität und damit einen Großteil der Stoffwechselvorgänge innerhalb der Frucht (Lammertyn et al. 2001; Watkins and Nock 2005; Tacken et al. 2010; Geyer und Praeger 2012). Charakteristisch für die CA-Lagerung ist die Veränderung der Gaszusammensetzung der Lagerraumatmosphäre. Die Absenkung von O_2 im Lagerraum wird durch die Zuführung von N_2 erreicht (Pull down) sowie teilweise auch durch die Fruchtatmung. Traditionell werden in der CA-Lagerung weitestgehend sichere und konstante Regelwerte eingestellt (Geyer und Praeger 2012).

Eine spezielle Form der CA-Lagerung verkörpert die Ultra Low Oxygen (ULO) Lagerung. Bei der ULO-Lagerung wird der O_2-Gehalt auf Werte von 1 kPa bis 2 kPa eingestellt. Die neuste Form der Lagerung ist die dynamisch CA-Lagerung (Dynamic Controlled Atmosphere = DCA). Bei der DCA-Lagerung werden O_2-Gehalte von ≤ 1 kPa realisiert (Schouten et al. 1998; Prange et al. 2013; Both et al. 2014; Köpcke 2015; Bessemans et al. 2016). Der niedrige O_2-Gehalt verringert die Fruchtatmung und beeinflusst die Ethylenbiosynthese, sodass während der Lagerung die Reifung verlangsamt wird (Lelièvre et al. 1997; Streif 2010; Pech et al. 2012). Die O_2-Gehalte von ≤ 1 kPa führen zu verlängerten Lagerungszeiten, verbessert die Qualitätserhaltung und verzögert das Auftreten von physiologischen Lagerungskrankheiten im Vergleich mit der herkömmlichen CA-Lagerung und Kühlhauslagerung (DeLong et al. 2007; Zanella et al. 2008; Gasser et al. 2010; Aubert et al. 2015; Weber et al. 2020). Während bei der CA/ULO-Lagerung konstante Regelwerte eingestellt werden, wird bei der DCA-Lagerung der O_2-Gehalt über die gesamte Lagerungszeit dynamisch an die jeweilige Lagerungsphase angepasst (Prange et al. 2013).

1.3 Chlorophyllfluoreszenz und Apfellagerung

In der DCA-Lagerung werden O_2-Gehalte von ≤ 1 kPa realisiert, womit die Äpfel nahe am Lower Oxygen Limit (LOL) gelagert werden können (Thewes et al. 2021). Der LOL beschreibt den untersten tolerierbaren Sauerstoffgehalt bei einer metabolischen Verschiebung vom vorwiegend aeroben zum vorwiegend fermentativen Stoffwechsel (Wright et al. 2012). Die Lagerung von Äpfeln unterhalb des LOL führt zur fruchteigenen Gärung mit der Anreicherung fermentativer Produkte. Zu den fermentativen Produkten, die in diesem Zusammenhang von Bedeutung sind, gehören Acetaldehyd, Ethanol und Ethylacetat (Pesis 2005; Bessemans et al. 2016). Für die Realisierung niedriger O_2-Gehalte im Kontext der DCA-Lagerung und die gleichzeitige Vermeidung wirtschaftlicher Ausfälle aufgrund von fruchteigener Gärung, kann die biologische Stressreaktion der Apfelfrucht mit der Methode der Chlorophyllfluoreszenz überwacht werden (Wright et al. 2008; 2010; 2012). Der Fluoreszenzparameter *Fo* hat sich als sensitiver Parameter zum Nachweis von Pflanzenstress bewährt (Harris und Heber 1993; Prange et al. 2002; Wright et al. 2008). Für die DCA-Lagerung stehen derzeit nur nicht-bildgebende Messverfahren zur Verfügung, um die Chlorophyllfluoreszenz zu messen. Hierzu gehören insbesondere das Harvest-Watch™ System (Satlantic Inc., Halifax, Nova Scotia, Kanada), das Mini-Apple-PAM™ System (Walz GmbH, Effeltrich, Deutschland) und der Fruit-Observer™ (Besseling, Oosterblokker, Niederlande). In der Apfellagerung wird die Chlorophyllfluoreszenz nur an wenigen Früchten im Lagerraum gemessen, die als repräsentative Fruchtprobe auszuwählen werden (Köpcke 2014). Das am häufigsten eingesetzte Fluoreszenzsystem für die Überwachung von Obst in der Lagerung ist das Harvest-Watch™ System. Mit diesem System wird eine Punktmessung an vier bis acht Früchten durchgeführt. Als Output wird ein Fluoreszenzwert für die gesamte Mischprobe ausgegeben (Prange et al. 2013; Wright et al. 2015; Boeckx 2018).

Abb. 1.3 zeigt die Verwendung der Chlorophyllfluoreszenz für die Identifizierung des LOL und die Detektion von Stress aufgrund von zu niedrigen O_2-Konzentrationen in der Lagerraumatmosphäre. Solange sich der Sauerstoff (rote Linie) für die Frucht in einer tolerierbaren Konzentration in der Lagerraumatmosphäre befindet, verbleibt *Fo* (blaue Linie) auf einem konstanten Niveau (*Fo*-Baseline). Sinkt der Sauerstoff jedoch unter eine kritische Grenze, steigt *Fo* nachhaltig an. Am Übergang von der *Fo*-Baseline zu einem nachhaltigen Anstieg von *Fo* kann der LOL in der Grafik abgelesen werden (Wright et al. 2008; 2010; 2012).

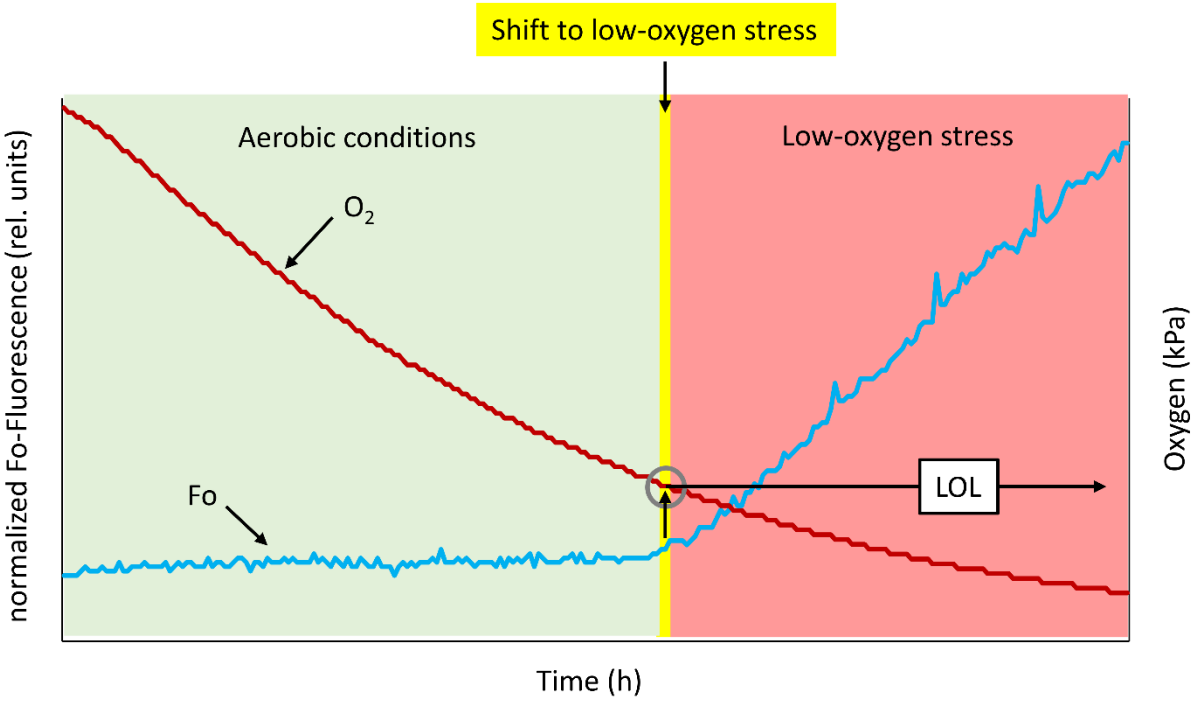

Abb. 1.3: Schematische Darstellung der Identifizierung des LOL am Apfel mittels Messung der Fluoreszenz (*Fo*) (blaue Linie) bei sinkendem O_2-Gehalt (rote Linie); *Fo* verbleibt auf einem konstanten Niveau (grüner Bereich) bis der LOL erreicht wird (gelber Bereich) und der nachhaltige Anstieg von *Fo* den Pflanzenstress sichtbar macht (roter Bereich) (Methode basierend auf Wright et al. 2008; 2010; 2012).

Die Chlorophyllfluoreszenz ist ein Phänomen der Photosynthese. Der Anstieg von *Fo* als Reaktion auf zu niedrige O_2-Konzentrationen in der Lagerraumatmosphäre ist mit respiratorischen Veränderungen in der Apfelfrucht verbunden (Gasser et al. 2008; 2010) und stellt somit ein indirektes Messverfahren dar. Es ist physiologisch unklar, warum *Fo* in Folge von O_2-Mangel ansteigt. Es wird ein Zusammenhang zwischen aerober Atmung (Mitochondrien), Fermentation (Cytosol) und Fluoreszenz (Chloroplasten) diskutiert (Wright et al. 2012). Wenn sich anaerobe Zustände entwickeln, lösen Pflanzen Signalwege aus und aktivieren fermentative Enzyme, um durch die alkoholische Gärung auch bei O_2-Mangel Energie zur Aufrechterhaltung der Lebenstätigkeit bereitstellen zu können (Pesis 2005; van Maris et al. 2006). In der Glycolyse wird Glucose zu Pyruvat abgebaut, wobei Nicotinamidadenindinukleotid (NAD^+) als Cofaktor fungiert und zu NADH + H^+ reduziert wird. Hierdurch werden zwei Moleküle Adenosintriphosphat (ATP) bereitgestellt. Es kommt zur Anreicherung von fermentativen Verbindungen, da Pyruvat zu Ethanol und CO_2 decarboxyliert wird. Während der Glykolyse wird NAD^+ regeneriert (Müller-Esterl et al. 2017). Unter O_2-Mangel könnte es aufgrund der Blockierung des Elektronenflusses zu einer Anreicherung von glykolytischen Reduktionsmittel (wahrscheinlich NADH) in den Mitochondrien und im Cytosol kommen. Hierdurch wird wahrscheinlich der Citrat-Zyklus gehemmt (Yang et al. 2014). Das überschüssige Reduktionsmittel könnte in die Chloroplasten transportiert werden (ob aktiv oder passiv ist unbekannt). Eine verstärke Ansammlung könnte zu einer übermäßigen Reduzierung des PQ-Pools führen. Folglich würde es zu einem Anstieg von *Fo* kommen (Wright et al. 2011; 2012; 2015). Des Weiteren wird diskutiert, ob die Akkumulierung von Fermentationsprodukten die Zellmembran oder die Organellen beeinflussen könnten und somit indirekt die Fluoreszenzausbeute verändert wird (Ke et al. 1994; Prange et al. 2005; Baker 2008; Wright et al. 2011). In die Diskussion wird auch der Xanthophyll-Zyklus eingebunden. Unter normalen atmosphärischen Sauerstoffbedingungen wird Zeaxanthin durch das Enzym Zeaxanthin-Epoxidase in Antheraxanthin umgewandelt. Diese Umwandlung kann bei O_2-Mangel gehemmt werden, wobei überschüssige Energie durch erhöhte Chlorophyllfluoreszenz abführt werden könnte (Wright et al. 2011; 2012).

2 Zielsetzung

Die Verwendung der Chlorophyllfluoreszenz für die Messung von Pflanzenstress wie beispielsweise Trocken-, Salz- oder Hitzestress hat sich in der landwirtschaftlichen und gartenbaulichen Forschung etabliert (Baker 2008; Matyssek und Herpprich 2019). In der Apfellagerung wird diese Methode zur Detektion von Stress aufgrund von zu niedrigen O_2-Konzentrationen in der Lagerraumatmosphäre verwendet, insbesondere für die Identifizierung des Lower Oxygen Limit (LOL) (Prange et al. 2013). In den vergangenen Jahren wurde beobachtet, dass einzelne Äpfel gleicher Sorte und Herkunft bei völlig identischen Lagerungsbedingungen ein unterschiedliches Stressverhalten hinsichtlich niedriger O_2-Konzentrationen gezeigt haben (Schlie et al. 2020). Die Ursachen hierfür sind weitestgehend unbekannt. Daher ist auch die Auswahl einer repräsentativen Fruchtprobe für die Chlorophyllfluoreszenzmessung erschwert, da in der Apfellagerung nur an wenigen Apfelfrüchten stellvertretend für den gesamten Lagerraumbestand die Fluoreszenz gemessen wird (Wright et al. 2012; Prange et al. 2013). Ferner werden in der Apfellagerung gegenwärtig nur nicht-bildgebende Chlorophyllfluoreszenz-Messverfahren eingesetzt. Die bildgebende Chlorophyllfluoreszenz liefert einen höheren Informationsgewinn gegenüber den nicht-bildgebenden Fluoreszenzmessverfahren (Lichtenthaler et al. 2005). Hierdurch besteht eine Wissenslücke, da nur wenige Erkenntnisse über die Fluoreszenzheterogenität auf der Apfelschale sowie zwischen einzelnen Apfelfrüchten bekannt sind. Des Weiteren ist die räumliche Verteilung der Chlorophyllfluoreszenz auf der Apfelschale hinsichtlich verschiedener Fluoreszenzparameter nicht beschrieben. Daher wird die Chlorophyllfluoreszenz an Apfelfrüchten mit einem bildgebenden Fluoreszenzmessverfahren untersucht. Die Untersuchungen werden in situ durchzuführt, um die Stressreaktion der Apfelfrüchte aufgrund von zu niedrigen O_2-Konzentrationen in der Lagerraumatmosphäre unter praxisnahen Bedingungen dokumentieren zu können.

Um das Potenzial der Chlorophyllfluoreszenz als sensorischer Parameter in der Apfellagerung zu untersuchen und weiterzuentwickeln, werden in dieser Arbeit folgende Zielsetzungen verfolgt:

I. Die Ursachen bezüglich des unterschiedlichen Stressverhaltens von einzelnen Äpfeln hinsichtlich niedriger O_2-Konzentrationen in der Lagerraumatmosphäre soll gefunden werden. Wenn diese Ursachen bekannt sind, soll es möglich sein, eine repräsentative Fruchtprobe für die Fluoreszenzmessung auszuwählen oder nur Früchte mit gleichem Stressverhalten zusammen zu lagern.

II. Die Fluoreszenzheterogenität auf bzw. zwischen einzelnen Apfelfrüchten sowie die räumliche Verteilung der Chlorophyllfluoreszenz auf der Apfelschale soll mit einem bildgebenden Fluoreszenzmessverfahren untersucht werden.

III. Der Anstieg der Grundfluoreszenz (*Fo*) auf der Apfelschale infolge von Stress, aufgrund von zu niedrigen O_2-Konzentrationen in der Lagerraumatmosphäre, soll visualisiert werden. Darüber hinaus soll eine Methode für die Identifizierung des LOL mit einem bildgebenden Fluoreszenzmessverfahren entwickelt werden.

IV. Die Reaktion der Chlorophyllfluoreszenzkinetik hinsichtlich niedriger O_2-Konzentrationen in der Lagerraumatmosphäre soll untersucht werden. In diesem Zusammenhang stellt sich die Frage, ob neben *Fo* auch weitere Parameter zur Erkennung von Stress bei Äpfeln verwendet werden können.

3 Publikationen

3.1 The Impact of Fruit Ripeness on the Lower Oxygen Limit, Chlorophyll Fluorescence and Fermentation Behavior in Apples

Research Paper

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CRediT author statement:

*Tim-Pascal Schlie***:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Visualization. *Thomas Rath*: Conceptualization, Validation, Formal analysis, Investigation, Writing - Review and Editing. *Dirk Köpcke:* Validation, Resources, Methodology, Validation, Writing - Review and Editing. *Werner Dierend*: Resources, Writing - Review and Editing, Methodology, Validation, Project administration, Funding acquisition.

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ORIGINAL ARTICLE / ORIGINALBEITRAG

The Impact of Fruit Ripeness on the Lower Oxygen Limit, Chlorophyll Fluorescence and Fermentation Behavior in Apples

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Abstract
Dynamic Controlled Atmosphere-Chlorophyll Fluorescence storage (DCA-CF) uses a fluorescence-based measurement method to detect fermentation in apples (*Malus* × *domestica* BORKH.) caused by low-oxygen levels at an early stage. In recent years, it has been observed that individual apples of the same variety and origin can exhibit different fermentation behavior when stored under completely identical conditions. The causes of the different fermentation behavior must be found in order to be able to use DCA storage optimally. This study aimed to find the causes of the different fermentation behaviors of individual apples. Our results show that fruit ripeness can affect the lower oxygen limit (LOL), especially immediately after harvest, when the starch degradation in the fruit is not yet complete. A significant increase in the LOL was observed in 'Elstar' (2020: 0.3 kPa, 0.6 kPa, 0.9 kPa; 2021: 0.3 kPa, 0.4 kPa, 0.6 kPa). 'Braeburn' also exhibited this behavior regarding the LOL at a lower level. The LOL could not be identified for some of the fruit (varying from 12.5% to 41.7% of the examined apples) previously stored in Ultra Low Oxygen (ULO) storage for 4 months. Also, the chlorophyll content in the apple skin influences the fluorescence measurement method. Within 2 weeks, the chlorophyll content in the apple skin was halved. If the chlorophyll content drops, the reliability of the fluorescence measurement also decreases. It turned out that apples with an $Fv/Fm < 0.7$ were unsuitable for valid LOL identification.

Keywords 'Braeburn' · Dynamic controlled atmosphere · 'Elstar' · Mini-Apple-PAM · Storage

Introduction

Dynamic Controlled Atmosphere (DCA) is increasingly used in modern long-term storage of apples (*Malus* × *domestica* BORKH.) because of proven benefits to fruit quality maintenance and shelf life. With DCA storage, the oxygen level is dynamically adjusted and set to values of ≤ 1 kPa O_2 in the long term (Zanella et al. 2008; Gasser et al. 2008). However, there is a risk of fermentation at oxygen levels of ≤ 1 kPa O_2 because the apples are stored close to the lower oxygen limit (LOL). The LOL represents the oxygen level that is still tolerated by the apple without developing disorders of fermentation (Prange et al. 2002; Wright et al. 2008, 2012). One option to monitor the fruit's fermentation behavior is the measurement of chlorophyll fluorescence (Prange et al. 2002; Wright et al. 2015). Other ways to identify the LOL are by determining fermentation products in the fruit and measuring the respiration quotient (RQ) (Thewes et al. 2020). The fluorescence parameter minimum fluorescence (*Fo*) is a sensitive indicator for low-oxygen stress. For this purpose, a representative fruit sample is selected on which the fluorescence is measured (DeLong et al. 2004; Prange et al. 2013). The fluorescence increases when the oxygen level drops below the LOL (Harris and Heber 1993; Wright et al. 2008). The cell metabolism changes from aerobic respiration to fermentation, which also changes photosynthetic activity in the fruit skin (chloroplasts) (Wright et al. 2010, 2012). The fermentative metabolism is thus made visible via an indirect method. In recent years, it has been observed in research and practice that individual apples of the same variety and origin can exhibit different fermentation behavior

Data Availability Statements The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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Table 1 Overview of the different harvest dates

Variety	Harvest 2020		Harvest 2021	
'Elstar'	(1)	01. Sep	(1)	6th Sep
	(2)	16. Sep	(2)	20th Sep
	(3)	28. Sep	(3)	4th Oct
'Braeburn'	(1)	22. Sep	(1)	27th Sep
	(2)	05. Oct	(2)	11th Oct
	(3)	19. Oct	(3)	25th Oct

when stored under completely identical conditions. This makes it difficult to select a representative sample for the fluorescence measurement (Schlie et al. 2020). Against this background, the aim of this study was to find the causes for the different fermentation behavior of individual apples. If these causes are known, it should be possible to select a representative sample or to store only fruits with the same fermentation behavior together.

Materials and Methods

Plant Materials

The German major variety 'Elstar, PCP' and the 1-methylcyclopropene (1-MCP) incompatible variety 'Braeburn, Mariri Red' were used for the investigations. The apple trees were grafted on M 9 rootstocks and grown in the experimental orchards of Fruit Research Center Jork, Germany (53° 31′ N, 9° 44′ E). The trees were planted in 2013 ('Elstar') and 2005 ('Braeburn'). The plant space was 3.50 m x 1.00 m. The apples were harvested on three different harvest dates listed in Table 1. Apples of 10 trees from a plot were harvested for each harvest date. Only the best quality fruits were selected. The harvest date was determined using the ripeness parameters firmness, starch index and total soluble solids (TSS) (further information see Sects. "Statistical Analysis" and "Different Harvest Dates") and the ripeness forecast from the Fruit Research Center in Jork. The 'Elstar' fruits were harvested with a fruit size of 65–75 mm (harvest date 1) and 70–80 mm (harvest date 2 and 3). The fruit size of 'Braeburn' was 65–75 mm (harvest date 1–3). The skin color of both varieties had a red content between 50% and 75% (manual selection). One part of the apples was briefly stored under cold storage (2 °C; ± 0.5 °C) until the experiments started. Another part of the apples was stored under ultra-low oxygen (ULO) (1.2 kPa O_2, < 0.1 kPa CO_2, 2 °C ± 0.5 °C).

Chlorophyll Fluorescence and LOL

The chlorophyll fluorescence measurements were performed using the Mini-Apple-PAM™ system (Walz, Effeltrich, Germany) based on pulse amplitude modulation (PAM) technology. The Mini-Apple-PAM™ system is able to measure the fluorescence of individual fruits (sensor-samples distance < 0.5 cm). A red light (λ_{max} = 625 nm; 0.1 µmol $m^{-2} s^{-1}$) was used to excite *Fo*. The sensor is located next to the red LED. The fluorescence parameter *Fo* was used to identify the LOL. *Fo* values were normalized (Wright et al. 2008). The *Fo* baseline was previously recorded by measuring Fo at oxygen levels of ≥ 1.5 kPa. The Fo baseline is needed to identify the increase in fluorescence clearly. The rise of *Fo* deviating from the Fo baseline made the LOL visible (Wright et al. 2012). Furthermore, a saturating pulse (max. 4000 µmol $m^{-2} s^{-1}$) was applied to determine maximum fluorescence (*Fm*). *Fm* was needed to calculate the maximum quantum yield of photosystem II (*Fv/Fm* = *(Fm – Fo) /Fm*) (Maxwell and Johnson 2000). Before the fluorescence measurements started, the apples were dark-adapted for 30 min. The fluorescence was measured at the equator of the fruit where the green-yellow ground color was dominant.

Experimental Design

The investigations were carried out in a storage room (2.5 °C; ± 0.5 °C). The Mini-Apple-PAM™ system was integrated into a gas-tight stainless steel box (140 cm × 60 cm × 50 cm), shown in Fig. 1. A total of 96 measurement points were available. These were distributed over four layers (block layout). A fan and a line system made of PVC tubes were integrated to ensure good airflow. In this way, a uniform air flow was ensured and it was verified that there was a uniform oxygen distribution in the stainless steel box by measuring the oxygen content at different levels (data not shown). The air atmosphere was reduced by supplying nitrogen (purity 99.8%). In the first step, the oxygen was reduced to about 3 kPa within 15 min. In the second step, the oxygen was reduced from 3 kPa to 0 kPa over 4–6 days. The oxygen concentration in the storage box was mea-

Table 2 The test variants of experiment A and the time of the *lower oxygen limit (LOL) test runs*

Variety	Variant	Cold storage LOL test run after harvest	ULO storage LOL test run after harvest
'Elstar'	Harvest date 1	1 Week	4 Months
	Harvest date 2 (control)	1 Week	4 Months
	Harvest date 3	1 Week	4 Months
'Braeburn'	Harvest date 1	3 Weeks	4 Months
	Harvest date 2 (control)	3 Weeks	4 Months
	Harvest date 3	3 Weeks	4 Months

ULO ultra-low oxygen

The Impact of Fruit Ripeness on the Lower Oxygen Limit, Chlorophyll Fluorescence and Fermentation Behavior in Apples

Fig. 1 The setup of the experiments with 96 fluorescence measuring points on four layers (**a**); Mini-Apple-PAM measurement device (**b**); Mini-Apple-PAM individual fruit measurement (**c**)

sured with the oxygen analyzer SCS 310 (Storage Control System, Paddock Wood, United Kingdom) (repeatability ± 0.02 kPa). The CO_2 concentration in the storage box was regulated using $Ca(OH)_2$ (1 kg) to values of < 0.1 kPa and checked with the CO_2 analyzer ICA 320 (Storage Control System, Paddock Wood, United Kingdom) (repeatability ± 0.1 kPa).

The experiments were split into two sections and carried out in several *LOL test runs*. Experiment A aimed to identify the LOL shortly after harvest and after several months of ULO storage ($n = 96$). The variants are shown in Table 2. The LOL test run took place 1 week after harvest for 'Elstar' and 3 weeks after harvest for 'Braeburn' (3 weeks of cooling before oxygen reduction) (Köpcke 2007).

Experiment B: Table 3 shows the test variants of experiment B. Regarding fermentation behavior and LOL, two different dates to start cold storage were examined. ($n = 48$). The *control* was stored under regular air (2 °C; ± 0.5 °C) immediately after harvest. The *delayed cold storage* variant was exposed to room temperature (~20 °C) for 12 days. After that, the apples were stored like the control. Both variants were examined in one *LOL test run*, so the samples were exposed to low-oxygen conditions for the same amount of time. In this way, the fruit's own fermentation products were more comparable.

Table 3 The test variants of experiment B and the time of the lower oxygen limit (LOL) test runs

Variety	Variant	Start cold storage	LOL test run after harvest	
			(2020/21)	(2021/22)
'Elstar'	Control	Immediately	5 Weeks	8 Weeks
	Delayed cold storage	12 days after harvest	5 Weeks	8 Weeks
'Braeburn'	Control	Immediately	8 Weeks	8 Weeks
	Delayed cold storage	12 days after harvest	8 Weeks	8 Weeks

Fruit Ripeness Measurements and Fermentation Analysis

The fruit ripeness was determined immediately after harvest ($n = 40$) and after each *LOL test run* (experiment A: $n = 96$; experiment B: $n = 48$). Some of the ripeness parameters are also quality parameters but are generally referred to as ripeness parameters in this study. The following parameters were examined. The firmness (kg cm^{-2}) was measured on each fruit using the Fruit Texture Analyzer (Güss, Cape Town, South Africa). The measurements were taken at the fruit's equator on the transition zone from the green to red side of the fruit. Furthermore, after the *LOL test runs*, the firmness was also measured at the chlorophyll fluorescence measuring zone. The starch index (1–10) was assessed using the CTIFL scale (CTIFL = Center Technique Interprofessionnel des Fruits et Légumes; France) using iodine-potassium iodide (Vaysse 2002). The TSS (°Brix)

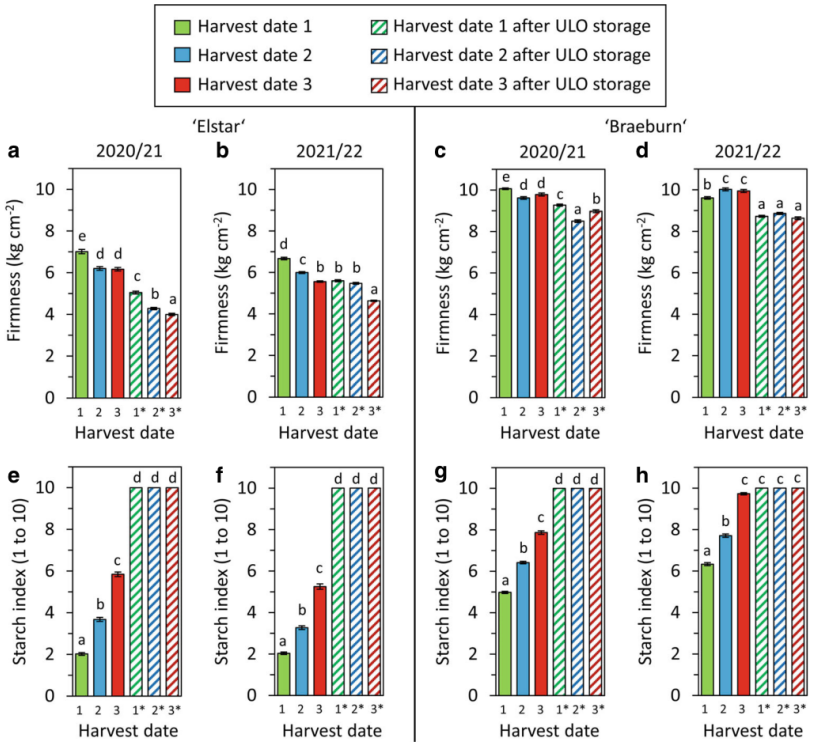

Fig. 2 Firmness (**a** to **d**) and starch index (**e** to **h**) of 'Elstar' and 'Braeburn' at the time of lower oxygen limit test runs in the season 2020/21 and 2021/22; Standard error ($p < 0.05$; $n = 96$). *ULO* Ultra Low Oxygen

were measured by refractometer PCE-DRW 2 (PCE Instruments, Meschede, Germany). Titratable acidity (TA; g L^{-1}) was measured by TitroLine alpha plus (Schott Instruments, Mainz, Germany) of the juiced and filtered sample with 0.1 N NaOH to an endpoint of pH 8.1 (1 ml consumed 0.1 N NaOH corresponded to 0.67 g L^{-1} malic acid). TA was evaluated block by block as a mixed sample ($n = 4$). The chlorophyll content was measured with a pigment analyzer (Control in Applied Physiology, Falkensee, Germany) on each individual fruit and reported as the Normalized Difference Vegetation Index (NDVI). The chlorophyll content was measured at the zone on the fruit, where later on, the chlorophyll fluorescence was measured during the *LOL test run*. The CLARUS 500 gas chromatography with headspace (Perkin-Elmer, Waltham, MA, USA) was used to perform the fermentation analysis. The filtered fruit juice (1 ml) was used to measure the fermentation products (acetaldehyde, ethanol). The measurements of fermentation products were carried block by block as a mixed sample ($n = 4$).

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics (Version 26) (Chicago, IL, USA) and Microsoft Excel 2016 (Redmond, WA, USA). The interval scaled data were analyzed by analysis of variance (ANOVA) and Bonferroni test ($p < 0.05$). The Levene test checked the equal variances. If equal variances could not be assumed, a Welch-ANOVA was performed, followed by a Games–Howell test. The ordinal scaled data were analyzed by the Kruskal-Wallis test ($p < 0.05$). The block mean data were used to calculate Spearman-Rho's rank correlation.

Results

Different Harvest Dates

Table 4 shows the fruit ripeness of the apples immediately after harvest in 2020 and 2021. The three harvest dates differed essentially in terms of the starch index and TA. The firmness of 'Elstar' decreased with each subsequent harvest date, while the firmness of 'Braeburn' remained at a stable level.

There was a certain amount of time between the harvest and the start of the experiments (see Sect. "Experimental Design"). This led to a further development of fruit ripening. Figure 2 shows the firmness (a to d) and starch index (e to h) of the varieties 'Elstar' and 'Braeburn', which were

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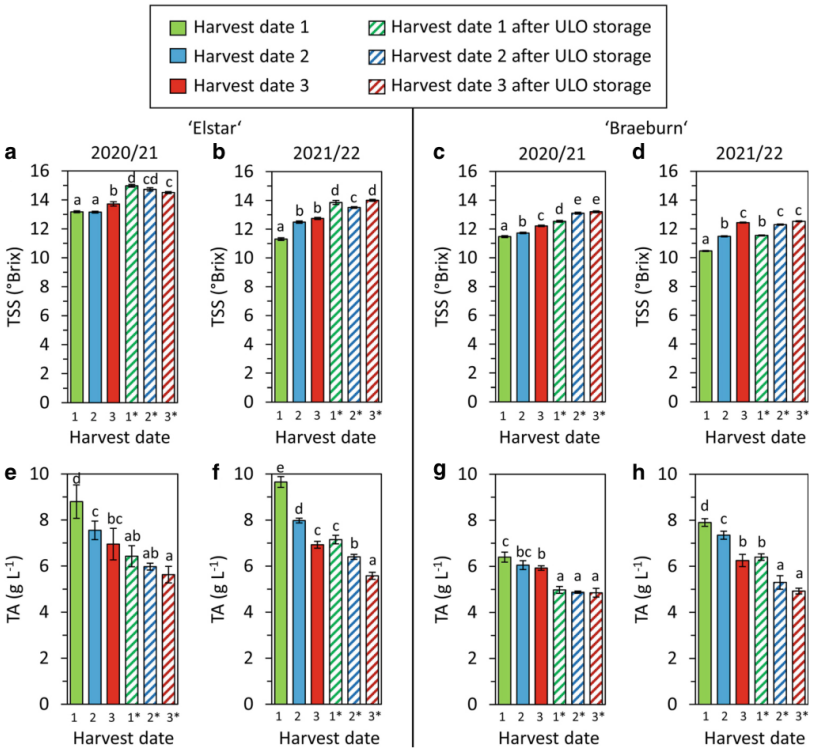

Fig. 3 TSS (**a** to **d**) and TA (**e** to **h**) of 'Elstar' and 'Braeburn' at the time of LOL test runs in the season 2020/21 and 2021/22; Standard error ($p < 0.05$; $n = 96$ (**a**-**d**), $n = 4$ (**e** to **h**)); *ULO* Ultra Low Oxygen

measured after the *LOL test run*. The firmness of 'Elstar' was 7 kg cm^{-2} and 4 kg cm^{-2}. The ULO 'Elstar' had the least firmness at the time of the LOL test runs (Fig. 2a,b). The firmness of 'Braeburn' was comparatively high, from 10.1 kg cm^{-2} to 9.6 kg cm^{-2}. The apples from the ULO storage showed less firmness but not less than 8.6 kg cm^{-2} (harvest date 3 after ULO; 2021/22) (Fig. 2c,d). The starch indices of the three harvest dates, which were examined close to the harvest, differed significantly. The apples from the ULO storage were starch-free at the time of the LOL test runs (e to h).

Table 4 Fruit ripeness at harvest date of 'Elstar' and 'Braeburn' in 2020 and 2021; firmness, starch index, total soluble solids (TSS; $n = 40$) and titratable acidity (TA; $n = 4$)

Variety	Season	Harvest date	Firmness (kg cm^{-2})	Starch index (1 to 10)	TSS (°Brix)	TA (g L^{-1})
'Elstar'	2020	1	7.6	1.2	13.7	10.1
		2	6.9	2.7	13.5	7.5
		3	6.4	4.5	13.9	6.6
	2021	1	6.9	1.1	11.3	10.3
		2	6.1	2.7	11.5	7.7
		3	5.7	4.3	12.6	7.4
'Braeburn'	2020	1	10.4	1.6	9.8	6.5
		2	10.1	3.3	10.6	6.2
		3	10.1	5.0	11.5	6.0
	2021	1	10.2	1.7	9.5	8.2
		2	10.6	3.9	10.5	7.4
		3	10.2	5.5	11.5	7.0

Figure 3 shows the TSS (a to d) and TA (e to h) of 'Elstar' and 'Braeburn' after the *LOL test runs*. Overall, higher TSS were measured for both varieties in the 2020/21 season than in the second year of the study. The TA was highest on harvest date 1 and decreased with each subsequent harvest date. The acid degradation continued in the ULO storage. The apples in the 2021/22 season tended to show higher TA than the first year of the study. The significant differences can be seen in Fig. 4.

Figure 4 (a to h) shows the results of LOL test runs of the three harvest dates and ULO storage. The LOL of 'Elstar' (2020) increased from 0.25 kPa O_2 to 0.6 kPa O_2 within 2 weeks and up to 0.9 kPa O_2 at harvest date 3. This gradual increase was also observed in 2021. The increases in LOL were significant in both years (Fig. 4a,b). The LOL of 'Braeburn' was identified after 3 weeks of pre-cooling under normal atmosphere cold storage. In 2020, the LOL of 0.27 kPa O_2 (harvest date 1), 0.33 kPa O_2 (harvest date 2)

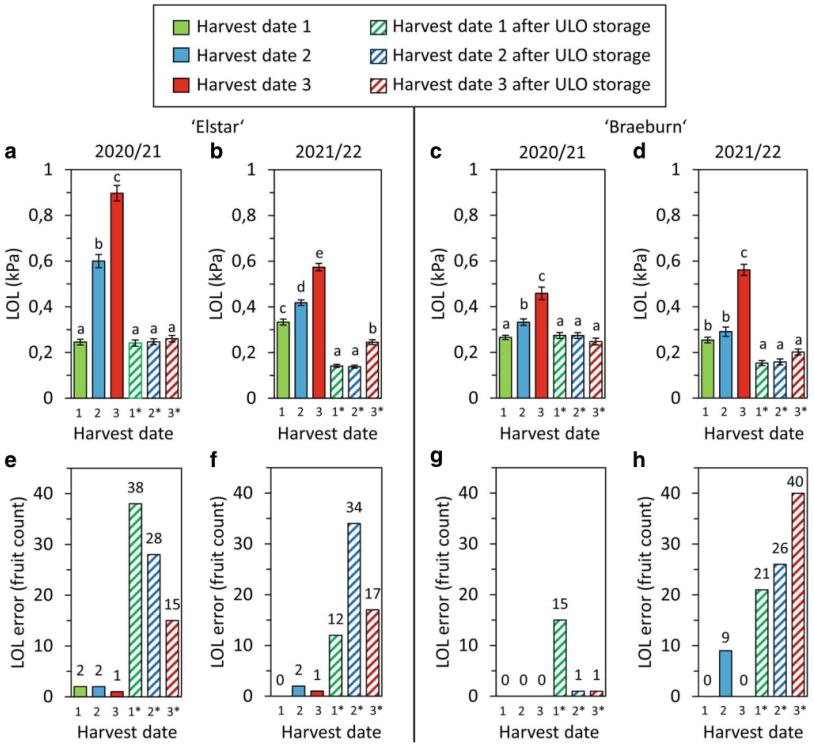

Fig. 4 The Lower Oxygen Limit (LOL) (**a** to **d**) and LOL error cases (**e** to **h**) of 'Elstar' and 'Braeburn' at three harvest dates and after 4 months of ULO storage in the season 2020/21 and 2021/22; Standard error ($p < 0.05$; $n = 96$ minus LOL error cases). *ULO* Ultra Low Oxygen

and 0.46 kPa O_2 (harvest date 3) were identified. In 2021, the LOL of harvest dates 1 and 2 ("Braeburn") did not differ significantly (Fig. 4c,d). LOLs from 0.2 kPa O2 to 0.3 kPa O2 were identified in the ULO-stored apples. However, it should be noted that there were failures (LOL error cases), especially with the ULO apples. Figure 4 (f to h) shows the number of fruits where no LOL could be identified. 'Elstar' failure ranged from 15.6% to 39.6% (2020/21) and 12.5% to 35.4% (2021/22) of the examined apples. A higher failure rate at 'Braeburn' (ULO) was seen in the first study year at harvest date 1 (16%) and in the second year (21.9% to 41.7% of the examined apples).

Figure 5 shows the chlorophyll content and *Fv/Fm* in the absence of low-oxygen stress (≥ 1.5 kPa). The chlorophyll content was measured at the point where the chlorophyll fluorescence had previously been measured. While an NDVI of 0.63 was measured on harvest date 1 (2020; 'Elstar'), the chlorophyll content decrease significantly to 0.3 within just 2 weeks (harvest date 2). In 2021, the chlorophyll content decreased significantly from 0.58 (harvest date 1) to 0.35 (harvest date 2). NDVI of –0.17 (2020) and 0.01 (2021) were measured in apples of harvest date 3. This rapid chlorophyll degradation in the fruit skin could also be observed in 'Braeburn' (2020: 0.61; 0.34; 0.17; 2021: 0.56; 0.26; 0.12). The decrease in chlorophyll content was significant. Only harvest date 3 ('Elstar', 2020) showed a NDVI of –0.17 after harvest and did not differ significantly from ULO apples (–0.18). Apples examined 1 week after harvest showed *Fv/Fm* of 0.76 to 0.70 (in the absence of low-oxygen stress). 'Elstar' and 'Braeburn' of ULO storage showed significantly lower *Fv/Fm* of 0.56 to 0.55.

Delayed Cold Storage

With the delayed cold storage variant, advanced fruit ripening could be provoked. Table 5 shows the results of experiment B. The delayed cold storage resulted in differences in ripeness and quality. In particular, the differences were achieved in terms of firmness and TA. In both varieties, there are significant differences in the LOL, and *Fv/Fm* between the two variants examined. The delayed cold storage variant had a high numbers of apples where the LOL could not be identified (up to 35% of the examined fruits).

The two variants were tested in the same *LOL test run*. Because the fruits were treated in exactly the same low-oxygen stress atmosphere, the level of fermentation products was comparable (Table 6). The information on fermentation products must be considered in the context of

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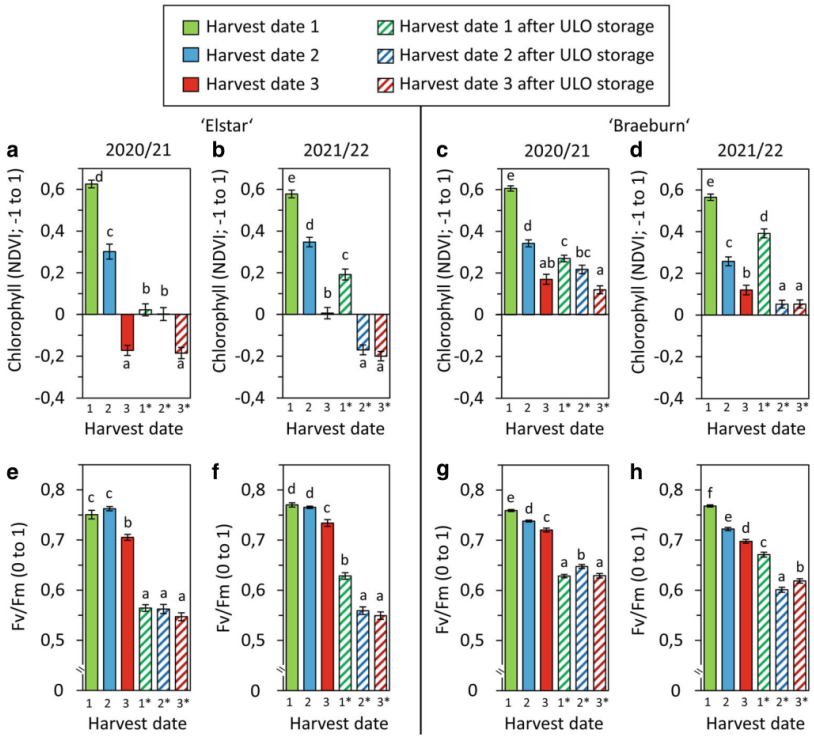

Fig. 5 Chlorophyll content as Normalized Difference Vegetation Index (NDVI) (**a** to **d**) and *Fv/Fm* in the absence of low-oxygen stress (≥ 1.5 kPa) (**e** to **h**) of 'Elstar' and 'Braeburn' at the time of LOL test runs in the season 2020/21 and 2021/22; Standard error ($p < 0.05$; $n = 96$). *ULO* Ultra Low Oxygen

the specific storage time ≤ 1 kPa O_2. Although both variants were exposed to low-oxygen conditions simultaneously, the variant delayed cold storage always showed significantly higher values for ethanol. In 2020 ('Elstar'), the ethanol values were twice as high as in the control. In 2021, ('Elstar') the ethanol values were even 5.5 times higher than in the control. In the case of the 'Braeburn', the values in the 2 test years were 1.5 and 2 times higher than in the control. In contrast, there were no significant differences in the acetaldehyde level.

Relationship Between Ripeness and Fluorescence

Figure 6 shows the relationship between *Fv/Fm* and the ripeness parameters firmness (a), starch index (b), TTS (c) and TA (d) of 'Elstar' and 'Braeburn' (data from both

Table 5 Firmness, starch index, total soluble solids (TSS), titratable acidity (TA), lower oxygen limit (LOL), LOL eror cases and *Fv/Fm* of the variants control and delayed cold storage ('Elstar' and 'Braeburn') in 2020 and 2021; Different small letters indicate statistically significant differences in the column for each variety separately ($p < 0.05$) ($n = 48$), TA ($n = 4$)

Variety	Season	Variant	Firmness (kg cm^{-2})	Starch index (1 to 10)	TSS (°Brix)	TA ($g L^{-1}$)	LOL (kPa)	LOL error cases (count)	*Fv/Fm* (0 to 1)
'Elstar'	2020	Control	5.4 $_a$	8.0 $_a$	14.3 $_a$	6.9 $_b$	0.39 $_b$	6	0.68 $_b$
		Delayed cold storage	4.5 $_b$	9.7 $_b$	14.6 $_a$	6.2 $_a$	0.28 $_a$	17	0.63 $_a$
	2021	Control	5.6 $_a$	6.2 $_a$	13.5 $_a$	8.0 $_b$	0.24 $_a$	1	0.75 $_b$
		Delayed cold storage	5.6 $_a$	8.4 $_b$	14.1 $_b$	6.7 $_a$	0.30 $_b$	15	0.47 $_a$
'Braeburn'	2020	Control	9.3 $_b$	9.5 $_a$	12.2 $_a$	5.7 $_b$	0.51 $_a$	1	0.69 $_b$
		Delayed cold storage	8.0 $_a$	9.9 $_a$	12.6 $_a$	5.3 $_a$	0.77 $_b$	0	0.64 $_a$
	2021	Control	9.7 $_b$	9.5 $_a$	12.3 $_a$	6.1 $_b$	0.41 $_a$	6	0.66 $_b$
		Delayed cold storage	7.4 $_a$	9.9 $_a$	12.9 $_a$	5.5 $_a$	0.51 $_b$	2	0.56 $_a$

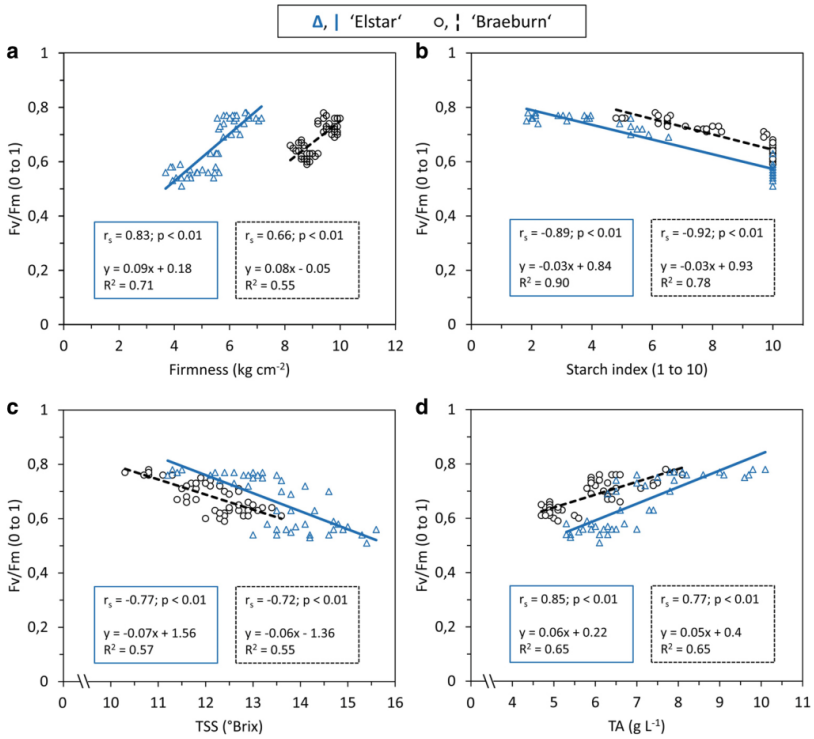

Fig. 6 Relationship between *Fv/Fm* and firmness (**a**), *Fv/Fm* and starch index (**b**), *Fv/Fm* and total soluble solids (*TSS*) (**c**), *Fv/Fm* and titratable acidity (*TA*) (**d**) using Spearman-Rho's rank correlation in 'Elstar' (*blue*) und 'Braeburn' (*black*). Correlation coefficient r_s, data represent means, $n = 48$ (*Fv/Fm* = maximum quantum yield of photosystem II)

Table 6 Fermentation products acetaldehyde and ethanol of the variants control and delayed cold storage ('Elstar' and 'Braeburn') after the lower oxygen limit test run in 2020 and 2021; Different small letters in the column indicate statistically significant differences ($p < 0.05$) ($n = 4$)

Variety	Season	Variant	Storage time 1 to 0 kPa O_2 (h)	Acetaldehyde (mg L^{-1})	Ethanol (mg L^{-1})
'Elstar'	2020	Control	43	4.4 $_a$	207.5 $_a$
		Delayed cold storage		6.9 $_a$	416.4 $_b$
	2021	Control	57	0.5 $_a$	13.0 $_a$
		Delayed cold storage		0.9 $_a$	72.0 $_b$
'Braeburn'	2020	Control	34	1.0 $_a$	34.2 $_a$
		Delayed cold storage		1.1 $_a$	53.9 $_b$
	2021	Control	80	0.3 $_a$	40.6 $_a$
		Delayed cold storage		0.5 $_a$	84.9 $_b$

study years). Firmness correlates significantly with *Fv/Fm* in both 'Elstar' ($r_s = 0.83$, $p < 0.01$) and 'Braeburn' ($r_s = 0.66$, $p < 0.01$). According to Cohen (1992), this is a strong effect. The firmness measured at the fluorescence measuring zone was used for the correlation analysis. The increase in starch index is negatively related to *Fv/Fm*. The correlation coefficient for 'Elstar' is $r_s = -0.89$ ($p < 0.01$) and for 'Braeburn' $r_s = -0.92$ (strong effect according to Cohen 1992). TSS also negatively correlated with *Fv/Fm* at 'Elstar' ($r_s = -0.77$, $p < 0.01$) and 'Braeburn' ($r_s = -0.72$, $p < 0.01$). The TA and *Fv/Fm* also correlated significantly in both varieties ('Elstar': $r_s = 0.85$, $p < 0.01$; 'Braeburn': $r_s = 0.77$, $p < 0.01$). According to Cohen (1992), these are also strong effects. In summary, the progressive ripeness of the fruits leads to a significant decrease in *Fv/Fm*.

Figure 7 shows the relationship between the fluorescence parameters *Fo* and the chlorophyll content (a) and between *Fv/Fm* and chlorophyll content (b). The degradation of the chlorophyll content in the apple skin has an impact on both, *Fo* and *Fv/Fm*. The chlorophyll content correlates significantly with *Fo* in 'Elstar' ($r_s = 0.83$, $p < 0.01$) and 'Braeburn' ($r_s = 0.51$, $p < 0.01$). Furthermore, low chlorophyll levels lead to low *Fv/Fm* values. The correlation coefficient for 'Elstar' is $r_s = 0.78$ ($p < 0.01$) and for 'Braeburn' $r_s = -0.77$

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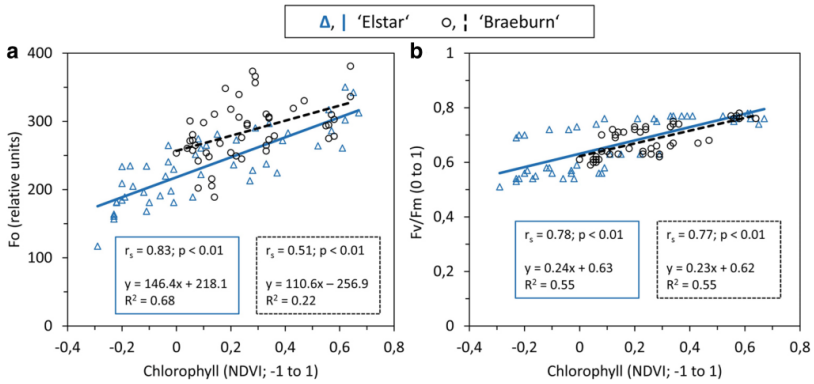

Fig. 7 Relationship between *Fo* and the chlorophyll content (**a**), *Fv/Fm* and the chlorophyll content (**b**) using Spearman-Rho's rank correlation; correlation coefficient r_s in 'Elstar' (*blue*) und 'Braeburn' (*black*). Data represent means, $n = 48$ (*Fo* = minimum fluorescence; *Fv/Fm* = maximum quantum yield of photosystem II)

($p < 0.01$). According to Cohen (1992), this are strong effects.

Discussion

In the recent history of fruit storage, chlorophyll fluorescence has been used as a sensory parameter to reveal physiological stress (Prange et al. 2013). The well-known fluorescence system in DCA storage is Harvest-Watch. With this system, six to eight fruits are measured with one sensor and the fluorescence is output as a normalized mean (Wright et al. 2008). The disadvantage is that no statement can be made about an individual fruit (Schlie et al. 2022). However, previous research indicated that there might be differences in fermentation behavior from fruit to fruit of the same variety and origin (Köpcke 2014; Schlie et al. 2020). One of the reasons for the different behavior of fruits could be the ripeness. Apples harvested immature with a starch index of around 1 (harvest date 1) showed a low LOL of 0.2 to 0.3 kPa O_2. However, if the harvest occurred 2 and 2 weeks later, the LOL increased significantly. Gasser et al. (2008) also observed differences in LOL at different harvest dates. Furthermore, Gasser and von Arx (2015) were able to show that the LOL was also dependent on the variety and could vary from year to year. Interestingly, the LOL increased in our study in the first few weeks of the post-harvest phase, in which the climacteric processes occur. This phase is characterized by physiological changes and increased metabolic activity, such as the increase in ethylene, hydrolysis of starch and the degradation of chlorophyll (Busatto et al. 2017; Paul et al. 2011; Wright et al. 2011). The increased metabolic activity likely resulted in a higher respiratory rate, leading to grown oxygen need and LOL (Wright et al. 2012). Furthermore, it cannot be ruled out that other factors could also have influenced the LOL, such as CO_2 gas diffusion through the fruit tissue (de Oliveira Anese et al. 2016) or the fruit's hormone balance (Pérez-Llorca et al. 2019). Whether a low LOL could be capped by early harvest with consistent DCA storage remains to be examined. Due to the increased fruit respiration, we suspect that capping the LOL in the climacteric phase is impossible. Using a dynamic oxygen level based on an online LOL answer could optimize DCA storage. However, there is a need for further research. The investigations with ULO apples showed that the LOL can fall during storage. Wright et al. (2012) suggest that apples may develop some adaptation to low-oxygen levels during long-term storage. However, the LOL data of the ULO apples from the present study should be interpreted with caution. The LOL could not be identified for part of the fruit. There were essentially two reasons for this. The first reason was fluctuating *Fo* signals at oxygen levels of ≥ 1.5 kPa O_2. However, reliable identification of the LOL requires a clear *Fo*-baseline as a reference (Wright et al. 2012). The second reason was that the *Fo* signal did not increase despite oxygen conditions of 0 kPa O_2. Fermentation products were detected in subsequent analyses of the affected individual fruits (data not shown). Weak or faulty fluorescence signals can indicate chlorophyll degradation or the beginning of senescence. The NDVI of 'Elstar' and 'Braeburn' was reduced by half within 2 weeks (harvest date 1 to 2). Some variants of 'Elstar' that were stored in the ULO even showed negative NDVI values. This indicates that the chlorophyll content has almost completely degraded because negative values imply water (Tucker and Sellers 1986). Identifying the LOL on individual fruits led to LOL error cases being noticed. The ripening of the fruit was explicitly promoted in the delayed cold storage variant. The delayed cold storage variant at 'Elstar' resulted in increased LOL error cases. In both varieties of the delayed cold storage variant, significantly higher ethanol levels were detected compared to the control. However, the apples were exposed to low-oxygen conditions simultaneously (individual storage time for each LOL test run). The treatment of room temperature before

cold storage accelerated the metabolism in the fruit, which also increased the accumulation of fermentation products.

In addition to *Fo*, the fluorescence parameter *Fv/Fm* was also recorded. *Fv/Fm* was determined at the beginning of the LOL test runs before low-oxygen stress was induced, so that the fruit's general stress perception could be determined. An unstressed plant leaf has an *Fv/Fm* value of 0.75 to 0.85 (Maxwell and Johnson 2000; Murchie and Lawson 2013). Song et al. (1997) found *Fv/Fm* values of 0.75 to 0.7 in unstressed apple fruits. Wright et al. (2012) measured on the sun-exposed side of 'Honeycrisp' a lower *Fv/Fm* (0.65) than on the shaded side (0.74). Our investigations found *Fv/Fm* values of 0.77 to 0.7 in apples examined 1 and 3 weeks after harvest, respectively. However, apples that had previously been in ULO storage for 4 months showed *Fv/Fm* values of <0.6 ('Elstar') and <0.7 ('Braeburn'), although low-oxygen stress was not present at the time of measurement. The view of the correlation analysis shows clearly a relationship between *Fv/Fm* and the chlorophyll content, as well as a correlation between *Fo* and the chlorophyll content. Furthermore, we were able to show that the various fruit ripening parameters (firmness, strength index, TSS, TA) correlate with *Fv/Fm*. Also, Song et al. (1997) were able to prove that there is a relationship between firmness and *Fv/Fm* as well as between starch index and *Fv/Fm*. Our results showed when chlorophyll degradation and fruit ripening had reached a critical point, the reliability of the fluorescence measurement became weaker and, thus, probably also the LOL. Therefore, we propose considering the parameter *Fv/Fm* when selecting a representative sample in the future. Apples with *Fv/Fm* < 0.7 should not be used for fluorescence measurement. The *Fv/Fm* parameter could be a helpful tool to detect the *LOL error cases* before DCA storage.

Conclusion

In the present study, the fermentation behavior of apples was described by measuring the LOL and the fruit's own fermentative products. Our results support the hypothesis that apples of the same variety and origin can have a different LOL with the same oxygen concentration in fruit storage. Based on individual fruit measurements, it was shown that the LOL was influenced by the fruit ripeness. In order to optimize DCA storage, only uniform fruits with the same ripeness should be stored together in one storage room. This also includes speedy harvesting and immediate storage of the apples. For implementation in large storage houses, this means more intensive coordination between farmers and storage house clerks. In addition, the chlorophyll content in the fruit skin was important. A low chlorophyll content led to weaker, sometimes fluctuating fluorescence signals, which had a massive impact on the reliability of the fluorescence method. In particular, in the case of apples with advanced fruit ripening, high numbers occurred in which the LOL could not be identified. Unfavorable fruit ripening limits the success of the fluorescence method. Another limitation of the fluorescence technique is that only a small number of representative apples in storage are used to measure chlorophyll fluorescence. Our results support the definition of what a representative sample might be. We propose not only to store apples with the same degree of ripeness under DCA storage but also to use the fluorescence parameter *Fv/Fm* as a standard value for selecting a representative sample. Apples with *Fv/Fm* < 0.7 should not be used for the fluorescence measurement.

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Author Contribution Tim-Pascal Schlie: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing—Original Draft, Visualization. Thomas Rath: Conceptualization, Validation, Formal analysis, Investigation, Writing—Review and Editing. Dirk Köpcke: Validation, Resources, Methodology, Validation, Writing—Review and Editing. Werner Dierend: Resources, Writing—Review and Editing, Methodology, Validation, Project administration, Funding acquisition.

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Conflict of interest T.-P. Schlie, T. Rath, D. Köpcke and W. Dierend declare that they have no competing interests.

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3.2 Detecting low-oxygen stress of stored apples using chlorophyll fluorescence imaging and histogram division

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Detecting low-oxygen stress of stored apples using chlorophyll fluorescence imaging and histogram division

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ABSTRACT

Currently, only non-imaging chlorophyll fluorescence measurements are used to identify the Lower Oxygen Limit (LOL) in Dynamic Controlled Atmosphere - Chlorophyll Fluorescence (DCA-CF) storage. The disadvantage of non-imaging fluorescence is that no statement can be made about the spatial heterogeneity of the sample. In contrast, chlorophyll fluorescence imaging can detect spatial heterogeneity of photosynthetic activity and has been established in research for some decades because the information benefit is higher. In this study, the chlorophyll fluorescence (F_o, F_m, F_v, F_v/F_m) of apples (*Malus* x *domestica*, BORKH.) was measured with a fluorescence imaging system in situ during storage. Intact apples of 'Braeburn' and 'Golden Delicious' were stored under low-oxygen stress conditions (< 1 kPa). The metabolic shift from aerobic to fermentative metabolism was made visible with the chlorophyll fluorescence imaging and was spatially localized on the sample. Furthermore, a method was developed to identify the LOL based on the chlorophyll fluorescence imaging combined with the histogram division method. This method considers the heterogeneity of the fluorescence and bundles the measured F_o data as histograms. Our results showed that the fluorescence imaging combined with the histogram division method can be a powerful tool for identifying the LOL.

1. Introduction

After harvest, apples (*Malus* x *domestica* BORKH.) are often stored in Controlled Atmosphere (CA). Types of CA storage are Ultra Low Oxygen (ULO; O_2 level 1 – 2 kPa) and Dynamic Controlled Atmosphere (DCA; O_2 level $\leq$ 1 kPa). Temperature, O_2, CO_2 and relative humidity are the controlled environmental conditions (Saltveit, 2003; Dilley, 2010; Prange et al., 2013; Both et al., 2014; Köpcke, 2015; Bessemans et al., 2016). The optimal storage conditions vary depending on the cultivar, harvest date, storage time and geographical location where the fruit were grown (Dilley, 2010; Watkins and Nock, 2012; Köpcke, 2015). The fundamental task of storage is to preserve fruit quality between harvest date and consumption (Abbott, 1999; Harker et al., 2002; Thewes et al., 2015). Numerous studies have shown that fruit quality can be preserved better in DCA storage compared to CA/ULO storage. DCA stored apples had higher firmness and acidity, fewer physiological disorders, enhanced shelf-life and greater emission of specific volatiles (DeLong et al., 2007; Lafer, 2008; Zanella et al., 2008; Gasser et al., 2010; Aubert et al., 2015; Köpcke, 2015; Tran et al., 2015; Both et al., 2017; Thewes et al., 2017; Weber et al., 2020).

Knowledge of the Lower Oxygen Limit (LOL) is essential for DCA storage. The LOL represents the lowest oxygen level that is tolerated by the fruit without developing disorders (Wright et al., 2012; 2015). One of several options to identify the LOL is using chlorophyll fluorescence (DeEll et al., 1998; Prange et al., 2003; DeLong et al., 2004). Studies showed that the minimum fluorescence (F_o) is a sensitive parameter for detecting stress in plants (Harris and Heber, 1993; Prange et al., 2002; Wright et al., 2008). If the oxygen level drops below the LOL, the plant cells' metabolism changes from aerobic to fermentation. Apparently, there is a relationship between aerobic respiration (mitochondria), fermentation (cytosol) and the increase in fluorescence, which emanates from the chloroplasts and Photosystem (PS) II (Gasser et al., 2008, 2010; Wright et al., 2011, 2012). Low-oxygen stress leads to reductant accumulation, which results in an over-reduction of the plastoquinone (PQ)

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pool and an increase in F_o (Harris and Heber, 1993; Wright et al., 2010, 2012). Storage conditions without low-oxygen stress show a relatively constant F_o-baseline. When the oxygen level is below the LOL, the minimum fluorescence increases based on the biological response of stored fruit (Zanella et al., 2005; Burdon et al., 2008; Gasser et al., 2008; Wright et al., 2011). The LOL must be identified anew for each storage season because the LOL varies among varieties, harvest dates and agrometeorological conditions where the fruit are grown (Prange et al., 2010; Weber et al., 2015; Gasser and von Arx, 2015). In DCA storage, apples are stored above the LOL plus a safety range of 0.2–0.3 kPa O_2 (Wright et al., 2011; Köpcke, 2014; Gasser and von Arx, 2015).

Non-imaging fluorescence systems for agricultural products are already available (e.g. Harvest-Watch™, Mini-Apple-PAM™). Currently, Harvest-Watch™ (Satlantic Inc., Halifax, Nova Scotia, Canada) is the most frequently used system in DCA-CF storage in Europe (Prange et al., 2013; Boeckx, 2018). To generate minimum fluorescence (F_α = theoretical F_o), the Harvest-Watch™ fluorometer used Pulse Frequency Modulation (PFM) technology. Four red light-emitting diodes (λ_{max} = 635 nm; 0.1–10 µmol m^{-2} s^{-1}) are used to excite the fluorescence at a sensor sample distance of 10 cm. The fluorescence is measured on a sample of six to eight fruit (Prange et al., 2003; DeLong et al., 2004; Wright et al., 2008). Another system is the Mini-Apple-PAM™ system (Walz GmbH, Effeltrich, Germany) that is based on Pulse Amplitude Modulation (PAM) technology. The single fruit is placed directly in front of the sensor with a sensor sample distance of < 0.5 cm (Köpcke, 2014). The shortcoming of such non-imaging fluorescence systems is that the measurements provide fluorescence values without spatial reference to the sample (Lichtenthaler et al., 2005). The fluorescence values are often normalized (Wright et al., 2008). In contrast fluorescence imaging makes it possible to measure the fluorescence for several kilopixels of the examined fruit simultaneously. The information gain of fluorescence can therefore be many times higher. The spatial heterogeneity of the sample becomes visible (Lichtenthaler et al., 2005; Kurenda et al., 2014) and local disorders are recognized at an early stage (Lichtenthaler et al., 2012).

The objective of this study was therefore to get more knowledge about the fluorescence heterogeneity of apples during storage (2.5 °C; ± 0.5 °C). Therefore, we measured chlorophyll fluorescence parameters (F_o, F_m, F_v, F_v/F_m) of 'Braeburn' and 'Golden Delicious' apples using a fluorescence imaging system to visualize the spatial distribution of the fluorescence on the sample. The fluorescence measurements were carried out both with sufficient oxygen levels and with low oxygen stress conditions. The second aim of this paper was to develop a method to identify the LOL, taking into account the spatial distribution of the fluorescence. For this purpose, the fluorescence data were analyzed and transformed into histograms and we tried to identify the LOL by means of a histogram division method.

2. Materials and methods

2. 1. Plant materials

'Braeburn' and 'Golden Delicious' apples (grafted on M9 rootstocks) were grown in the experimental orchards of Fruit Research Center Jork, Germany (53° 31′ N, 9° 44′ E). The apples were produced in integrated fruit production and harvested in 2020 at the commercial picking date ('Braeburn' 5 Oct; 'Golden Delicious' 21 Sep). Immediately after harvest, the apples were stored in cold storage (2.5 °C; ± 0.5 °C) until the experiment started. Fruit ripeness and quality were examined by analysis of firmness, Total Soluble Solids (TSS), Titratable Acidity (TA) and starch index (all n = 40; Table 1) two to four days after the apples had cooled down. Fruits with a specific fruit size were considered for the experiments. Firmness (N) was measured using the Fruit Texture Analyzer (Güss, Cape Town, South Africa) at the transition zone between the sun and shade side of the fruit. TSS and TA were analyzed after apples were juiced and filtered. TSS (%) was measured by refractometer

Table 1
Fruit size (mm), firmness (N), starch index (1–10), TSS (%) and TA (g L^{-1}) of apples; average and standard deviation for both 'Braeburn' and 'Golden Delicious' apples are shown (all n = 40).

Variety	Fruit size (mm)	Firmness (N)	Starch Index (1 – 10)	TSS (%)	TA (g L^{-1})
'Braeburn'	65–75	98.5 ± 3.9	2.9 ± 0.7	11.3 ± 0.2	5.9 ± 0.3
'Golden Delicious'	75–85	78.2 ± 6.9	3.6 ± 0.6	13.5 ± 0.3	5.7 ± 0.3

PCE-DRW 2 (PCE Instruments, Meschede, Germany). TA was evaluated by the titration (Schott Instruments, Mainz, Germany) with 0.1 N NaOH until pH reached 8.1 (1 ml consumed 0.1 N NaOH equal to 0.67 g L^{-1} malic acid). The starch index was assessed using iodine-potassium iodide (Lugol iodine). After an exposure time of ten minutes, the starch index (1 – 10) was rated using the CTIFL scale (CTIFL = Center Technique Interprofessionnel des Fruits et Légumes; France). The 1 stands for no starch degraded (completely dark), and the 10 for interface free of starch (no coloring).

2. 2. Chlorophyll fluorescence imaging

Chlorophyll fluorescence imaging was performed using the Open FluorCAM 701 MF (Photon Systems Instruments, Brno, Czech Republic). The FluorCAM had four LED panels (light-emitting diodes) positioned at an angle of 45° to the sample plane. The lights framed the sample plane from four directions. Two types of illumination were used. The minimum fluorescence (F_o) was measured with orange-red light (λ_{max} = 621 nm; 0.1 µmol m^{-2} s^{-1}). The maximum fluorescence (F_m) was measured with white saturating light (max. 1 000 µmol m^{-2} s^{-1} at 420–650 nm). Similar LED panels were placed opposite to each other. A Charged Coupled Device (CCD) camera detected the chlorophyll fluorescence in series of images at 12-bit resolution in 512 × 512 pixels and 50 images per second. A far-red filter (695–770 nm) was used to block reflective light. The camera was justified vertical to the sample. The shutter opening time was set to 10 µs (PAM mode). The intensity of the measuring lights to the base plate plane (area of 9 cm × 9 cm) was checked with the LI-COR LI-190R PAR sensor (Lincoln, Nebraska, USA). The following fluorescence parameters were examined: minimum fluorescence (F_o; fluorescence intensity measured in the dark-adapted state, when all PS II reaction centers are open), maximum fluorescence (F_m; fluorescence intensity measured in the dark-adapted state during the application of a saturating pulse of light), variable fluorescence ($F_v = F_m - F_o$; fluorescence measured in the dark-adapted state, when non-photochemical processes are minimum) and maximum quantum yield of PS II photochemistry measured in the dark-adapted state (F_v/F_m) (Maxwell and Johnson, 2000; Baker, 2008). The fluorescence images are displayed in pseudo-colors, with the fluorescence intensity ranging from blue (low intensity) to red (high intensity). The color codes are given for each fluorescence parameter. The Open FluorCAM system was also described in Nedbal et al. (2000) and Kurenda et al. (2014).

2. 3. Experimental design

The FluorCAM was installed in a cold storage room (2.5 °C; ± 0.5 °C). A black plastic box (polystyrene; ≈ 28 L) in which the fruit was kept was placed under the FluorCAM. This box was locked gas-tight with a transparent acrylic glass lid (8 mm). The transmission loss of the acrylic glass pane was 6%, according to our experiments (data not shown). The fruit was placed directly under the CCD camera with a sample-camera distance of 10 cm. The oxygen content was reduced by adding nitrogen (purity 99.8%) and regulated with a mass flow controller. The oxygen concentration was measured with SCS 310 oxygen analyzer (Storage Control System, Paddock Wood, United Kingdom)

(Repeatability: ± 0.02 kPa). The oxygen data were recorded every minute via data logger. Calcium hydroxide ($Ca(OH)_2$) (250 g) was also stored in the box to keep CO_2 in the storage atmosphere at a low level [$Ca(OH)_2 + CO_2 \rightarrow CaCO_3 + H_2O$] ($CO_2$ was not recorded). In the first instance, fluorescence was measured under normoxia conditions in order to establish a pre-stress baseline. After that, low-oxygen stress was created up to an oxygen level of 0.1 kPa (± 0.02 kPa). The fluorescence measurements were carried out every 30 min, the oxygen level in the storage was measured simultaneously. Before starting the fluorescence measurements, the apples were adapted to the dark for 30 min. The fluorescence was measured on one side of the fruit with red and green color components ('Braeburn') or green and yellow components ('Golden Delicious'), respectively. The experiments were carried out with five replicates for each variety (n = 5). One repetition equals one fruit. To get more information about the setup see Fig. 1.

2. 4. Method of identifying the LOL

The fluorescence parameter F_o was used to identify the LOL (Harris and Heber, 1993; Prange et al., 2002). The fluorescence imaging enables the generation of fluorescence data for each picture element (pixel) of an image. Thus, several 10,000 individual fluorescence information are available for each image (Lichtenthaler et al., 2012). The data must be meaningfully summarized so that the heterogeneity of F_o can be taken into account. Therefore, the F_o data (relative units) of each fluorescence image at time t was analyzed and transformed into a histogram (H_t). To recognize differences between two fluorescence images, the histogram division was calculated (see also Wang et al., 2009). The data were distributed into k intervals with a constant width of 25 frequencies. The maximum number of histogram intervals was 40. This means that only F_o values up to a maximum of 1 000 (relative units) are taken into account.

$$\boldsymbol{H_t} = \begin{pmatrix} h_{t,\ k=1} \\ . \\ . \\ . \\ h_{t,\ k=40} \end{pmatrix} \quad (1)$$

H_t = histogram at time t.
k = histogram intervals.

In order to identify low-oxygen stress with the histogram division method, a reference is required that was measured in the pre-oxygen stress stage. The reference histogram (H^*) must be recalculated of each series of measurements. It results from the average of five fluorescence measurements in the pre-oxygen-stress stage:

$$H^* = \frac{1}{5}\sum_{t=1}^{5} H_t \quad (2)$$

H^*=reference histogram.
H_t=histogram at time t.

The histogram division (Δd_t) was calculated by dividing the nth histogram at time t by the reference histogram (H^*). The intervals of the nth histogram at time t are divided by those of the reference histogram with the same bandwidth. The values of the quotients were added up to Δd_t. If a histogram interval is not occupied, it has the value zero. If it is occupied, it has a positive value. Thus, k can never be a negative value. However, in order to prevent division with the denominator zero, the intervals of h^* are increased by $k + 1$.

$$\Delta d_t = \sum_{k=1}^{40} \frac{h_{t,\ k}}{h^*_{k+1}} \quad (3)$$

Δd_t= histogram division quotient at time t.
h_t= intervals of nth histogram at time t.
h^*= intervals of reference histogram.
k = histogram intervals.

In general, the increase of F_o causes the histogram intervals to shift to the right based on h^*. The increase in F_o also means that the previously unfilled intervals gain in importance. The histogram intervals that were previously small or unoccupied become larger. The previously large histogram intervals become smaller. The histogram shift to the right becomes visible with the histogram division method.

2. 5. Statistical analysis

The descriptive statistics (average, standard deviation, histogram) and all graphics were performed using Microsoft Excel 2016 (Redmond, Washington, USA). The chlorophyll fluorescence data and pseudo-color images were evaluated with the software FluorCAM 7 (Photon Systems Instruments, Brno, Czech Republic).

3. Results

3. 1. Degree of fluorescence heterogeneity

Fig. 2 shows the fluorescence parameters F_o, F_m, F_v, and F_v/F_m while the oxygen level decreases (storage temperature 2.5 °C; ± 0.5 °C). The gray areas of the diagrams represent the standard deviation (SD). F_o was constant at oxygen levels from 2 kPa to 1 kPa and increased at low oxygen levels (< 1 kPa). In contrast, F_m remained at a constant level regardless of the oxygen level. F_v and F_v/F_m decreased with reducing oxygen level. F_o, F_m and F_v of 'Braeburn' had constant SD of ± 33%, ± 30% and ± 31% for both oxygen levels above and below 1 kPa. The SD of the parameter F_v/F_m increased from ± 4.2% to ± 7.7% over the course of treatment. 'Golden Delicious' had SD of ± 29% (F_o) and ± 27% (F_m and F_v) for both oxygen levels above and below 1 kPa. The SD of the parameter F_v/F_m increased from ± 3% to ± 5.3%. The ratio of average to the SD for F_o, F_m and F_v was 3:1 (rounded down).

The differences in the levels of chlorophyll fluorescence (F_o, F_m, F_v and F_v/F_m) at oxygen levels of 20.2 kPa and 0.2 kPa are shown in Fig. 3. The oxygen level was gradually reduced over a period of 9.5 h. Pseudo-colors represent the fluorescence with the intensity ranging from blue (low intensity) to red (high intensity). The increase of F_o, the constancy of F_m and the decrease of F_v and F_v/F_m are shown clearly. The pseudo-color images show that the increase in F_o-fluorescence was not uniform over the entire fruit. The decrease in F_v/F_m was also not uniform. Overall, the higher fluorescence values appeared at the central area of the fruit. The images (Fig. 3; A to I) show the fluorescence (F_o,F_m, F_v, F_v/F_m) of one sample here as a representative example.

Fig. 1. The structure of the setup: The FluorCAM with four LED panels, CCD camera and apple fruit. The oxygen analyzer's shown at the bottom left side. The box was closed with an acrylic plate (not shown).

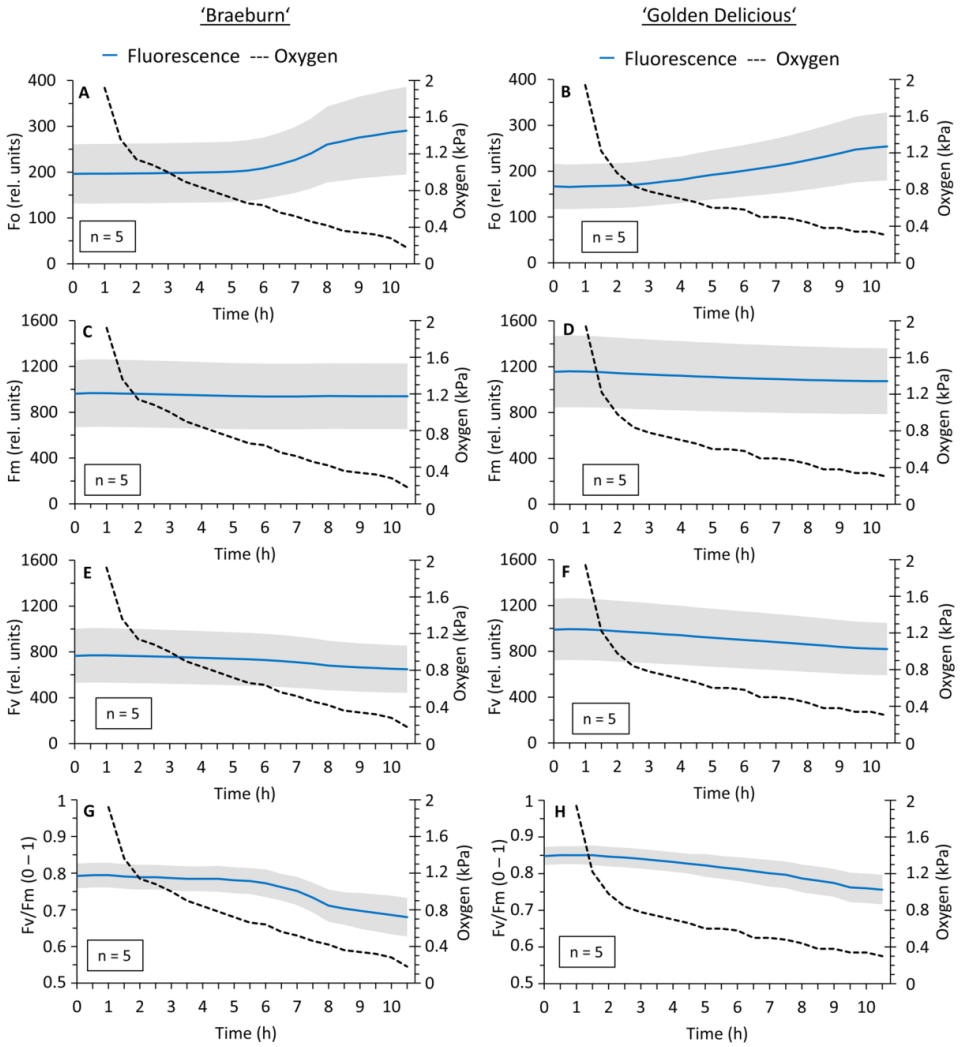

Fig. 2. The chlorophyll fluorescence parameters F_o (A; B), F_m (C; D), F_v (E; F), and F_v/F_m (G; H) are shown, the average as a solid line and the standard deviation as a gray area. The measurements were made during storage (2.5 °C; ± 0.5 °C) and with decreasing oxygen levels (dashed line) for both 'Braeburn' (left) and 'Golden Delicious' (right) (all n = 5).

3. 2. Histogram division method

The Calculation of Δd_t for histograms B to E (Fig. 4) equals values from 12 to 13 (B,12; C,12; D,12; E,13). No increase in F_o was measured at oxygen levels from 5 kPa to 0.7 kPa. Calculating Δd_t for histograms F to J shows an increase in Δd_t (F,18; G, 27; H, 317; I, 12412; J, 13186). The shift in the histograms (F to J) to the right becomes visible at oxygen values < 0.7 kPa. The previously small or unfilled histogram intervals are gaining in importance. The previously high histogram intervals are getting smaller. The increase in F_o is also visible macroscopically in the pseudo-color images (f to j). Fig. 5 shows the course of Δdt of the entire measurement series of one sample (Fig. 4) here as a representative example. As long as F_o does not increase, there is no histogram shift to the right. The Δd_t baseline was established (black line with circles) and ran parallel to the x-axis. Reaching the critical oxygen value is reflected in an increase in Δd_t. The simultaneous measurement of the oxygen content (dashed line) allows reading the LOL on the graph. The results of the LOL individual fruit identification of 'Braeburn' and 'Golden Delicious' are shown in Table 2.

4. Discussion

The previous investigations of DCA-CF storage are based on the application of non-imaging fluorescence. One of the problems associated with the use of non-imaging fluorescence is the heterogeneity of plant samples. There are differences in pigment levels and chlorophyll fluorescence ratios between sun and shade leaves (Lichtenthaler et al., 2007; Sarijeva et al., 2007) and also in fruit (Merzlyak et al., 2008). Non-imaging fluorescence is based on single-point measurements and the heterogeneity of photosynthetic activity makes this approach error-prone (Lichtenthaler et al., 2005). Chlorophyll fluorescence imaging overcomes this problem (Lichtenthaler et al., 2005; Gorbe and

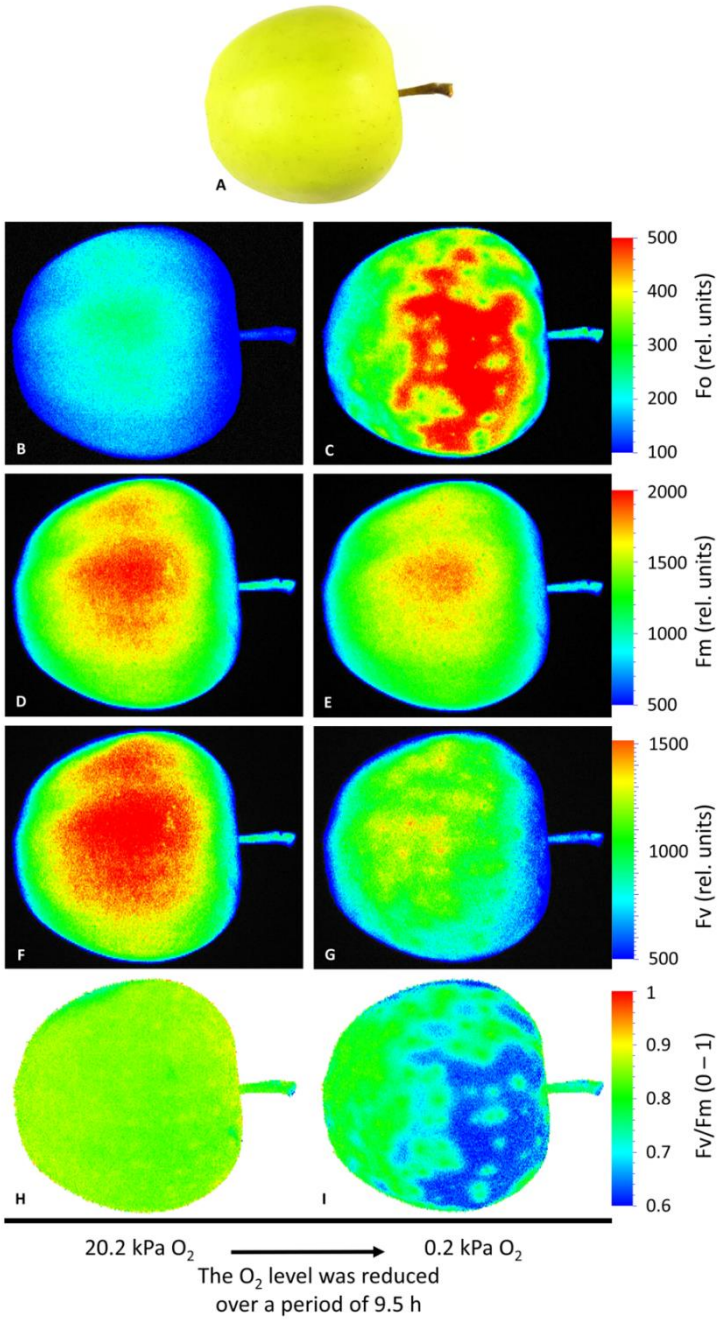

Fig. 3. 'Golden Delicious' in real life (A) and representative chlorophyll fluorescence images (pseudo-colors) of the parameters F_o (B; C), F_m (D; E), F_v (F; G), and F_v/F_m (H; I) during storage (2.5 °C; ± 0.5 °C) and the oxygen level of 20.2 kPa (left) and 0.2 kPa (right). The color codes are indicated on the right-hand side and range from blue (minimum value) to red (maximum value).

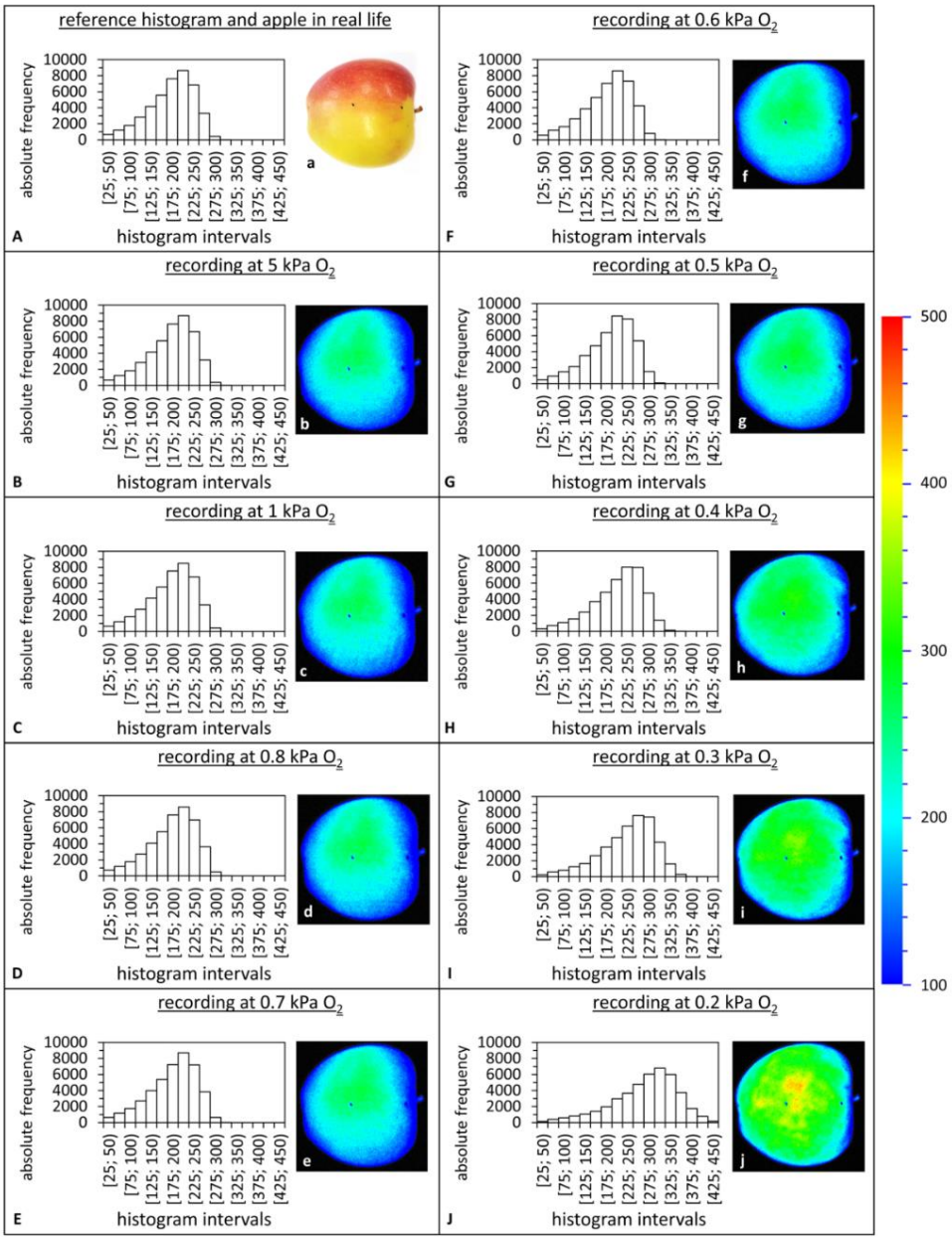

Fig. 4. As a representative example, an extract of recordings from a series of measurements is shown. The F_o data (rel. units) were distributed into k intervals with a constant width of 25 frequencies. The maximum number of histogram intervals is 40. The intervals which are not occupied are not shown. The reference histogram (A) is based on the average of five fluorescence measurements above the LOL. The image next to the reference histogram shows the 'Braeburn' apple in real life (a). The histograms (B to J) show the F_o data of the pseudo-color images (b to j) that are shown directly to the right. The color code on the right applies to all images shown.

Calatayud, 2012). The imaging fluorescence makes the spatial heterogeneity of the photosynthetic activity of leaves and fruit visible (Baker, 2008; Nedbal and Whitmarsh, 2010). The photosynthetic activity can be extremely heterogeneous at the leaf surface and between the leaves (Baker et al., 2001; Lichtenthaler et al., 2007; Sarijeva et al., 2007). Especially when plants or parts of plants are exposed to environmental stresses in the case of drought, water stress, chilling or attack by pathogens e.g. (Hogewoning and Harbinson, 2007; Sperdouli and Moustakas, 2011; Rousseau et al., 2013). The heterogeneous distribution of the chlorophyll fluorescence was demonstrated in our investigations. The

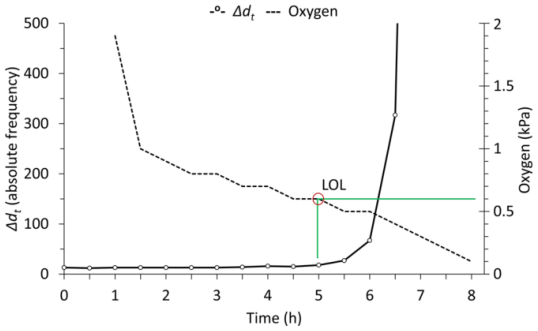

Fig. 5. The identifying of the LOL using the histogram division method on a 'Braeburn' apple as a representative example. The Δd_t (absolute frequency) is shown as a black line with circles and oxygen (kPa) as a dashed line; the LOL is marked with a circle.

Table 2
The LOL was identified on individual fruit using the histogram division method for both 'Braeburn' and 'Golden Delicious'; average, standard deviation (SD), minimum (MIN) and maximum (MAX) are shown (all n = 5).

	LOL (kPa)	SD (kPa)	MIN (kPa)	MAX (kPa)
'Braeburn'	0.6	± 0.1	0.5	0.7
'Golden Delicious'	0.7	± 0.1	0.5	0.8

fluorescence heterogeneity exists both between the apples (Fig. 2) and within a fruit (Figs. 3 and 4) above and below the LOL. Wright et al. (2012) found a SD of ± 31.9% (F_o), ± 46% (F_m), ± 55.8% (F_v) and ± 15.3% (F_v/F_m) at 'Honeycrisp' with non-imaging fluorescence measurements (n = 16). Our experience with non-imaging fluorescence measurements also confirms these results (data not shown). A comparison of imaging and non-imaging fluorescence was not performed in this study. However, both methods are described in the literature and have been established in research for decades (Baker et al., 2001; Lichtenthaler et al., 2005; Baker, 2008; Nedbal and Whitmarsh, 2010; Gorbe and Calatayud, 2012). The parameter F_v/F_m is a value between 0 and 1 and healthy plants or parts of plants range from 0.75 to 0.85. In the event of stress or damage of photosystem II, F_v/F_m is reduced (Bjorkman and Demmig, 1987; Bolhar-Nordenkampf et al., 1989). Wright et al. (2012) measured F_v/F_m values between 0.65 and 0.74 above the LOL. However, our measurements showed slightly higher values ('Braeburn' 0.76; 'Golden Delicious' 0.83) and F_v/F_m decreased below the LOL (0.63 and 0.72). The treatment with low-oxygen stress in our experiments was probably too short for a clearer decrease of F_v/F_m because experience has shown that this parameter reacts more slowly to stress (Prange et al., 2002). Overall, most of the higher fluorescence values (F_o, F_m, F_v) come from the central area of the fruit. This is likely an effect of the fruit's rounded topography. The central area of the fruit, vertically under the camera, showed higher fluorescence values than the edge areas. This is a typical phenomenon with rounded objects, which also Ciscato et al. (2001) and Lichtenthaler et al. (2012) observed. The chlorophyll fluorescence imaging makes visible what is partially hidden by non-imaging fluorescence techniques. Often non-imaging fluorescence systems cannot recognize the heterogeneity of fluorescence, which remains hidden to a certain extent (Lichtenthaler et al., 2005; Baker, 2008). Especially, when the non-imaging fluorometer measures a sample of four to eight fruit simultaneously with only one sensor (Prange et al., 2003; DeLong et al., 2004; Wright et al., 2008). The non-imaging fluorescence measurement of several apples lying next to each other could intensify the topographical effect. The positioning below the sensor cannot be carried out evenly. The topographic effect remains undetected because only the average fluorescence of the fruit sample is shown as output. However, this value is often normalized. In this context, the origin and the spatial relation of the fluorescence signal are unknown. In contrast, chlorophyll fluorescence imaging can detect spatial heterogeneity of photosynthetic activity and fluorescence. The spatial heterogeneity of the examined sample becomes visible (Lichtenthaler et al., 2005; Lichtenthaler et al., 2012; Kurenda et al., 2014).

The results of Prange et al. (2002) can be confirmed with the imaging fluorescence that F_o is suitable for detecting low oxygen stress in apples. The F_o value of apples (173 and 218) is also comparable with our experience (Wright et al., 2012). However, to consider the heterogeneity of F_o to identify the LOL, the availability of a suitable method is necessary. For this purpose, the histogram division method has been proposed. The histogram division method is based on a study of the localization of vehicle license plates (Wang et al., 2009). The method was changed and simplified for this study because the FluorCAM only outputs fluorescence values and not color channels or gray-scale images normally used in image processing. Furthermore, the apples in the storage are non-moving objects, in contrast to vehicles with license plates. The histogram division method bundles all F_o data (original values) and takes the heterogeneous fluorescence distribution into account. This method enables the fluorescence images to be compared with one another. As long as the fluorescence does not increase, the histograms are similar. The Δd_t baseline is established. The increase in Δd_t indicates low-oxygen stress. The LOL can be read in the graphic (Fig. 5). This corresponds to the definition of the LOL (Wright et al., 2010, 2012). In image processing, the histogram difference is also often calculated in order to compare two images with one another. It is a positive number that is calculated using the absolute value or the square (Rabin et al. 2008; Shakeri et al., 2017; Kumar et al., 2018). The use of this method is also conceivable. However, a decision was made against the difference method because the shift to the right is only partially visible. The low fluorescence values of the reference histogram are rated higher by making them positive with the absolute value or square. This is inconvenient and would result in a flat spike of Δd_t. The increase in fluorescence would be masked. The non-imaging DCA-CF techniques evaluate fluorescence from a small amount of fruit. In fact, this is seen as a major limitation of the technology compared to other DCA techniques such as Respiration Quotient (measurements of CO_2 production and O_2 uptake) and alcohol measurements (fruit juice or room air). DCA-CF imaging aims to overcome these limitations by installing the camera over a fruit crate in the storage room to measure a variety of fruit. In this way, the heterogeneity could be determined better because more than 6–8 fruits are measured. The number of repetitions in this study is low but sufficient to describe the method proposed. However, further research is needed for this. Furthermore, it is still unclear how fluorescence imaging systems affect fruit performance and quality during storage.

The comparison of results regarding the LOL from different studies is difficult. The LOL is cultivar-specific and can vary depending on the year, harvest date and agrometeorological conditions where the fruit are grown. The fruit quality of the samples used in this study corresponds to the recommendations for northern Germany. Our experience shows that the LOL results for 'Braeburn' and 'Golden Delicious' are plausible (2020 season). Furthermore, the LOL is also dependent on the storage temperature and storage time (Prange et al., 2010; Weber et al., 2015). Gasser and von Arx (2015) examined 'Topaz' and identified different LOL in three consecutive years (0.21 kPa, 0.18 kPa and 0.56 kPa). The storage temperature is a factor that can affect the level of the LOL. Wright et al. (2010) investigated the influence of temperature on the LOL ('Honeycrisp'). The apples were stored at 20 °C, 10 °C, 3.5 °C and 0 °C and different LOL were identified (0.72 kPa, 0.33 kPa, 0.22 kPa and 0.08 kPa). The proposed method is not limited to identify the LOL of apples and could be used in many ways. The non-imaging fluorescence has also been used on other types of fruit, such as kiwi, pears and avocados (Prange et al. 2002; Yearsley et al., 2003; Vanoli et al., 2010).

Furthermore, the histogram division method can also be transferred to detect other stress factors that play a role in fruit storage. These include high CO_2 levels, low temperatures, testing of 1-Methylcyclopropene (1-MCP) treatment, water loss (Prange et al., 2010; 2012) and physiological disorder of the fruit (Saquet and Streif, 2002; Lötze et al. 2006). The imaging fluorescence is superior because the location of the disorder is recorded. Ciscato et al. (2001) used different fluorescence emissions to detect stress caused by physiological disorders. It was possible to visualize internal disturbances of the 'Jonagold' fruit before macroscopic symptoms appeared. Another problem that often occurs in storage practice is the different fermentation behavior of fruits in relation to low oxygen levels. In this phenomenon, apples of the same cultivar and origin have different LOL, if the fruit maturity is heterogeneous at harvest time (Schlie et al., 2020). The selection of a representative sample to identify the LOL is difficult. The use of fluorescence imaging would not solve this problem but at least reduce it since dozens of fruit could be measured simultaneously with one system. Suppose there is an increase in F_0 on any part of the measured area, the histogram will change and Δd_t will increase immediately.

5. Conclusion

In the present study, the chlorophyll fluorescence of apples was measured with a fluorescence imaging system in situ during storage. A high degree of fluorescence heterogeneity has been demonstrated. The new methodology to identify the LOL using chlorophyll fluorescence imaging combined with the histogram division method has been introduced. This method bundles all F_o data and enables the heterogeneity of the sample to be taken into account and incorporated into the process. Our results showed that the fluorescence imaging combined with the histogram division method can be a powerful tool for identifying the LOL in the DCA-CF storage. A combination of fluorescence imaging and digital image processing would be possible. The camera could be installed above the fruit boxes in the storage room. In this way, the LOL of dozens of apples can be identified by one camera system simultaneously. In contrast to non-imaging fluorescence techniques, the information benefit in terms of fluorescence data is higher. In general, cameras and image processing are becoming increasingly cheaper. Our results show that the fluorescence imaging plus histogram division differs from existing non-imaging methods. However, these results are insufficient to assess how their use affects fruit performance and quality during DCA-CF storage. Further research is needed.

CRediT authorship contribution statement

Tim-Pascal Schlie: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Thomas Rath:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – review & editing. **Werner Dierend:** Resources, Writing – review & editing, Project administration, Funding acquisition. **Dirk Köpcke:** Validation, Resources, Writing – review & editing.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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3.3 Recording of Low-Oxygen Stress Response Using Chlorophyll Fluorescence Kinetics in Apple Fruit

Research Paper

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RESEARCH

Recording of Low-Oxygen Stress Response Using Chlorophyll Fluorescence Kinetics in Apple Fruit

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Abstract
Long-term storage of apples (*Malus* x *domestica*, Borkh.) is increasingly taking place under Dynamic Controlled Atmosphere (DCA). The oxygen level is lowered to ≤ 1 kPa O_2 and the apples are stored just above the Lower Oxygen Limit (LOL). Low oxygen stress during controlled atmosphere storage can lead to fermentation in apples if oxygen levels are too low. Chlorophyll fluorescence can be used to detect low-oxygen stress at an early stage during storage. The currently available non-imaging fluorescence systems often use the minimal fluorescence (*Fo*) parameter. In contrast, the use of chlorophyll fluorescence kinetics is insufficiently described. Therefore, this study aimed to gain more knowledge about the response of chlorophyll fluorescence kinetics to low oxygen stress in apples using a fluorescence imaging system. The results show that the kinetic fluorescence curves differ under aerobic and fermentation conditions. The fermentative conditions initiated a decrease in fluorescence intensity upon application of the saturation pulses during exposure to actinic light. This result was made at 18 °C and 2 °C ambient temperatures. Interestingly, the kinetic curve changed at 2 °C before fermentation products accumulated in the apples. Non-photochemical quenching (*NPQ*) decreased under fermentation conditions in the dark phase after relaxation. Upon entering the dark relaxation phase after Kautsky induction, $\phi PSII$ began to increase. Under atmospheric oxygen conditions, $\phi PSII$ reached values of 0.81 to 0.76, while under fermentation, $\phi PSII$ values ranged from 0.57 to 0.44.

Keywords Controlled Atmosphere · Fermentation · Lower Oxygen Limit · Pseudo-Color Image · Post-Harvest · Storage

Introduction

Commercially produced apples (*Malus* x *domestica* Borkh.) are often stored under Controlled Atmosphere (CA) several months after harvest. In the CA storage rooms, the atmospheric gas composition (O_2 and CO_2) is changed in addition to the temperature reduction (1 to 5 °C) (Dilley, 2010; Prange et al., 2013). Depending on the variety and storage time, the O_2 level is reduced to between 1 kPa and 2 kPa and the CO_2 level is increased to 0.5 kPa to 4 kPa (Saltveit, 2003; Both et al., 2014; Köpcke, 2015). In preserving the quality of the apples, a decrease in the oxygen level is essential. Dynamic Controlled Atmosphere (DCA) is the latest form of CA storage (Prange et al., 2013). In the DCA storage, the oxygen level (≤ 1 kPa) is set very close to the Lower Oxygen Limit (LOL), the lowest limit the fruit can tolerate without developing disorders (Zanella et al., 2008; Gasser & von Arx, 2015; Thewes et al., 2020). The LOL indicates the oxygen level where metabolism changes from predominantly aerobic to fermentation (Wright et al., 2011, 2012, 2015). The storage below the LOL leads to the accumulation of fermentation products, especially acetaldehyde, ethanol and ethyl acetate. These fermentation products are undesirable in high concentrations for commercial marketing (Pesis, 2005; Wright et al., 2015). Several technologies have been developed to determine optimal oxygen levels by monitoring fruit metabolism in real-time and identifying the LOL during DCA storage. Four different DCA systems are currently being used or tested in fruit storage (Thewes et al., 2021). Fruit monitoring by analyzing undesirable fermentation products that can be measured in the fruit juice.

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(Schouten et al., 1998; Veltman et al., 2003). Furthermore, the respiratory quotient (RQ) can be determined by monitoring the oxygen consumption of the fruit and the CO_2 production of the fruit (Weber et al., 2015, 2020; Bessemans et al., 2016). Another method to measure fruit respiration during storage is based on only monitoring the fruit's CO_2 production. In the presence of fermentation processes, the intensity of CO_2 production changes (Thewes et al., 2020; Büchele et al., 2023). In addition, chlorophyll fluorescence measurement can be used to detect low-oxygen stress and identify the LOL. The fluorescence measurements are usually performed with non-imaging sensors (Wright et al., 2008; Prange et al., 2013). The minimal fluorescence (*Fo*) has turned out to be a sensitive parameter for detecting low-oxygen stress in plants and fruit (Harris & Heber, 1993; Prange et al., 2002; Wright et al., 2008). The change of chlorophyll fluorescence as a response to low-oxygen stress in apples is associated with respiratory changes (Gasser et al., 2008, 2010). A connection between aerobic respiration (mitochondria), fermentation (cytosol) and fluorescence (chloroplasts) is suspected (Wright et al., 2012). However, the regulation mechanisms of fermentation in apples have not been conclusively clarified (Boeckx et al., 2019). It is hypothesized that low-oxygen stress might lead to cytosolic reductant excess. The reductant would consequently accumulate in the chloroplasts, where it is used to reduce the plastoquinone (PQ) pool and consequently increases *Fo* (Wright et al., 2011, 2012, 2015).

Information on the spatial distribution of the fluorescence signal is required for different and special analyzes with regard to low-oxygen stress (Lichtenthaler et al., 2012; Schlie et al., 2022). Therefore, the objective of this study was to get more knowledge about the response of chlorophyll fluorescence kinetics to low-oxygen stress in apples, especially relating to the spatial distribution. The usage of kinetic chlorophyll fluorescence for the early detection of low-oxygen stress was investigated, combined with whether other parameters besides *Fo* could be used to detect low-oxygen stress in apples. For this purpose, fluorescence parameters were examined with a fluorescence imaging system under different oxygen conditions and temperatures on individual apples.

Materials and Methods

Plant Materials

The investigations were carried out with the apple variety 'Elstar'. The apples were cultivated in the experimental orchards of Fruit Research Center Jork, Germany (53° 31' N, 9° 44' E). The apples had a fruit size of 70 to 80 mm, firmness of 59,4 N (±3.8) (using Fruit Texture Analyzer, Güss, Cape Town, South Africa), Total Soluble Solids of 11.5% (±0.6%) (using Refractometer PCE-DRW 2, PCE Instruments, Meschede, Germany) and starch index of 3 (±0.7) (using the CTIFL scale = Center Technique Interprofessionnel des Fruits et Légumes; France) (Vaysse, 2002) at harvest. Fruit ripeness was measured immediately after harvest (all n = 40).

Experimental Setup

The schematic representation of the experimental setup is shown in Fig. 1. Chlorophyll fluorescence were performed using the Open FluorCAM 701 MF (Photon Systems Instruments, Brno, Czech Republic), including the FluorCAM 7 software. The Open FluorCAM was essentially made up of a Charged Coupled Device (CCD) camera, a filter (695 to 770 nm), four LED panels and an embedded in metal construction. The CCD camera (12-bit resolution in 512 × 512 pixels, 50 images s^{-1}) was justified vertically to the fruit sample. The shutter opening time was set to 10 µs (PAM = Pulse Amplitude Modulation). Two types of LED panels (orange-red and white) were positioned around and aligned at a 45° angle to the samples. The same types of light were placed opposite each other. A mini storage box (black; polystyrene; ≈ 28 L) was installed below the FluorCAM. The fruit sample was placed in the box directly under the CCD with a sample-camera distance of 10 cm. The storage box was locked with a transparent acrylic glass lid (8 mm). The oxygen was measured with SCS 310 oxygen analyzer (Storage Control System, Paddock Wood, United Kingdom), including data logger. A compressed gas tank with N_2 can be connected to the storage box to achieve low oxygen levels. In addition, a relief valve prevents damage from overpressure. Fluorescence measurements were performed in a climate chamber with temperature control. The setup was similarly used in Schlie et al. (2022). The fluorescence measurements were performed on the green side of the fruit skin and placed on its side (Merzlyak et al., 2008; Lichtenthaler et al., 2012).

Chlorophyll Fluorescence and Kinetics

The fluorescence measurements are carried out using different light sources. Orange-red light (λ_{max} = 621 nm; 0.1 µmol m^{-2} s^{-1}) was used as the measuring light (ML) and white light (420 to 650 nm; 1000 µmol m^{-2} s^{-1}) was used for the saturation pulse (SP). Furthermore, orange-red light (λ_{max} = 621 nm; 100 µmol m^{-2} s^{-1}) was used as actinic light (AL) (Nedbal et al., 2000; Nedbal & Whitmarsh, 2004). The intensity of the light (base plate area of 9 cm × 9 cm) was checked with the LICOR LI-190R PAR sensor (Lincoln,

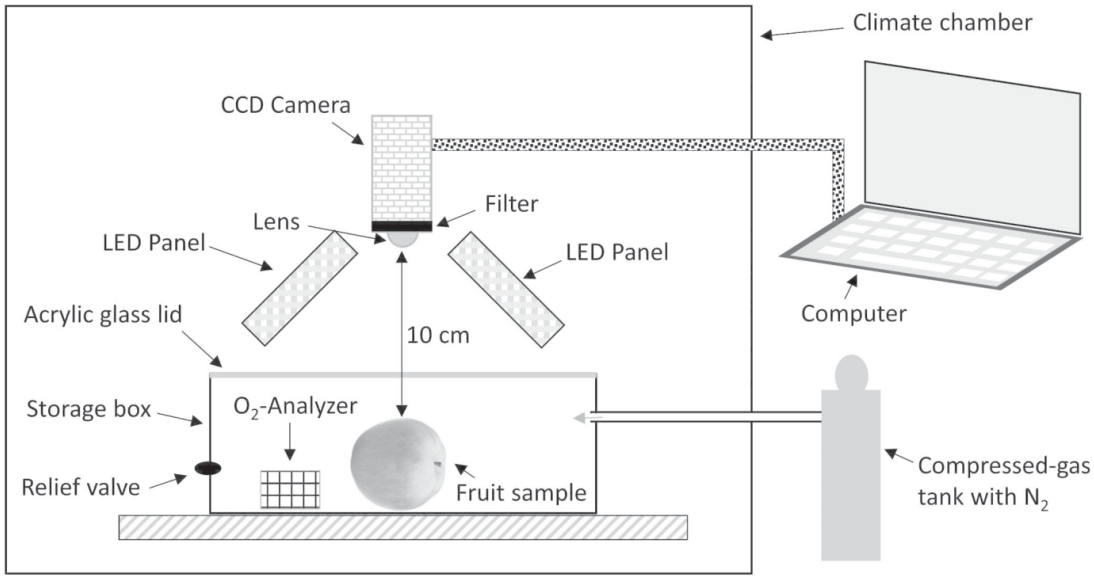

Fig. 1 Schematic representation of the experimental setup

Nebraska, USA). Because the storage box was closed with transparent acrylic glass to realize different oxygen conditions, the light measurements were carried out in the presence of this acrylic glass lid. In order to describe the influence of the acrylic glass lid on the results, spectrometer measurements (Ocean HDX, Ocean Optics, Florida, USA) were carried out with and without the lid for the wavelengths involved. The methodological result for this is shown in Fig. 2. The transmission loss was up to 6%.

The fluorescence measurements were carried out using a standard protocol of the FluorCAM 7 software, which was also used in modified form by Pineda et al. (2008) and Cen et al. (2017). Figure 3 shows the chronological sequence of the protocol. Before the fluorescence measurement was started, the fruit sample was dark-adapted for 60 min so that the reaction centers opened (Murchie & Lawson, 2013). The minimal fluorescence (*Fo*) was measured using the orange-red ML (duration: 5.04 s). The maximal fluorescence (*Fm*) was measured by applying the SP (duration: 0.8 s). A dark pause of 10 s followed. At 15.84 s the actinic light was switched on and exposure lasted 60 s. During the actinic light phase, five SP (duration per SP: 0.8 s) were used to measure *Fm_L1*, *Fm_L2*, *Fm_L3*, *Fm_L4* (L stands for light) and *Fm_Lss* (*Lss* stands for light steady-state). Immediately (0.08 s) before each SP was given, the terminal fluorescence (*Ft*) was measured (*Ft_L1*, *Ft_L2*, *Ft_L3, Ft_L4, Ft_Lss*). A dark relaxation phase followed for 40 s after Kautsky induction. During the dark relaxation phase, 3 SP (duration per SP: 0.8 s) were used to measure *Fm_D1*, *Fm_D2* and *Fm_D3* (D stands for dark) and immediately (0.08 s) before each SP was given, *Ft_D1*, *Ft_D2* and *Ft_D3* was measured.

The three-dimensional fruit sample was output as a two-dimensional pseudo-color image. The fluorescence image provided several 10,000 individual fluorescence information. However, it should be noted that the two-dimensional depiction of a three-dimensional sample using an imaging method always involves the risk of incorrect values because the data are not angle-corrected. The central area of the apple, which was vertically below the camera, showed higher fluorescence values than the fruit's edge areas. For this reason, only the data from the center of the image that was recorded vertically under the camera were used for the evaluation. Starting from the center of the image, an area of 2880 pixels was selected. The 90th percentile value was calculated on the basis of the selected fluorescence data. The fluorescence values corrected in this way were used to calculate the parameters listed in Table 1.

Oxygen Treatment and Low-Oxygen Stress

The variants examined are shown in Table 2. The examinations were carried out at 18 °C (±0.5 °C) and 2 °C (±0.5 °C). Before the experiments started, the fruit samples were acclimatized to the respective treatment temperature for three days. The oxygen treatment carried out into three variants and was generated step by step. First, the fruit sample was exposed to atmospheric oxygen (~20.9 kPa)

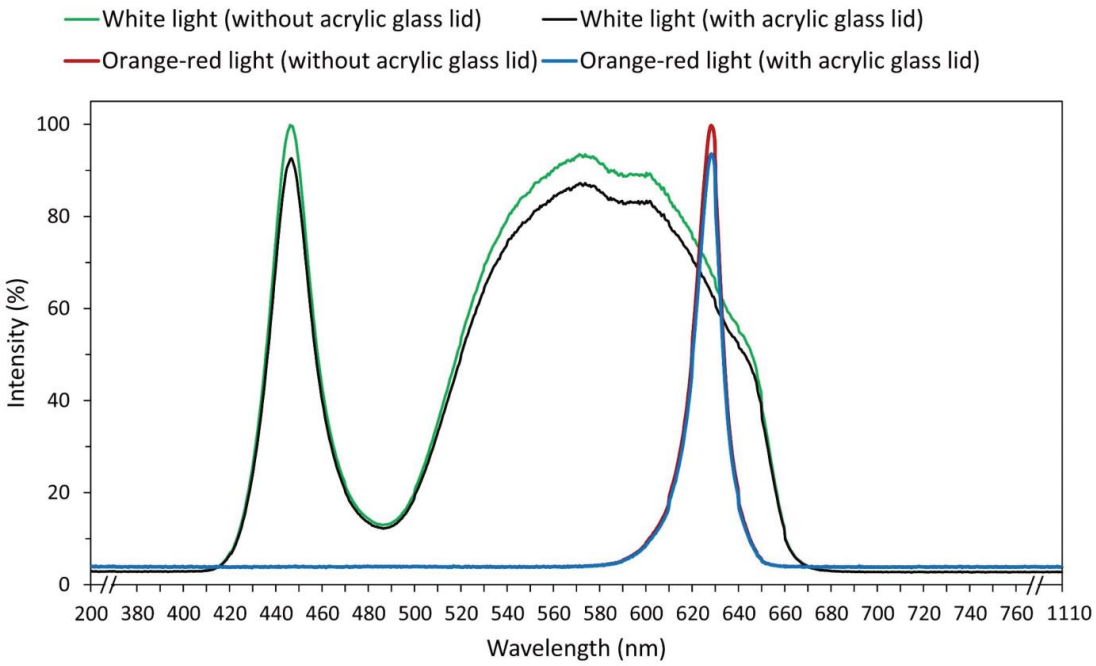

Fig. 2 Emission spectra of the light sources involved in the fluorescence measurement; white light measured without the acrylic glass lid (green), white light with acrylic glass lid (black), orange-red light without the acrylic glass lid (red); orange-red light with acrylic glass lid (blue)

for three hours. Following this, nitrogen (purity 99.8%) was added to the storage box for about seven to ten minutes until an oxygen level of 1 kPa to 0.5 kPa was reached. The fruit sample was treated with this oxygen level (1 kPa to 0.5 kPa) for 3 h. The oxygen level was close to low-oxygen stress and represents DCA storage (Wright et al., 2012). In the last step, the oxygen level was reduced to <0.02 kPa (seven to ten minutes addition of nitrogen) to generate low-oxygen stress and fermentation (Pesis, 2005). The level was held for three hours. The experiments were performed with individual apples so that each fruit sample (=repetition) was sequentially run through all three oxygen treatments ($n=5$).

Fermentation Analysis

The fermentation analysis was performed using the CLARUS 500 gas chromatograph (Perkin-Elmer, Waltham, USA). For the fermentation analysis of the fermentation products acetaldehyde, ethanol and ethyl acetate, 1 ml of the filtered fruit juice was transferred into a headspace vial (5 ml). The headspace vials were closed with a rubber stopper and a metal ring and placed in the autosampler of the gas chromatograph (exactness of the analysis of the fermentative products ± 1 mg L^{-1}). Each individual fruit sample was analyzed for fermentation products after completion of the oxygen treatments described in Sect. 2.5. In addition, apples which were not involved in the oxygen treatment experiments were examined for fermentation products as a control ($n=5$). This analysis was done immediately after the apples were harvested.

Statistical Analysis

All statistical analyses were performed using SPSS 27 (Chicago, Illinois, USA) and Microsoft Excel 2016 (Redmond, Washington, USA). The results were calculated by analysis of variance (ANOVA) and Bonferroni test ($p<0.05$). In addition, the homogeneity of error variances was checked by the Levene test.

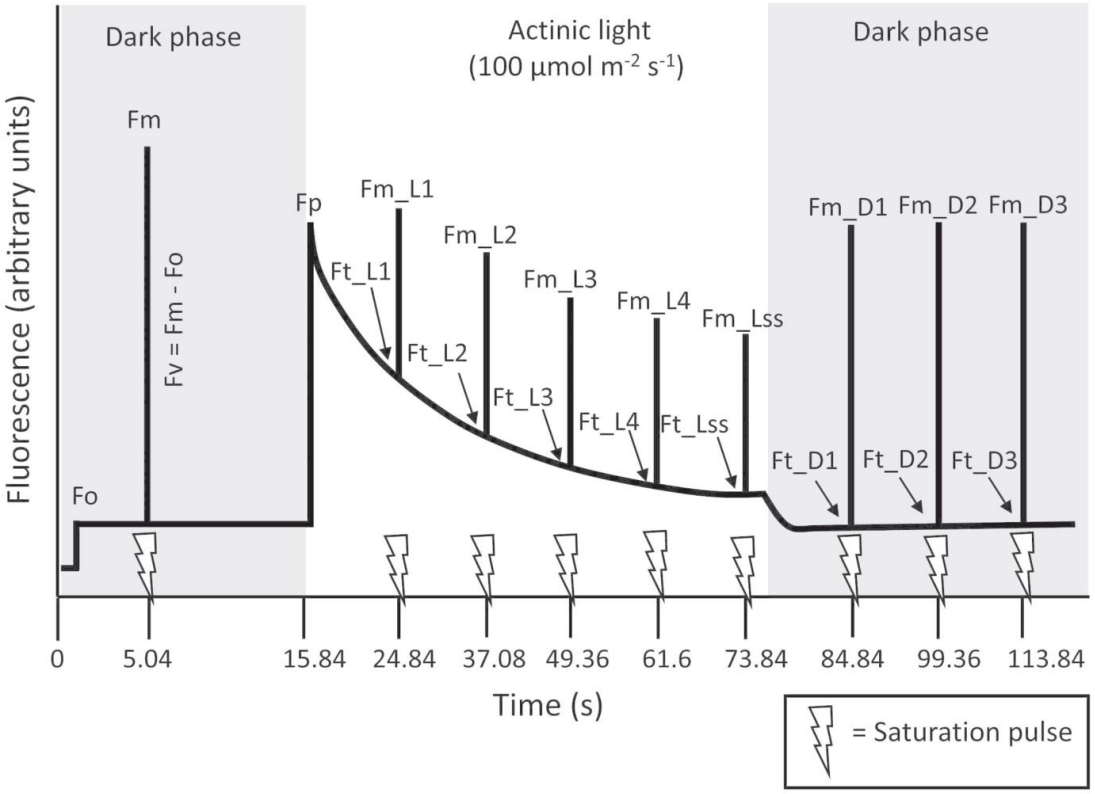

Fig. 3 Schematic representation of the fluorescence protocol; the white flashes indicate the use of saturation pulses (1000 µmol m^{-2} s^{-1}); *Fo* = minimal fluorescence; *Fm* = maximal fluorescence; *Fv* = variable fluorescence; *Fp* = peak fluorescence; *Ft* = terminal fluorescence; L = light; Lss = light steady-state; D = dark

Results and Discussion

Dark-Adapted Fluorescence and Pseudo-Color Images

Table 3 shows the basic parameters of the dark-adapted fluorescence measurement *Fo* and *Fm* as well as *Fv/Fm* and *Fv/Fo* under the three investigated oxygen conditions at 18 °C and 2 °C. The fermentation resulted in a significant increase in *Fo*. At the temperature of 18 °C, the increase occurred during the shift to low-oxygen stress (1 to 0.5 kPa). Given the temperature of 2 °C, the significant increase in *Fo* only occurred during fermentation. Significant differences between the three oxygen conditions at 18 °C could also be determined for the parameters *Fv/Fm* and *Fv/Fo*. At the temperature of 2 °C, significant differences were found between the control and fermentation as well as shift to low oxygen stress and fermentation. With the decrease in oxygen content, *Fm* tended to decrease at 18 °C and 2 °C, but not significantly. Similar results, especially for *Fo* and *Fv/Fm*, were reported by Wright et al. (2008) and Wright et al. (2012) in apple fruit, using non-imaging fluorescence measurements. The pseudo-color images in Fig. 4 show the change and variation in fluorescence on the apple skin. Furthermore, the pseudo-color images, especially from *Fo* and *Fm* show that the fluorescence in the center of the image is higher than in the outer areas. The central area of the apple, which was vertically below the camera, showed higher fluorescence values than the fruit's edge areas, which was also observed in Lichtenthaler et al. (2012) and Schlie et al. (2022). This observation was considered when evaluating the data (90th percentile value). The change in fluorescence is clearly visible at 18 °C between all three variants, especially for *Fo* (1a to 1c) and *Fv/Fm* (3a to 3c). In contrast, the fluorescence at 2 °C changed under almost entirely fermentative conditions (4a to 4c; 6a to 6c). Our measurements showed *Fv/Fm* values of 0.83 to 0.85 at 20.9 kPa O_2. The drop began at 18 °C in the shift to low-oxygen stress

Table 1 Overview of the calculated fluorescence and quenching parameters with formulas (based on Oxborough and Baker, 1997; Maxwell and Johnson, 2000; Baker, 2008; Murchie and Lawson, 2013)

Parameter	Formula	Description
Fv/Fm	*(Fm - Fo)/Fm*	Maximum PSII quantum yield, dark-adapted
Fv/Fo	*(Fm - Fo)/Fo*	Ratio of variable to minimum fluorescence
NPQ_L1	*(Fm - Fm_L1)/Fm_L1*	Non-photochemical quenching induced in light (*L1*)
NPQ_L2	*(Fm - Fm_L2)/Fm_L2*	Non-photochemical quenching induced in light (*L2*)
NPQ_L3	*(Fm - Fm_L3)/Fm_L3*	Non-photochemical quenching induced in light (*L3*)
NPQ_L4	*(Fm - Fm_L4)/Fm_L4*	Non-photochemical quenching induced in light (*L4*)
NPQ_Lss	*(Fm - Fm_Lss)/Fm_Lss*	Steady-state non-photochemical quenching in light (*Lss*)
NPQ_D1	*(Fm - Fm_D1)/Fm_D1*	Non-photochemical quenching relaxing in dark (*D1*)
NPQ_D2	*(Fm - Fm_D2)/Fm_D2*	Non-photochemical quenching relaxing in dark (*D2*)
NPQ_D3	*(Fm - Fm_D3)/Fm_D3*	Non-photochemical quenching relaxing in dark (*D3*)
ϕPSII_L1	*(Fm_L1 - Ft_L1)/ Fm_L1*	PSII quantum yield induced in light (*L1*)
ϕPSII_L2	*(Fm_L2 - Ft_L2)/ Fm_L2*	PSII quantum yield induced in light (*L2*)
ϕPSII_L3	*(Fm_L3 - Ft_L3)/ Fm_L3*	PSII quantum yield induced in light (*L3*)
ϕPSII_L4	*(Fm_L4 - Ft_L4)/ Fm_L4*	PSII quantum yield induced in light (*L4*)
ϕPSII_Lss	*(Fm_Lss - Ft_Lss)/ Fm_Lss*	Steady-state PSII quantum yield in light (*Lss*)
ϕPSII_D1	*(Fm_D1 - Ft_D1)/ Fm_D1*	PSII quantum yield relaxing in dark (*D1*)
ϕPSII_D2	*(Fm_D2 - Ft_D2)/ Fm_D2*	PSII quantum yield relaxing in dark (*D2*)
ϕPSII_D3	*(Fm_D3 - Ft_D3)/ Fm_D3*	PSII quantum yield relaxing in dark (*D3*)

Table 2 Test variants of the experiments for measuring chlorophyll fluorescence kinetics

Variant	Temperature (°C)	O_2 Treatment (kPa)
Atmospheric O_2 (control)	18	~ 20.9
Shift to low-O_2 stress	18	1 to 0.5
Fermentation	18	< 0.02
Variant	Temperature (°C)	O_2 Treatment (kPa)
Atmospheric O_2 (control)	2	~ 20.9
Shift to low-O_2 stress	2	1 to 0.5
Fermentation	2	< 0.02

Table 3 The fluorescence parameters *Fo*, *Fm*, *Fv/Fm* and *Fv/Fo* of the fruit samples of two different temperatures (18 °C and 2 °C) under atmospheric O_2 (20.9 kPa O_2), shift to low-O_2 stress (1 kPa O_2 to 0.5 kPa O_2) and fermentation (< 0.02 kPa O_2); Data based on the 90th percentile value; Means and standard error; (n = 5); (a.u. = arbitrary units)

Different small letters grouped within a column indicate statistically significant differences for each temperature separately ($p < 0.05$; Bonferroni test)

Variant	Temperature (°C)	*Fo* (a. u.)	*Fm* (a. u.)	*Fv/Fm* (0 to 1)	*Fv/Fo* (a. u.)
Atmospheric O_2 (control)	18	$220_a \pm 14$	$1243_a \pm 95$	$0.83_c \pm 0.001$	$4.8_c \pm 0.1$
Shift to low-O_2 stress	18	$312_b \pm 26$	$1149_a \pm 94$	$0.73_b \pm 0.03$	$3.2_b \pm 0.4$
Fermentation	18	$561_c \pm 37$	$1019_a \pm 90$	$0.45_a \pm 0.03$	$0.9_a \pm 0.1$
Variant	Temperature (°C)	*Fo* (a. u.)	*Fm* (a. u.)	*Fv/Fm* (0 to 1)	*Fv/Fo* (a. u.)
Atmospheric O_2 (control)	2	$226_a \pm 6$	$1510_a \pm 60$	$0.85_b \pm 0.01$	$5.9_b \pm 0.5$
Shift to low-O_2 stress	2	$235_a \pm 6$	$1419_a \pm 57$	$0.84_b \pm 0.01$	$5.2_b \pm 0.5$
Fermentation	2	$528_b \pm 52$	$1369_a \pm 53$	$0.63_a \pm 0.03$	$2.2_a \pm 0.4$

and reached 0.45 (*Fv/Fm*) on fermentation. At 2 °C, *Fv/Fm* initially remained stable and decreased during fermentation (0.63). Hägele et al. (2016) showed on fresh-cut salads that plant stress could be detected with chlorophyll fluorescence imaging. Cutting lettuce (stress) resulted in initial but partially reversible stress within the first hours after plant tissue injury. In contrast to the control (entire lettuce leaves), *Fv/Fm* of cut lettuce decreased during storage, which revealed increased stress levels (Hägele et al., 2016). Measurements of *Fv/Fm* allow assessment of photosynthetic activity as an indicator of cell and tissue vitality in fresh lamb's lettuce (*Valerianella olitoria*) (Schlüter et al., 2009). For unstressed

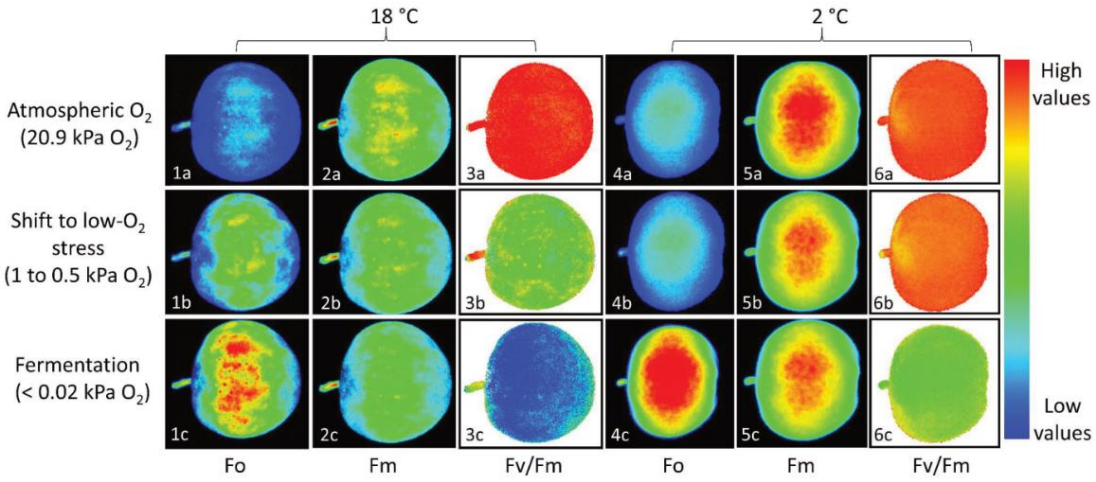

Fig. 4 Pseudo-color images of representative fruit samples of two different temperatures (18 °C and 2 °C) under atmospheric O_2 (20.9 kPa O_2), shift to low-O_2 stress (1 kPa O_2 to 0.5 kPa O_2) and fermentation (< 0.02 kPa O_2); Color codes range from red (high values) to blue (low values); Images are not angle corrected

leaves, *Fv/Fm* is ~0.83 (Murchie & Lawson, 2013). Other authors give a value of 0.75 to 0.85 for healthy plants or parts of plants (Bjorkman & Demmig, 1987; Bolhar-Nordenkampf et al., 1989). The presence of stress can damage photosystem II and leads to a decrease in *Fv/Fm* (Long et al., 1994; Wright et al., 2012). Nutrient deficiencies also led to a reduction in *Fv/Fm* in *Citrus Huanglongbing* (Cen et al., 2017).

Fluorescence Kinetics

The chlorophyll fluorescence kinetic curves are shown in Fig. 5. The data shown are based on the 90th percentile value. Only the kinetic data of the center of the image were taken into account (compare 2.3). The curves at both temperatures 18 °C (a to c) and 2 °C (d to e) were comparable. The peaks during the phase with AL (15.84 to 75.84 s) were interesting. While the application of SP led to increased fluorescence under atmospheric oxygen and the shift to low-oxygen stress, the opposite was observed during fermentation. Fluorescence kinetics changed dramatically before anaerobic metabolism led to the accumulation of ethanol and acetaldehyde (Fig. 5; Table 4). When switching to fermentation, the peaks decreased and the SP did not increase fluorescence intensity. Instead, a brief drop in fluorescence at a low level was observed. The development of the fluorescence kinetic curve during the fermentation showed no further increase in the fluorescence intensity due to the application of the SP. Quite the contrary, the peaks collapsed during the fermentation. This indicates that the primary quinone electron acceptor (Q_A) of photosystem II was already fully reduced (closed) before the SP was applied (Maxwell & Johnson, 2000). Apparently, the light intensity of the AL combined with low-oxygen stress was sufficient to reduce Q_A completely. Wright et al. (2011) showed on spinach leaves that the increase in *Fo* and the decrease in *qP* at oxygen values below the LOL indicate an over-reduced state of the PQ pool. Anaerobic conditions also led to an excessive reduction of the PQ pool in leaves of *Pisum sativum*, thereby inhibiting the photosynthetic electron transport chain (Tóth et al., 2007). The measurements of the fluorescence induction kinetics at *Thalassiosira pseudonana* also show a high degree of reduction in the chloroplast stroma as well as an intense electron pressure in the PQ pool (Cruz et al., 2011). Fluorescence kinetics can also be used to identify other stress factors. Tian et al. (2021) used fluorescence kinetics for the early detection of salt stress in *Arabidopsis*. Furthermore, fluorescence imaging can also be used to detect apple scab. However, it turned out that the use of thermal imaging was the more efficient tool (Belin et al., 2013). Yao et al. (2018) showed that fluorescence kinetics could detect drought stress in Arabidopsis. However, the investigations on drought-stressed Arabidopsis leaves showed a different development of fluorescence kinetics. During exposure to AL, the fluorescence intensity decreased more significantly in the phases between the SP (Yao et al., 2018).

Figure 6 presented *NPQ* (a and c), and *ϕPSII* (b and d) under atmospheric oxygen conditions (line with green rhombuses), shift to low-oxygen stress (line with yellow triangles) and fermentation (line with red circles). The respective stations of the quenching analysis (*L1*, *L2*, *L3*, *L4*, *Lss*, *D1*, *D2*, *D3*) are shown. *NPQ* started with a value below 0.15

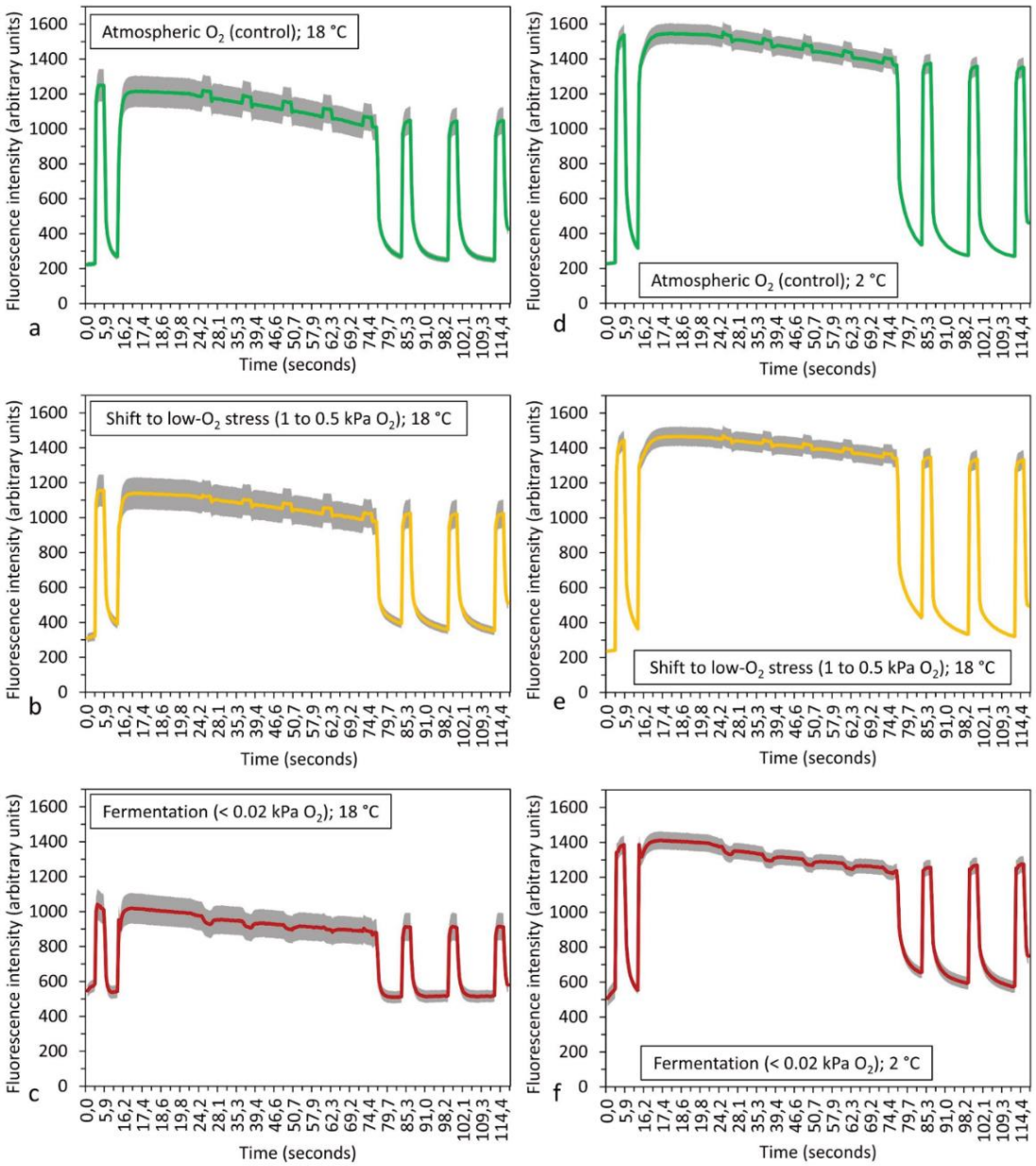

Fig. 5 Chlorophyll fluorescence kinetic curves of apples under atmospheric O_2 (20.9 kPa O_2) (green line), shift to low-O_2 stress (1 kPa O_2 to 0.5 kPa O_2) (yellow line) and fermentation (< 0.02 kPa O_2) (red line) and two temperatures (18 °C and 2 °C); Mean (line), standard error (gray area); Data based on the 90th percentile value; (n = 5)

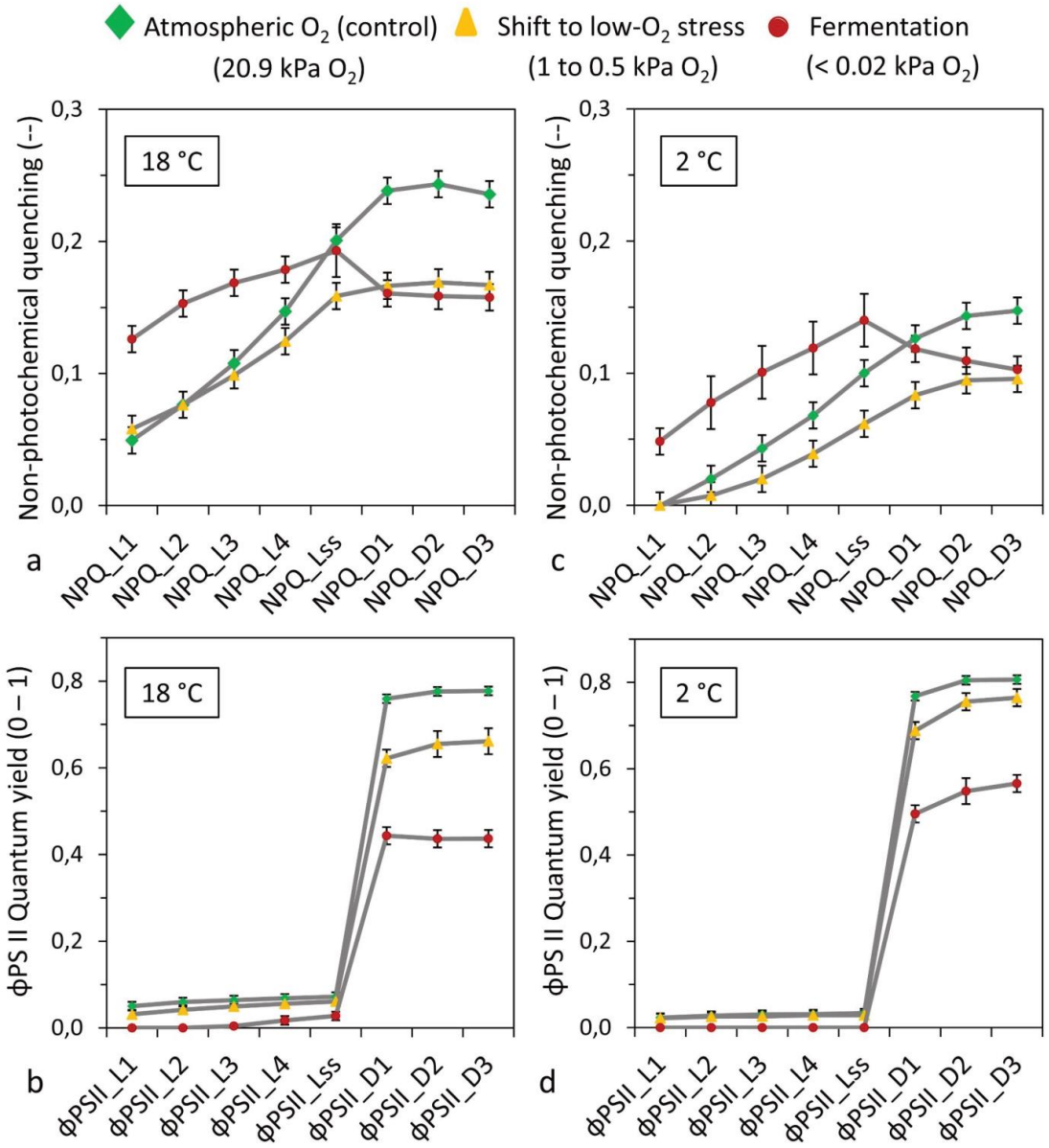

Fig. 6 The kinetic development of the chlorophyll fluorescence parameters Non-photochemical quenching (*NPQ*) and *ϕPSII* Quantum yield under atmospheric O_2 (20.9 kPa O_2) (green rhombuses), shift to low-O_2 stress (1 kPa O_2 to 0.5 kPa O_2) (yellow triangles) and fermentation (< 0.02 kPa O_2) (red circles) and two temperatures (18 °C and 2 °C); Data based on the 90th percentile value; Means and standard error; (n = 5)

and increased with every SP applied. When the steady-state (*Lss*) is reached, *NPQ* continues to grow under atmospheric conditions in the dark relaxation after Kautsky induction (*D1* to *D3*). During the shift to low-oxygen stress, *NPQ* also increased. However, under fermentation conditions, *NPQ* decreased again after reaching steady state. In general, *NPQ* was determined at a low level in this study. This could be related to the fact that the fluorescence measurements were

performed on the shaded side of the fruit. Chen and Cheng (2007) showed that sun-exposed apple skin had higher heat dissipation capacity and larger *NPQ* than the shaded apple skin. The non-photochemical quenching is used by plants to protect themselves from the adverse effects of high light intensity and results in increased thermal energy output (Horton & Ruban, 2005). The *ϕPSII* quantum yields during light adaptation (*L1*, *L2*, *L3*, *L4*, *Lss*) were below 0.1 at both temperatures (18 °C and 2 °C). With the entry into the dark relaxation phase after Kautsky induction, *ϕPSII* began to rise. *ϕPSII_D1* to *ϕPSII_D3* reached the value of 0.81 to 0.76 under atmospheric oxygen conditions. However, with the shift to low-oxygen stress, the *ϕPSII* decreased 0.66 to 0.62 at 18 °C and 0.76 to 0.69 at 2 °C. Under fermentation conditions, the *ϕPSII* was further reduced to 0.44 (18 °C) and 0.57 to 0.5 (2 °C). *ϕPSII* is affected by the amount of electron acceptors ($NADP^+$). Hence *ϕPSII* decreases in the case of limited NADPH consumption (Baker & Rosenqvist, 2004). The changes of *ϕPSII* could thus be attributed to the down-regulation of PSII and differences in the electron flux reduction capacity of PSII as well (Delalieux et al., 2009). There seems to be a difference in fluorescence yield between fruit and leaf because the chlorophyll content in the apple skin decreases in the post-harvest phase (Blackhall et al., 2020). Although apples also contain photosynthetic pigments, these are not primarily responsible for photosynthesis (Merzlyak et al., 2008). The decrease *of Fv/Fm* and *ϕPSII* during treatment under low-oxygen stress conditions supports the assumption that low-oxygen stress may be at least indirectly associated with PSII.

Table 4 shows the fermentation products found in the apples at harvest and after the low-oxygen treatment. The acetaldehyde and ethanol content at harvest and low-oxygen treatment at 2 °C did not differ significantly. The experiments at 18 °C resulted in a 100 times higher ethanol production in the apples. The acetaldehyde content was significantly increased compared to the harvest and the low-oxygen treatment at 2 °C. Ethyl acetate was only detected in small amounts (< 1 mg L^{-1}) at 18 °C. In the investigations at an ambient temperature of 2 °C, the kinetics changed even before fermentative products accumulated in the apple. The higher temperature likely led to efficient metabolism and a higher respiratory rate compared to 2 °C. On 'Honeycrisp' apples, it could be shown that the LOL is temperature-dependent. LOL from 0.22 kPa to 0.72 kPa at temperatures from 3.5 to 20 °C were found (Wright et al., 2010). In addition, the treatment time of 3 h with the oxygen value of < 0.02 kPa O_2 was too short for the accumulation of high fermentation products at 2 °C (Pesis, 2005). The products of anaerobic metabolism (ethanol, acetaldehyde and ethyl acetate) can degrade in apple fruit (Wood et al., 2022). Therefore, when comparing fermentation products, the temporal context of storage must always be considered. The absolute level of fermentation products is related to how long the apples were stored under low-oxygen conditions (Toivonen & DeEll, 2001; Pesis, 2005).

Table 4 Fermentation products (acetaldehyde, ethanol, ethyl acetate) in apples after harvest (without treatment) and after low-oxygen treatment; means and standard error; (n = 5)

	Acetaldehyde (mg L^{-1})	Ethanol (mg L^{-1})	Ethyl acetate (mg L^{-1})
Harvest	0.6_a ± 0.1	1.0_a ± 0.2	< 1 --
Fermentation (18 °C)	3.5_b ± 0.9	102.0_b ± 19.3	< 1 --
Fermentation (2 °C)	0.4_a ± 0.1	1.4_a ± 0.2	< 1 --

Different small letters grouping within a column indicate statistically significant differences ($p < 0.05$; Bonferroni test)

Low-oxygen stress was visualized using fluorescence kinetics and imaging. This extends the early detection of low-oxygen stress in the post-harvest of apples. However, it should be noted that the two-dimensional depiction of a three-dimensional sample using an imaging method always involves the risk of incorrect values. Therefore, the fluorescence data from the center of the image was used and the 90th percentile value was calculated. The influence of storage time was not evaluated because the experiments were performed with freshly harvested apples. At the time of the experiments, the chlorophyll content in the apple skin was still comparatively high (Schlie et al., 2023). It is known that chlorophyll content can affect fluorescence measurement in apples. Furthermore, the present study cannot make any statements about the adaptation of the fruit to low-oxygen levels during storage (Wright et al., 2012). Therefore, the reactions of fruit concerning the proposed parameters can also be different. We know that the number of repetitions with five apples per variant was small but sufficient to describe the presented methodology. The method was developed in this study. However, it remains to be shown whether this method contributes to more accurate results than non-imaging fluorescence methods in DCA storage experiments.

Conclusion

The study shows that the application of chlorophyll fluorescence kinetics in storage is possible. It has been shown that low-oxygen stress could be detected with fluorescence kinetics. The results show that there are other parameters besides *Fo* that could be used to detect low-oxygen stress in apples. At low temperatures (2 °C), the fluorescence kinetics, as well as *Fv/Fm* and *ϕPSII*, changed even before fruit's

own ethanol was accumulated. Detection of low-oxygen stress using fluorescence kinetics provides a reference for further studies on stored fruit. However, it must be considered that the experiments were carried out with freshly harvested fruit. Therefore, no statement can be made about the influence of the storage time concerning the proposed parameters.

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Declarations

Declaration of Competing Interest The authors declare that there is no conflict of interest.

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4 Generelle Diskussion

Die in der vorliegenden Arbeit präsentierten Ergebnisse liefern neue Erkenntnisse zur Chlorophyllfluoreszenz als sensorischer Parameter in der Apfellagerung. Die Generelle Diskussion ergänzt die bereits diskutierten Aspekte in den Kap. 3.1 bis 3.3. Die wesentlichen Ergebnisse der vorliegenden Arbeit sind nachfolgend aufgeführt.

1. Der Reifegrad der Apfelfrüchte, welcher mittels Fruchtfleischfestigkeit, Stärkeabbauwert, %-lösliche Trockensubstanzanteil und Säuregehalt beschrieben wird, beeinflusst den LOL signifikant. Insbesondere besteht der Einfluss direkt nach der Ernte, wenn der Stärkeabbau in den Früchten noch nicht abgeschlossen ist. An einem Teil der Apfelfrüchte, die zuvor vier Monate unter ULO-Bedingungen lagerten, konnte der LOL nicht identifiziert werden. Hierbei variierte der Anteil dieser Früchte ohne LOL Identifizierung von 12,5 % bis 41,7 % der untersuchten Äpfel (Kap. 3.1).
2. Der Chlorophyllgehalt in der Apfelschale beeinflusst die Methode der Fluoreszenzmessung. Mit abnehmenden Chlorophyllgehalt sink die Zuverlässigkeit der Fluoreszenzmessung rapide (Kap. 3.1).
3. Ein hoher Grad an Fluoreszenzheterogenität kann auf der Schale von einzelnen Apfelfrüchten bzw. zwischen den einzelnen Apfelfrüchten nachgewiesen werden. Mithilfe des bildgebenden Fluoreszenzmessverfahrens wird die räumliche Verteilung der Chlorophyllfluoreszenz (*Fo, Fm, Fv, Fv/Fm*) auf einzelnen Apfelfrüchten bei unterschiedlichen O_2-Konzentrationen in der Lagerraumatmosphäre dargestellt und beschrieben (Kap. 3.2).

4. Ferner kann der Anstieg der Grundfluoreszenz auf der Apfelschale infolge von Stress, aufgrund von zu niedrigen O_2-Konzentrationen in der Lagerraumatmosphäre, visualisiert werden. In diesem Zusammenhang ist eine Methode zur Identifizierung des LOL für ein bildgebendes Fluoreszenzmessverfahren entwickelt worden. Die neue Methode greift auf die gesamten Daten der Grundfluoreszenz eines jeden Messvorganges zurück und berücksichtigt somit die Fluoreszenzheterogenität der Fruchtprobe für die Identifizierung des LOL (Kap. 3.2).
5. Die Reaktion der Chlorophyllfluoreszenzkinetik kann für die Detektierung von Stress aufgrund von zu niedrigen O_2-Konzentrationen in der Lagerraumatmosphäre bei Äpfeln eingesetzt werden. Unter fermentativen Bedingungen zeigen die kinetischen Kurven eine Abnahme der Fluoreszenzintensität bei Anwendung der Sättigungsimpulse während der Einwirkung von aktinischem Licht (Kap. 3.3).
6. Außerdem sind die Parameter *Fv/Fm* sowie *ϕPSII_D1, ϕPSII_D2* und *ϕPSII_D3* für die frühzeitige Detektierung von Stress aufgrund von O_2-Mangel geeignet (Kap. 3.3).

Die nicht-bildgebende Fluoreszenzmessung hat sich bisher in der Apfellagerung behauptet und wird insbesondere in der DCA-Lagerung eingesetzt, um Stress aufgrund von zu niedrigen O_2-Konzentrationen in der Lagerraumatmosphäre frühzeitig erkennen und den LOL identifizieren zu können. In der DCA-Lagerung wird das Harvest-Watch^{TM} System häufig verwendet. Dieses System basiert auf der Pulsfrequenzmodulationstechnologie (PFM) und generiert den Fluoreszenzparameter *Fα* (Prange et al. 2013). Wright et al. (2008) vergleichen *Fα* mit *Fo* hinsichtlich der Reaktion auf O_2-Mangel bei der Apfelsorte ‘Honeycrisp‘. *Fo* wird mittels Pulsamplitudenmodulation (PAM) erzeugt. Das Fluoreszenzverhalten von beiden Fluorometertypen ist vergleichbar, obwohl sich *Fα* nicht völlig analog zu *Fo* zeigt. Sowohl *Fo* als auch *Fα* steigen an, wenn der O_2-Gehalt unterhalb verträglicher Werte für ‘Honeycrisp‘ sinkt. In der vorliegenden Arbeit ist die Fluoreszenz stets mit der PAM Technik mit rotem Licht (621 nm oder 625 nm) angeregt worden.

Ein Nachteil des nicht-bildgebenden Harvest-Watch™ Systems besteht darin, dass die Fluoreszenz als Punktmessung an vier bis acht Früchten durchgeführt wird. Als Output wird ein Fluoreszenzwert für die gesamte Fruchtprobe ausgegeben (Prange et al. 2013; Wright et al. 2015). Somit ist unklar, welchen Anteil die Einzelfrucht an der Fluoreszenzausbeute hat. Es werden keine Information über die Heterogenität der Fluoreszenz bereitgestellt. Das Mini-Apple-PAM™ System bietet die Möglichkeit Einzelfrüchte zu messen, jedoch handelt es sich hierbei auch um eine Punktmessung. Die Erfahrungen mit nicht-bildgebenden Fluoreszenzsystemen zeigen, dass diese Systeme bei der Messung von *Fo* nicht immer zuverlässig sind (Lichtenthaler et al. 2021). Geringe Chlorophyllgehalte in der Apfelschale können zu schwachen oder schwankenden *Fo*-Fluoreszenzsignalen führen. Die Fluoreszenzmessungen an Apfelfrüchten mit geringen Chlorophyllgehalten können mitunter scheinbar stabile Fluoreszenzsignale zeigen. Jedoch kann teilweise beobachtet werden, dass die *Fo*-Fluoreszenz auch unter fermentativen Bedingungen nicht ansteigt (O_2 von <0,1 kPa in der Lagerraumatmosphäre). Das birgt die Gefahr der fehlerhaften Interpretation von *Fo*. Hier besteht die Möglichkeit, dass der Anwender das scheinbar stabile *Fo*-Fluoreszenzsignal mit Reflektion (des Anregungslichtes) verwechselt. Hieraus könnte aufgrund von fehlerhafter O_2-Steuerung in der Apfellagerung unter Umständen hohe wirtschaftliche Schäden entstehen (Geyer und Praeger 2012). Daher ist für die sichere Handhabung der Fluoreszenz in der Apfellagerung ein Tool notwendig, um die Zuverlässigkeit von *Fo* verbessert abschätzen zu können. In den Untersuchungen mit nicht-bildgebenden Fluoreszenzsensoren wird *Fv/Fm* unter nicht-gestressten Bedingungen (≥ 1,5 kPa O_2) gemessen. Hierbei zeigen Früchte mit geringen Chlorophyllgehalten signifikant niedrigere *Fv/Fm*-Wert. Der Chlorophyllgehalt korreliert positiv mit der Abnahme von *Fv/Fm* als auch mit der Abnahme von *Fo*. Hieraus könnte geschlussfolgert werden, dass mittels *Fv/Fm* indirekt die Höhe des Chlorophyllgehaltes in der Apfelschale abgeschätzt werden kann. Dies ist umso bedeutender für die DCA-Lagerung, da der Chlorophyllgehalt in der Apfelschale die Methode der Fluoreszenzmessung massiv beeinflusst hat.

Die Änderungen des Gehalts an photosynthetisch aktivem Chlorophyll haben Zsom et al. (2001) beispielsweise bei Paprika mittels Fluoreszenzbildgebung (*Fo, Fm, Fv, Fv/Fm*) charakterisiert (Zsom et al. 2021). Insgesamt zeigen die Ergebnisse, dass Apfelfrüchte mit höheren Chlorophyllgehalten auch höhere *Fo* Werte aufweisen. Ähnliches haben Li et al. (2006) an Gerste und Nowicka (2020) an Algen gezeigt. Allerdings müssen bei der Betrachtung von Äpfeln sortenspezifische und saisonale Unterschiede berücksichtigt werden (Prange et al. 2010; Gasser and von Arx 2015).

Der Einsatz der bildgebenden Chlorophyllfluoreszenz ist nicht neu, sondern seit Jahrzehnten in der Forschung etabliert (Nedbal und Whitmarsh 2004; Lichtenthaler et al. 2005). Jedoch wird bisher die Verwendung der bildgebenden Chlorophyllfluoreszenz in der Apfellagerung in der Literatur unzureichend beschrieben. Die bildgebende Chlorophyllfluoreszenz wird in der vorliegenden Arbeit verwendet, um die Fluoreszenzheterogenität auf bzw. zwischen einzelnen Apfelfrüchten sowie die räumliche Verteilung der Chlorophyllfluoreszenz auf der Apfelschale zu untersuchen. Hierbei kann ein hoher Grad an Fluoreszenzheterogenität sowohl auf als auch zwischen einzelnen Apfelfrüchten nachgewiesen werden. Lichtenthaler et al. (2012) haben vier Fluoreszenzbanden (F440 blau, F520 grün, F690 rot, F740 dunkelrot) an hellgrünen Äpfeln (‘Braeburn‘) mit einem bildgebenden Fluoreszenzmessverfahren untersucht. Hierbei haben sie die Veränderungen der Fluoreszenzausbeute in den vier Fluoreszenzbanden und das Fluoreszenzverhältnisse während der Lagerung (4 °C; unter Normalatmosphäre) und Reifung bewertet. Die blauen und grünen Fluoreszenzbanden steigen während der Lagerung an. Die dunkelrote Bande zusammen mit dem Chlorophyllgehalt verringert sich kontinuierlich. Hingegen steigt die Intensität der roten Fluoreszenzbande F690 zunächst an (nach drei Monaten) und verringert sich anschließend (nach sechs Monaten). Aufgrund des unterschiedlichen Verhaltens der verschiedenen Fluoreszenzbanden bei Äpfeln während der Lagerung schlagen Lichtenthaler et al. (2012) vor, stets die Abbildung von mehreren Fluoreszenzbanden vorzunehmen, um die Fehleranfälligkeit der Chlorophyllfluoreszenz-technik zu reduzieren (Lichtenthaler et al. 2012).

Die Anregung verschiedener Fluoreszenzbanden wäre auch in der DCA-Lagerung denkbar. Jedoch ist die praktische Umsetzung vermutlich aufwändig. Yoo et al. (2014) setzen die bildgebende Chlorophyllfluoreszenz ein, um einen Indikator zur Beurteilung der Frische von Apfel- und Kiwifrüchten zu finden. Die kühlgelagerten Früchte (4 °C; unter Normalatmosphäre) zeigen stabile *Fv/Fm*-Werte von ca. 0,8 auch nach 30 Tagen Lagerung. Dagegen zeigen die verschiedenen mit Hitze (42 °C und 25 °C) behandelten Varianten nach 6 bis 16 Tagen eine Reduzierung von *Fv/Fm*. Ein ähnliches Verhalten zeigen die Apfel- und Kiwifrüchte bei den Parameter *ϕPSII* und *NPQ* unter Hitzestress. Die 4 °C Variante zeigt allerdings stabile *ϕPSII* und *NPQ* Werte (Yoo et al. 2014). Herppich et al. (2020) untersuchen die Auswirkungen von Heißwasserbehandlungen auf die Unversehrtheit des Photosyntheseapparats. Für ‘Fuji‘, ‘Granny Smith‘ und ‘Greenstar‘ erweisen sich Temperaturen über 60 °C als kritisch (bei ‘Braeburn‘ bereits ab 55 °C), wobei die Fluoreszenzmessungen eine Abnahme von *Fv/Fm* zeigen (Herppich et al. 2020). In weiteren Bereichen der Nacherntephase von Äpfeln wird mit bildgebenden Fluoreszenzmessverfahren experimentiert, beispielsweise um Stippigkeit vor dem Auftreten visueller Symptome frühzeitig erkennen zu können (Ciscato et al. 2001; Lötze et al. 2006) oder auch das frühzeitige Erkennen von *Gloeosporium*-Fruchtfäulen (Pieczywek et al. 2018). Der Einsatz der bildgebenden Fluoreszenz wird auch zur Detektierung von Stress an Zitrusfrüchten eingesetzt. Obenland und Neipp (2005) stellen dar, dass mithilfe der Parameter *Fo*, *Fm* und *Fv/Fm* geschädigte Bereich auf der Fruchtschale von grünen Zitronen infolge einer Heißwassertauchung (55 °C für 5 Minuten) identifiziert werden können, bevor sichtbare Symptome auftreten. Nedbal et al. (2000b) können bei Zitronen mittels bildgebender Chlorophyllfluoreszenz mit Schimmelpilz befallene Bereiche auf der Fruchtoberfläche infolge der physiologischen Beeinträchtigungen von nicht befallenen Bereichen unterscheiden.

Neben der Chlorophyllfluoreszenz gibt es weitere Verfahren, um Stressreaktionen von Kernobst aufgrund von zu niedrigen O_2-Konzentrationen in der Lagerraumatmosphäre zu detektieren (Thewes et al. 2021). Hierzu zählt die Überwachung von Fermentationsindikatoren. Hierbei werden unerwünschter Fermentationsprodukte im Fruchtsaft gemessen oder über Indikatoren in der Lagerraumluft identifiziert (Schouten et al. 1998; Veltman et al. 2003; Thewes et al. 2022a). Diese Methoden sind mit einem größeren Aufwand und Analysen verbunden. Zudem geben sie häufig Informationen über Fermentationsereignisse im Lagerbestand im Kontext einer Rückschau an (Eslami-Jahromi et al. 2021). Des Weiteren kann mit der Ermittlung des Atmungsquotienten (Respiratory Quotient = RQ) Stress bei Apfelfrüchten nachgewiesen werden. Bei diesem System werden der O_2-Verbrauch und die CO_2-Produktion der Früchte berücksichtig. Unter fermentativen Bedingungen kommt es zu signifikanten Änderungen der O_2-Verbrauchsrate und der CO_2-Produktionsrate (Weber et al., 2015; Bessemans et al., 2016; Weber et al., 2020; Thewes et al. 2022b). In neueren Studien wird diese Methode weiterentwickelt, da Undichtigkeiten in den Apfellageräumen (O_2 Eintrag von außen) die RQ Messergebnisse beeinflusst haben. In der weiterentwickelten Methode wird während der Lagerung die Fruchtatmung nun ausschließlich aus Basis der CO_2-Produktionsrate der Früchte überwacht. Die CO_2-Produktionsrate ändert sich bei Anwesenheit von Fermentationsprozessen charakteristisch im Vergleich zur CO_2-Produktion unter aeroben Lagerungsbedingungen (Thewes et al. 2020; Büchele et al. 2023). Gasser et al. (2021) haben die Methoden Chlorophyllfluoreszenz und RQ miteinander verglichen. Hierbei haben sie eine Konvergenz dieser beiden Methoden hinsichtlich der Identifizierung von Stressreaktionen aufgrund von zu niedrigen O_2-Konzentrationen in der Lagerraumatmosphäre bei den Sorten ‘Red Delicious‘ und ‘Granny Smith‘ festgestellt (Gasser et al. 2021). Ferner erlangen Verfahren, wie die Anwendung der Spektroskopie im sichtbaren Licht (400 nm bis 750 nm) und nahen Infrarot (750 nm bis 2500 nm) in der Nacherntephase (Lagerung) zunehmend an Bedeutung, jedoch bisher ohne konkrete Praxisanwendungen (Camps et al. 2007; Guo et al. 2020; Janssen et al. 2020; Walsh et al. 2020).

Die eingeführte Methode der Histogramm Division berücksichtigt die Heterogenität der Fluoreszenz, um den LOL zu identifizieren. Jedoch muss berücksichtigt werden, dass bisher nur die Fluoreszenz von Einzelfrüchten bildgebend gemessen wurde. Daher sollte im nächsten Schritt die Messung der bildgebenden Fluoreszenz auf die (offene) Fruchtoberfläche einer Apfelkiste ausgeweitet werden. Die entwickelte Methode zur Identifizierung des LOL kann hierfür verwendet werden. Ferner ist zu berücksichtigen, dass die Fluoreszenzmessungen an den Einzelfrüchten in einem Zeitintervall von sieben bis zehn Stunden erfolgten. Daher bleibt offen, wie sich die Fluoreszenzheterogenität auf der Apfeloberfläche bei Langzeitmessungen (Wochen bis Monate) im Lagerraum verhält. Ebenso stellt sich die Frage, welche Auswirkungen die Messungen der bildgebenden Chlorophyllfluoreszenz auf die Fruchtqualität und die Lagerführung haben. Histologische Untersuchungen hinsichtlich des Chlorophyllgehaltes der Apfelschale wurden im Rahmen der vorliegenden Arbeit nicht durchgeführt und sollten in weiteren Forschungsarbeiten erarbeitet werden. Darüber hinaus sollte in weiteren Forschungsarbeiten geprüft werden, ob laserbasierte Fluoreszenzmesssysteme in Kombination mit Bildverarbeitung verwendet werden können. Bei der Entwicklung einer bildgebenden Praxisanwendung sollten weitere Fluoreszenzparameter neben *Fo* gemessen werden. Die Kombination von mehreren Fluoreszenzparametern sowie die Methode der Fluoreszenzbildgebung bietet die Möglichkeit, die Fehleranfälligkeit von nicht-bildgebenden Fluoreszenzmessverfahren zu überwinden und die Gefahr von Fehlinterpretationen zu verringern (Lichtenthaler 2021).

5 Schlussfolgerung

Die vorliegende Arbeit zeigt welche Ursachen für das unterschiedliche Stressverhalten von einzelnen Äpfeln bei niedrigen O_2-Konzentrationen in der Lagerung verantwortlich sind. Der Reifegrad der Äpfel, welcher mittels Fruchtfleischfestigkeit, Stärkeabbauwert, %-löslicher Trockensubstanzanteil und Säuregehalt beschrieben wird, beeinflusst den LOL signifikant. Hierbei besteht der Einfluss insbesondere direkt nach der Ernte, wenn der Stärkeabbau in den Äpfeln noch nicht abgeschlossen ist. Neben der Fruchtreife muss auch der Chlorophyllgehalt in der Apfelschale berücksichtigt werden, da das Chlorophyll die Methode der Fluoreszenzmessung massiv beeinflusst. Da die Fluoreszenz nur an wenigen Äpfeln gemessen wird, stellvertretend für den gesamten Lagerraumbestand, ergeben sich hieraus besondere Anforderungen für die DCA-CF-Lagerung. Hierzu gehört eine frühzeitige Ernte im optimalen Erntefenster (Chlorophyll in der Apfelschale) sowie die sortenreine Lagerung von Äpfeln mit gleichem Stressverhalten. Ferner zeigen die Ergebnisse, dass der Fluoreszenzparameter *Fv/Fm* die Auswahl einer repräsentativen Fruchtprobe für die Fluoreszenzmessung unterstützt. Äpfel mit $Fv/Fm < 0{,}7$ sind für die Fluoreszenzmessung nicht zu berücksichtigen.

Die Messungen mit dem bildgebenden Fluoreszenzmessverfahren zeigen einen hohen Grad an Fluoreszenzheterogenität, sowohl auf als auch zwischen einzelnen Apfelfrüchten. Die Fluoreszenzbilder der Äpfel beinhalten einen Rundheitseffekt, der aufgrund der heterogenen räumlichen Verteilung der Chlorophyllfluoreszenz (*Fo*, *Fm*, *Fv*) deutlich wird. Dieser Rundheitseffekt wird in der neuen Methode zur Identifizierung des LOL für ein bildgebendes Fluoreszenzmessverfahren berücksichtigt. Die Methode greift auf die gesamten Fluoreszenzdaten eines jeden Messvorganges zurück und gibt die Daten als Histogramm wieder, womit die Fluoreszenzheterogenität der Fruchtprobe einbezogen wird. Der Anstieg der Grundfluoreszenz (*Fo*) infolge von Stress aufgrund von zu niedrigen O_2-Konzentrationen in der Lagerraumatmosphäre führt zu einer Rechtsverschiebung der Histogramme.

Damit können geringfügige Änderungen der Fluoreszenz umgehend auf Basis einer großen Datengrundlage (mehrere 10 000 Fluoreszenzinformationen) erkannt werden, im Gegensatz zur Punktmessung der nicht-bildgebenden Fluoreszenz. Des Weiteren reagiert die Chlorophylfluoreszenz wesentlich sensitiver auf Stressereignisse als andere DCA Verfahren. Der Anstieg der Fluoreszenz erfolgt fast in Echtzeit, während Verfahren zur Messung von Fermentationsprodukten in Raumluft oder Fruchtsaft bzw. die RQ-Ermittlung nur Informationen über Fermentationsereignisse im Kontext einer Rückschau liefern.

Darüber hinaus zeigt die Reaktion der Chlorophyllfluoreszenzkinetik (Apfelschale) charakteristische Änderungen bei niedrigen O_2-Konzentrationen in der Lagerraumatmosphäre. Unter fermentativen Bedingungen zeigen die kinetischen Fluoreszenzkurven eine Abnahme der Fluoreszenzintensität bei Anwendung der Sättigungsimpulse während der Einwirkung von aktinischem Licht. Die Ergebnisse belegen, dass neben *Fo* auch weitere Fluoreszenzparameter zur Erkennung von Stress bei Äpfeln verwendet werden können. Die Parameter *Fv/Fm*, *ϕPSII_D1*, *ϕPSII_D2* und *ϕPSII_D3* sind für die frühzeitige Detektierung von Stressreaktionen bei O_2-Mangel geeignet. Die simultane Verwendung von mehreren Fluoreszenzparametern erweitert die Früherkennung von O_2-Mangel im Nacherntebereich von Äpfeln. Die Kombination von der Fluoreszenzbildgebung und das Verwenden von mehreren Fluoreszenzparametern reduziert die Fehleranfälligkeit von Fluoreszenzmessverfahren und verringert somit die Gefahr von Fehlinterpretationen.

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Auszeichnungen und Stipendien

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Publikationsliste

Journal und Manuskript

Schlie, T. P., Köpcke, D., Rath, T., Dierend, W. (2020). Die Bedeutung von Eigenschaften der einzelnen Apfelfrucht für die DCA-CF-Lagerung bei der Sorte ‘Elstar, PCP‘. *Erwerbs-Obstbau*, 62, 57–60. https://doi.org/10.1007/s10341-019-00463-z

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Ogniwek, T. P., Rath, T., Dierend, W. (2017). Strahlungsbasierte Bekämpfung von Fruchtfäuleerregern am Apfel. In: Schriftenreihe Band 32 der 51. Gartenbauwissenschaftliche Jahrestagung der Deutsche Gartenbauwissenschaftliche Gesellschaft e.V. (DGG), Seite 70.

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