Enhancement of aroma and flavour volatiles in apple juice

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Abstract

Aroma typical to apples develops during ripening and is comprised of a large range of volatile chemical compounds from several chemical classes. Previous research has established that exposing apples to hypoxic conditions induces changes in volatile concentrations; acetaldehyde and ethanol accumulate to high concentrations and after return to aerobic conditions ethyl esters increase and non-ethyl esters decrease. The present study investigated the effect of short term-hypoxic treatments on the enhancement of ethyl esters and decrease in non-ethyl esters with respect to: organoleptic changes in apple aroma induced by exposure to hypoxia; the influence of temperature and time at 0 °C before treatment on the magnitude of enhancement of ethyl esters after exposure to hypoxia; the effect of cultivars and ripeness stage on types and quantities of ethyl esters enhanced after exposure to hypoxia. Brief periods of hypoxia at ambient temperatures have potential for disinfestation treatments or as pre-treatments to maintain fruit quality during extended storage.

Volatile compounds were extracted from 20 mL aliquots of apple juice with an equal volume of diethyl ether:*n*-pentane (2:1 v/v), vigorously stirred for 3-5 seconds, frozen at -18 °C to separate solvent and aqueous phases, concentrated with a fast stream of oxygen free nitrogen (200 $mL \cdot min^{-1}$) to 200 µL and analysed by gas chromatography. Apple juice could be held in ice or air up to 256 minutes without loss of volatile compounds. Loss in solvent washes was 76.5% for octyl acetate and recoveries during concentration of the solvent extract ranged from 2.5% for ethyl acetate to 86.4% for *trans*-2-hexenal. Solvent extraction was simpler, faster, extracted more compounds, and had better reproducibility than dynamic headspace extracts obtained using Tenax® traps.

Nine cultivars of apples, Cox's Orange Pippin, Fuji, Golden Delicious, Granny Smith, Pacific Rose, Red Delicious, Royal Gala, Splendour and Southern Snap were exposed to 100% carbon dioxide for 24 h at 20°C. Apples exposed to hypoxia had concentrations of acetaldehdye, ethanol, ethyl acetate and ethyl esters consistently enhanced while concentrations of acetate esters and aldehydes were depressed. Maximum ethyl ester enhancement occurs within 2 to 3 d after removal from hypoxia. Exposure to hypoxia for 24 h at 20 °C did not affect rates of softening or induce physiological damage. Cultivars varied considerably in response to hypoxic treatment with Cox's Orange Pippin and Golden Delicious having the least and Fuji and Red Delicious the greatest enhancement in ethyl esters. Fruit exposed to hypoxia had larger odour unit scores than control fruit suggesting that such changes in volatile concentration may affect the aroma and/or flavour.

Fuji and Royal Gala apples were exposed to 100% CO_2 for 24 h, at 10, 15, 20 or 25 °C and maintained at treatment temperature for up to 14 d. Carbon dioxide and ethylene production and firmness were proportional to temperature but were unaffected by exposure to hypoxia. Ethyl esters were enhanced at all temperatures at differential rates according to cultivar. Apples treated and maintained at 10 °C had the greatest overall enhancement of ethyl esters and the

least decrease in other esters compared to apples at 15, 20 or 25 °C. This enhancement in volatiles persisted for up to 10 d after removal from hypoxia. Best maintenance of apple quality after treatment with hypoxia is at low temperatures suggesting that apples treated with hypoxia and maintained below 15 °C would have enhanced volatile concentration.

Noncooled Fuji and Royal Gala apples at preclimacteric to postclimacteric ripeness stages were exposed to 100% CO_2 for 24 h at 20 °C for up to 14 d. A batch of the same fruit were placed at 0 °C, removed to 20 °C and exposed to hypoxia at monthly intervals for up to 5 months. Exposure to hypoxia decreased carbon dioxide production in Fuji apples at the preclimacteric and rising climacteric stages and at the climacteric. Respiration rate, ethylene production and volatile concentration of RG apples were not affected by exposure to hypoxia at any stage of ripeness or period at 0 °C. After exposure to hypoxia Fuji apples had enhanced ethyl esters at the preclimacteric and rising climacteric stages and after being at 0 °C for up to 5 months. Volatile concentrations were lower in apples maintained at 0 °C compared to noncooled apples. Apples at 0 °C had the greatest enhancement of ethyl esters after hypoxia suggesting that exposure to low temperatures did not just slow volatile biosynthesis but had an additional effect on volatile biosynthesis.

Apple aroma consists of mainly low molecular weight esters produced by esterification of alcohol's by alcohol acyl CoA transferase (AAT) where acyl CoA's are substrates. Increased esterification activity in apples returned to air, following a hypoxic treatment, is due possibly to enhanced AAT activity or to competitive inhibition of other alcohols by ethanol. Concentrations of acetate and ethyl esters from skin disks of Cox's Orange Pippin, Fuji, Golden Delicious, Granny Smith, Pacific Rose, Red Delicious, Royal Gala, Splendour and Southern Snap apples exposed to 100% CO_2 for 24 h at 20 °C, were compared to disks from control fruit, after addition of C_2 to C_6 alcohols, either individually, or as a mixture in equimolar amounts to the disks. Ethanol added as an individual alcohol induced high ethyl acetate concentrations, but when added as part of a mixture, little ethyl acetate was produced indicating substrate preference was for longer chain alcohols. Apple cultivars had four patterns of change in ester production after exposure to hypoxia: increased acetate and ethyl esters; increased acetate esters and decreased or no change in ethyl esters; no change or decreased acetate esters and increased ethyl esters; no change or decreased acetate esters and decreased or no change in ethyl esters, implying that AAT activity is affected differentially by hypoxia. Hypoxia induces changes in capacity to produce esters which last up to 7 d indicating that pre-storage treatments using hypoxia has the potential to change the aroma profile of apples.

Juice of Fuji and Royal Gala apples exposed to a brief period of hypoxia (100% CO_2 for 24 h at 20 °C) and ripened at 20 °C for up to 8 d, was analysed by taste panels using quantitative descriptive analysis. Hypoxia induced large increases in ethyl esters including ethyl butanoate and ethyl-2-methyl butanoate in Fuji apples but not in Royal Gala apples. There was no difference in average panellist scores for sensory characteristics for Fuji and Royal Gala apples at any sampling time.

The lack of difference may have been due to large variation between panellist's assessment of sensory characteristics and/or inability to assess aroma, flavour and sweetness independently. A number of individual volatiles correlated with aroma in juice from apples exposed to hypoxia, including hexan-1-ol, butyl acetate, 2 methyl butyl acetate and propyl butanoate for Fuji; and ethanol, ethyl acetate, propyl acetate and propyl butanoate for Royal Gala. Multivariate analysis indicated that panellists associated increased ethyl esters with off flavour rather than more intense apple aroma. This could have been due to juice from apples exposed to hypoxia having a different apple-like character than control fruit which did not fit the definition of apple aroma used to train panellists.

The enhanced ethyl ester concentrations in fruit exposed to hypoxia are probably due to large increases in ethanol concentration that competitively inhibited formation of non-ethyl esters. Golden Delicious and RG did not have enhanced concentration of ethyl esters and/or decreases in acetate ester concentration even though fermentation volatiles were enhanced to high concentrations and ethyl acetate increased to concentrations similar to those found in fruit which had enhanced ethyl esters. The mechanism producing ethyl acetate and ethyl esters in GD and RG was probably different from that in CO, FU, PR, RD, SP, SS cultivars. Therefore, after exposure to hypoxia, additional factors influence changes in volatile concentration other than the increased pool of substrate available for esterification.

A possible mechanism by which hypoxia affects ester biosynthesis is that under hypoxic conditions cytoplasmic pH falls below the optimum of 7 to 8, inducing increased ADH activity and synthesis and producing large increases in ethanol concentration. Ester biosynthesis is suppressed during hypoxia leading to increased alcohol and aldehyde concentrations creating a pool of substrates that could be rapidly utilised by AAT on return to aerobic conditions. It is possible that AAT activity or concentration changes are induced by hypoxic conditions. The different capacity of apple cultivars to esterify alcohols from control and hypoxic treated fruit may be due to changes in substrate specificity of either, or both, newly induced ADH and AAT.

Exposure to hypoxia consistently caused increases in ethyl esters in several apple cultivars. The practical uses for treatments where apples are exposed to hypoxia for 24 h include: disinfestation treatments, manufacture of apple juice concentrates, enhancement of aroma in apples maintained in long term air or controlled atmosphere storage and as a tool for examining volatile biosynthesis.

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I dedicate this thesis to my son Alexander for whom this all seems worthwhile.

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List of Abbreviations

-p	without addition of precursor
+p	with addition of precursor
AAT	Alcohol acylCoA transferase
ACP	Anaerobic compensation point
ADH	Alcohol dehydrogenase
ATP	Adenosine triphosphate
C	Control
CA	Controlled atmosphere
C_2H_4	Ethylene
CoA	Coenzyme A
CO_2	Carbon dioxide
CO	Cox's Orange Pippin
d	Day
FID	Flame ionisation detector
FU	Fuji
GC-MS	Gas chromatography-mass spectroscopy
GD	Golden Delicious
GLC	Gas liquid chromatograph
GS	Granny Smith
h	Hour
LDH	Lactate dehydrogenase
LOX	Lipoxygenase
N_2	Nitrogen
NADH	Nicotinamide adenine dinucleotide-reduced
O_2	Oxygen
PDH	Pyruvate dehydrogenase
PR	Pacific Rose
QDA	Quantitative descriptive analysis
RD	Red Delicious
RG	Royal Gala
RH	Relative humidity
SP	Splendour
SPME	Solid phase microextraction
SS	Southern Snap
TCA	Tricarboxylic acid