Impact of flavour solvent, (propylene glycol or triacetin) on vanillin, 5-hydroxymethyl-furfural, 2,4-decadienal, 2,4-heptadienal, structural parameters and sensory perception of shortcake biscuits over accelerated shelf life testing

NI YANG [1], JOANNE HORT [1], ROBERT LINFORTH [1], KEITH BROWN [2], STUART WALSH [2] AND IAN D. FISK [1]*

[1]Division of Food Sciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire, LE12 5RD, UK

[2]Aromco Ltd, Bell Farm Industrial Park, Nuthampstead, Hertfordshire, SG8 8ND, UK

* Corresponding Author

Division of Food Sciences, University of Nottingham, Sutton Bonington Campus, Sutton Bonington, Loughborough, Leicestershire, LE12 5RD, United Kingdom.

Tel: +44 (0)115 951 6222. Fax: +44 (0)115 951 6142.

E-mail: joanne.hort@nottingham.ac.uk

ABSTRACT

The influence of choice of flavour solvent, propylene glycol (PG) or triacetin (TA), was investigated during accelerated shelf life (ASL) testing of shortcake biscuits. Specifically, the differential effect on the stability of added vanillin, the natural baked marker compound 5-hydroxylmethyl-furfural (HMF), specific markers of oxidative rancidity (2,4-decadienal, 2,4-heptadienal), and the structural parameters of hardness and fracturability. Significantly more HMF was formed during baking of biscuits prepared with TA, these biscuits were also more stable to oxidative degradation and loss of vanillin during ageing than biscuits prepared with PG. Fresh TA biscuits were significant more brittle than fresh PG biscuits. There was no impact of solvent choice on hardness. Sensory evaluation of hardness, vanilla flavour and oily off-note was tested during ASL testing. There was no significant impact of storage on sensory ratings for either the PG or TA biscuits.

Keywords: Aroma; biscuit; shelf life; flavour solvent

Highlights:

- Shortcake biscuits were manufactured with flavour solvent and vanillin
- Vanillin was lost during accelerated shelf life testing
- Oxidative breakdown products were generated during accelerated shelf life testing
- Biscuits were more stable when propylene glycol was replaced with triacetin

INTRODUCTION

A consistent and stable flavour within a food or drink is important for manufacturers to maintain both brand identity and to facilitate repeat purchase (Heenan, Hamid, Dufour, Harvey, & Delahunty, 2009). Vanillin (4-hydroxy-3-methoxybenzaldehyde) is a common flavour ingredient for food products (Sinha, Sharma, & Sharma, 2008) and its stability has been studied comprehensively in many food systems including dairy products (Anklam, Gaglione, & Muller, 1997; Gassenmeier, 2003; Graf & De Roos, 1996). Bakery products are also a major application route for vanillin and, to date, only limited data has been published on the stability of vanillin in this sector. When comparing bakery products, some are consumed shortly after production (breads, cakes or pastries) and some, such as biscuits, have a relatively long shelf-life (Kilcast & Subramaniam, 2000), and therefore require a more stable flavour preparation.

The physical and sensory characteristics of commercial biscuits vary depending on the particular recipe and manufacturing process. Short-cake biscuits are produced using sugar, flour and shortening, and produce a product that has a crumbly texture as a result of the inhibition of formation of protein (gluten) strands by its high fat content (Chevallier, Della Valle, Colonna, Broyart, & Trystram, 2002).

During the preparation of biscuit products, vanillin is dissolved in a flavour solvent to make a vanilla flavouring, which is then added into the dough and mixed to enhance dispersal. Propylene glycol (1,2-propanediol, PG) is one of the most widely used flavour solvents as it has a broad range of applications and is relatively inexpensive; a common alternative is triacetin (1,2,3-triacetoxypropane, TA) which

46 can be used for specific applications where propylene glycol is ineffective. For
47 example TA is used to facilitate the addition of flavour during chewing gum
48 manufacture (Potineni, 2008).

49 PG is a colourless organic diol with a boiling point of 188 ^{o}C and vapour pressure
50 of 0.129. TA is a triester of glycerol and acetic acid, has a higher boiling point
51 (260 °C) and is less volatile (vapour pressure = 0.00248) than PG.

52 During baking, there are a large number of complex interacting chemical reactions
53 and physical processes that lead to the generation of the final biscuit flavour.
54 Chemical processes include Maillard chemistry, caramelisation and lipid oxidation
55 (Ait Ameur, Rega, Giampaoli, Trystram, & Birlouez-Aragon, 2008). Physical
56 processes may include volatilisation, physical binding, physical entrapment and the
57 localisation and redistribution of reactants and aroma compounds across the matrix
58 (de Roos, 2006). The broad range of these reactions makes the prediction of the final
59 chemical composition of baked products complex, although through retrospective
60 analysis, key physical and chemical traits can be understood and managed.

61 Aroma compounds present in solid foods are, by definition, volatile and therefore
62 have the potential to be lost through volatilisation. In addition, aroma compounds may
63 react with themselves, interact with structures within a product (Fernández-Vázquez,
64 Linforth, Hort, Hewson, Vila, Heredia Mira, et al., 2013) or other ingredients (Ian D.
65 Fisk, Boyer, & Linforth, 2012; I. D. Fisk, Linforth, Taylor, & Gray, 2011; Kant,
66 Linforth, Hort, & Taylor, 2004; Tietz, Buettner, & Conde-Petit, 2008), oxidative
67 degradation may occur, as initiated by free radicals, metal ions or oxygenated species
68 (Chapman, Rosenberry, Bandler, & Boor, 1998; Frankel, 1985), and migration
69 between phases of the food matrices (Given, 2009) and the product headspace may

occur to varying degrees (I. D. Fisk, Kettle, Hofmeister, Virdie, & Silanes Kenny, 2012) over different time points (Yu, Macnaughtan, Boyer, Linforth, Dinsdale, & Fisk, 2012). During storage, the more mobile components of a food may interact with the packaging which may lead to changes in the concentration of flavour precursors, desirable food flavours and off-notes.

5-Hydroxylmethyl-furfural (HMF) is a commonly formed compound during baking and is often used as a marker of the thermal degradation of sugars (Ait Ameur, Rega, Giampaoli, Trystram, & Birlouez-Aragon, 2008). HMF can be subsequently lost over time due to volatility and has the ability to bind with other compounds.

During product ageing, the process of lipid oxidation will lead to the generation of a wide range of hydroperoxides which can then degrade to form secondary oxidation products. In the example of linolenic acid, volatile secondary products such as 2,4-decadienal (Ullrich & Grosch, 1987) and 2,4-heptadienal (Dixon & Hammond, 1984) may be formed and can therefore be used as marker compounds for the progression of lipid oxidation. These compounds were found in foods with oxidised and stale flavours (Saison, De Schutter, Uyttenhove, Delvaux, & Delvaux, 2009), and can contribute an oily off-note (Josephson & Lindsay, 1987).

The focus of this study was to examine the influence of flavour solvent (PG or TA) on the chemical and physical stability and key sensory attributes of shortcake biscuits. The specific objective was to explain the interaction of flavour solvent and time (ageing) on the stability of added vanillin, a natural baked marker compound (HMF), specific markers of oxidative rancidity (2,4-decadienal, 2,4-heptadienal), structural parameters (hardness, fracturability), and the sensory perception of hardness, vanilla flavour and oily off-note on standardised shortcake biscuits.

94

MATERIALS AND METHODS

Chemicals

Food-grade vanillin, propylene glycol (PG) and triacetin (TA) were supplied by Aromco Ltd (Nuthampstead, UK). Two simple vanilla flavours were made by mixing vanillin (10 % w/w) with PG or TA as the flavour solvent, in both cases vanillin was soluble in the flavour solvent. Both flavourings were made on the day of application. The standard application dosage for both flavourings was 0.2 % w/w in the biscuit dough (i.e. 200 ppm of vanillin was added initially).

The internal standard (IS) consisted of 3-heptanone (≥ 98 %, Acros Organics, New Jersey, USA) for GC-MS detection and acetovanillone (≥ 98 %, SAFC Supply Solutions, St. Louis, USA) for HPLC detection. Methanol (HPLC Grade ≥ 99.9 %) was purchased from Fisher Scientific UK Ltd, Loughborough, UK. Remaining chemicals, unless specified, were purchased from Sigma Aldrich, UK: 5-hydroxylmethyl-furfural (HMF, purity ≥ 99 %), acetic acid (purity ≥ 99.85 %), 2,4-decadienal (85 % purity) and 2,4-heptadienal (purity > 88 %).

Preparation of Standard Biscuits

A fat base was made from 15 g shortening, 15 g icing sugar, 2 g invert sugar, 0.3 g skimmed milk powder, 0.3 g salt, 0.3 g lecithin. Shortening was supplied by Cardowan Creameries Ltd (Glasgow, UK), and other ingredients were supplied by C Holland & Sons Ltd (Royston, UK). All the above ingredients were blended by a spade blender (Hobart, Windsor, UK) for 2 min of continuous mixing. A water base was prepared by dissolving 0.14 g sodium bicarbonate and 0.03 g ammonium bicarbonate (C Holland & Sons Ltd, Royston, UK) in 11 g tap water. Standard dough

was made by adding biscuit flour (56 g, Rank Hovis, High Wycombe, UK) into the fat base and then gradually adding the water base by continuous mixing with a spade blender until the dough was smooth. Biscuit flour contained 9% protein. The flavouring was then added and the mixture further blended for 2 min.

Two batches of dough were made – one with vanillin-PG flavouring added and another with vanillin-TA flavouring added. Each dough preparation was rolled to 40 mm thickness using a Pastry Brake (Seewer Rondon, Burgdorf, Switzerland) and shaped by a model cutter (36 mm diameter, round with fluted edge) to produce individual biscuits. The biscuits with PG and TA flavouring were positioned in alternating rows, with equal separating distances on the same tray to reduce baking variation. Biscuits were baked (Deck Oven, Sveba-dahlen, Fristad, Sweden) at 230 °C for 8 min, dried (100 °C) for 3 min, and then the baking tray was removed from the oven to allow the biscuits to cool (25 °C) for 10 min. Biscuits at the edge of the tray were discarded to minimise baking variation that was known to occur in these positions (Yang, Hort, Linforth, Taylor, Brown, Walsh, et al., 2011). All biscuits were stored in sealed non-permeable aluminium bags.

Shelf-life Test

Control samples were stored at -80 °C and the remaining sample sets were stored at 20 °C, 32.5 °C and 45 °C in scientific ovens (Sanyo Scientific Oven, Loughborough, UK). Triplicate samples were removed weekly from each storage condition and stored in sealed non-permeable aluminium bags at -80 °C. All samples were tested together at the end of the storage test in a randomised order for instrumental analysis.

Biscuits for sensory analysis were baked at the same time as those for instrumental analysis. Half of them, control samples, were stored at -80 °C, the other half were divided into 3 and stored at 45 °C in the laboratory oven (Sanyo Scientific Oven, Loughborough, UK) for 3, 6, and 8 weeks respectively, these were subsequently tested as detailed below. Three sessions of sensory tests were carried out for the stored biscuits at each of the time points. Control samples were removed from the freezer four hours before each test to allow them to thaw at room temperature, and the test biscuits were also allowed to cool to room temperature.

Sample Extraction

Intact biscuit (3 g) was ground and a 1 g sample was weighed for extraction. Every biscuit sample was extracted with 10 ml methanol, and 100 µl of the internal standard (IS) was added prior to extraction. The IS consisted of 3-heptanone (123 µl) and acetovanillone (100 mg) in 100 ml methanol. The mixed samples were placed on a roller mixer (Thermo Scientific, Tube roller Spiramix 10) for 30 min, and then centrifuged at 1300 x g for 20 min at 5 °C (Thermo CR3i Multifunction Centrifuge, KeyWrite-DTM). The upper solvent layer was isolated and 1 ml of the extract was filtered (Nylon Syringe-Filter 4 mm 0.4 µm) into 2 ml amber vials, capped with Teflon coated lids and analysed by HPLC and GC-MS as appropriate.

Quantification of non-volatile compounds (HPLC)

A HPLC (Alliance® Waters 2095, Waters Corporation, Massachusetts, USA) fitted with a Photodiode Array Detector (PDA, Waters 996) was used. Compounds were measured at an absorption wavelength of 270 nm and separated by a C18 column (C18 Techsphere, 250 x 4.6 mm, Thermo Scientific, Manchester, UK). The

instrumental settings were as follows: injection volume 10 µl; flow rate 1 ml per min; gradient elution with A) water (1 % acetic acid) and B) methanol (ramped from 20 % B v/v to 50 % B v/v over 30 min then to 100 % B v/v over 1 min and held for 2 min). The chromatography data was analysed by Millenium[32] software (Waters, USA).

A calibration curve was prepared using standard solutions in methanol at three concentration within the range 0-20 µg/ml. Samples were analysed in triplicate, coefficient of determination (R^2) exceeded 0.99 in all cases and in the instances whereby analyte concentration was outside of the linear calibration region, samples were diluted, further details are provide in Yang (2012). Concentration was calculated from the ratio of the peak area of the compound of interest to the peak area of the internal standard in both samples and standards. Retention times for 5-hydroxy-methyl-furfural, vanillin and acetovanillone were 5.11 min, 15.00 min and 17.93 min respectively.

Quantification of volatile compounds (GC-MS)

Separation and detection of the aroma compounds of interest was achieved with splitless injection of the methanol extract (1 µL) using a Trace GC Ultra (Thermo Scientific, Manchester, UK) coupled with a DSQII mass spectrometer (Thermo Scientific). The inlet temperature was 200 °C and data acquisition was started at 5 min in full scan mode with a mass range of 30-250 m/z, scan rate 500 $m/z.s^{-1}$. Separations were performed on a ZB-Wax column - length 30 m, inner diameter 0.25 mm, and film thickness 1 µm (Phenomenex Inc., Macclesfield, UK).

The initial oven temperature was set at 40 °C for 1 min then increased at 8 °C min^{-1} to 250 °C and held at 250 °C for 6 min. Concentrations of analyte were

187 calculated using ‘Xcalibur’ software (Thermo Scientific, UK) and identification was
188 verified by comparing to the retention times and mass spectrum of authentic standards.
189 Retention time and specific m/z used for quantification are detailed below for each
190 analyte: 3-heptanone (8.48, 114 m/z); 2,4-heptadienal (15.04, 81 m/z); propylene
191 glycol (16.34, 45 m/z); 2,4-decadienal (20.19, 81 m/z); triacetin (23.59, 103 m/z);
192 5-hydroxy-methyl-furfural (29.54, 126 m/z) and vanillin (30.05, 152 m/z), although
193 for 5-hydroxy-methyl-furfural and vanillin HPLC was used for quantification.

194 **Texture Analysis**

195 A 3-point bending rig (HDP/3PB) was used with TA XT texture analyser (Stable
196 Micro Systems Ltd, Surrey, UK) to measure force in compression using a heavy duty
197 platform (HDP/90). The inner gap distance between two plates was 17.5 mm and the
198 upper blade linked to the probe moved vertically with 3.25 mm either side of the
199 plate. The biscuit was placed on the top of two plates centrally. Pre-test speed was 1.0
200 mm/s, test speed was 3.0 mm/s and post-test speed was 10.0 mm/s. The force exerted
201 to break the biscuit is defined as hardness (g) and the distance to the point of break is
202 termed the resistance of the sample to bend - fracturability (mm).

203 **Sensory Evaluation**

204 In order to define the key attributes that could change significantly during biscuit
205 storage, a preliminary trial comparing fresh biscuits with biscuits stored for 7 weeks at
206 45 °C was informally conducted by 3 subjects in our laboratory to define the sensory
207 attributes that were followed later. Three attributes were identified as changing on
208 storage: brittleness (fracturability), vanilla flavour and an oily off-note.

209 Paired comparison tests (BS ISO 5495:2005) were performed by 30 biscuit

consumers (aged 21-31 years old, 14 male, 16 female) to determine if sensory changes had occurred in both the TA and PG biscuits during storage. Prior to the test, the definition and test method for each attribute was clarified to the panellists. Since fracturability is difficult for panellists to understand, a more 'brittle' biscuit was described as being more easily broken down on the first bite, which is related to fracturability measurement. Panellists were instructed to bite the middle of the biscuit with their front teeth to make the assessment and then expectorate any biscuit entering the oral cavity. Vanilla flavour was demonstrated by asking panellists to smell the difference between a jar of blank biscuits and a jar of vanilla flavoured biscuits. A standard vanilla reference was available if the panellist was unsure of the vanilla flavour. The final attribute, on oily off-note, was illustrated by allowing panellists to smell aged biscuits previously stored at 45 °C for 12 weeks, which had the oily off-note. To control portion size, the panellists were asked to eat the entire biscuit during testing.

Each panellist compared biscuits stored for 3, 6 and 8 weeks to a control for each attribute for both the PG and TA samples. Half of the panellists carried out paired comparisons for PG biscuits first and the other half for the TA biscuits, according to a randomised balanced design. A five minute break was taken by the panellists after the first 3 evaluations and they were asked to cleanse their palate with water (Evian, Danone, France) between samples. All biscuits were presented in 30 ml medicine cups labelled with random 3 digit codes. All tests were designed in 'Fizz' software and carried out in sensory booths at 18 ± 1 °C under red light to minimise the effect of any variation in biscuit colour. Data was collected using Fizz sensory software (Biosystems, Cergy-Pontoise, France).

Data Analysis

ANOVA, followed by Tukey's multiple comparison tests where appropriate, was used to determine if differences existed across storage time and temperature (α = 0.05). Paired comparison tests were analysed using the binomial statistics element of the statistical package (Fizz, Biosystems, Cergy-Pontoise, France).

RESULTS AND DISCUSSION

Stability of Added Aroma - Vanillin

Over the eight week storage trial there was a significant ($p < 0.001$) reduction in vanillin concentration in all samples, which was accelerated at elevated temperatures (45 °C, 32.5 °C) and is shown in Figure 1. There was also a significant difference between the flavour solvents; with biscuits prepared with TA as the carrier solvent retaining significantly more vanillin over the shelf life test period than those prepared with PG.

One potential explanation may be the formation of PG-acetal from propylene glycol and the aldehyde, vanillin (Coleman, 2006; Elmore, Dodson, & Mottram, 2011). Whilst PG-acetal may be generated and therefore present in the flavour stock, it is unlikely to develop further in the dry product. Furthermore, there was no difference in the total vanillin concentration in the fresh samples (TA and PG) at day zero (103 ppm and 107 ppm respectively).

Another possible explanation could be the physicochemical properties of PG and TA. TA has a higher boiling point than PG and TA is less volatile which may account for higher retention of the solvent during heating and storage but is unlikely to impact the loss of vanillin.

In a monophasic system, the stability of aldehydes has previously been shown to be more stable in an oil matrix (medium chain triglyceride) than in an aqueous matrix during storage (Leclercq, Reineccius, & Milo, 2006). TA (Log P = 0.36) is more hydrophobic than PG (Log P = -0.78) and this may also partially account for the enhanced stability (de Roos, 2006). Although the absolute differences are relatively

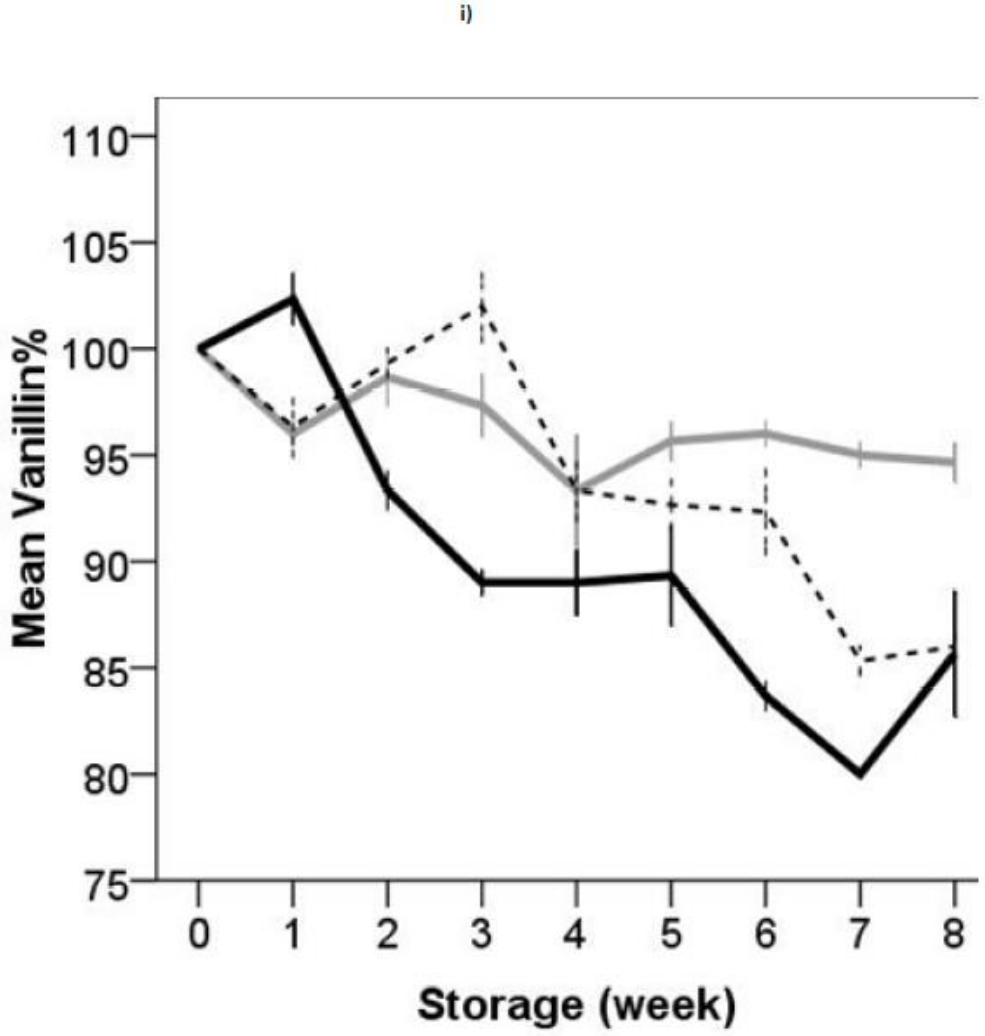
small compared to the large amount of shortening present.

Another hypothesis for the differences in flavour stability may be changes in the microstructure of the biscuit produced by solvent migration during baking, this is further discussed in the general discussion section.

Stability of Baked Biscuit Aroma - HMF

5-hydroxymethylfurfural (HMF) is a key intermediate of the browning process and is present in many cooked products. In commercial cookies HMF concentration may range from 0.5 to 74.6 ppm (Ait Ameur, Trystram, & Birlouez-Aragon, 2006), its formation and final concentration being dependent on the local microchemistry, process temperatures, and available reactants (Yang, Fisk, Linforth, Brown, Walsh, Mooney, et al., 2012). The average HMF level in the fresh biscuits prior to storage was 1.7 and 1.9 ppm for PG and TA respectively (Fi

Figure 1: Vanillin concentration normalized to week 0 (%) in biscuits prepared with a) PG and b) TA then stored for 8 weeks at 20 °C (grey line), 32.5 °C (dotted line) and 45 °C (black line). Data points are displayed ± standard error, n=3.



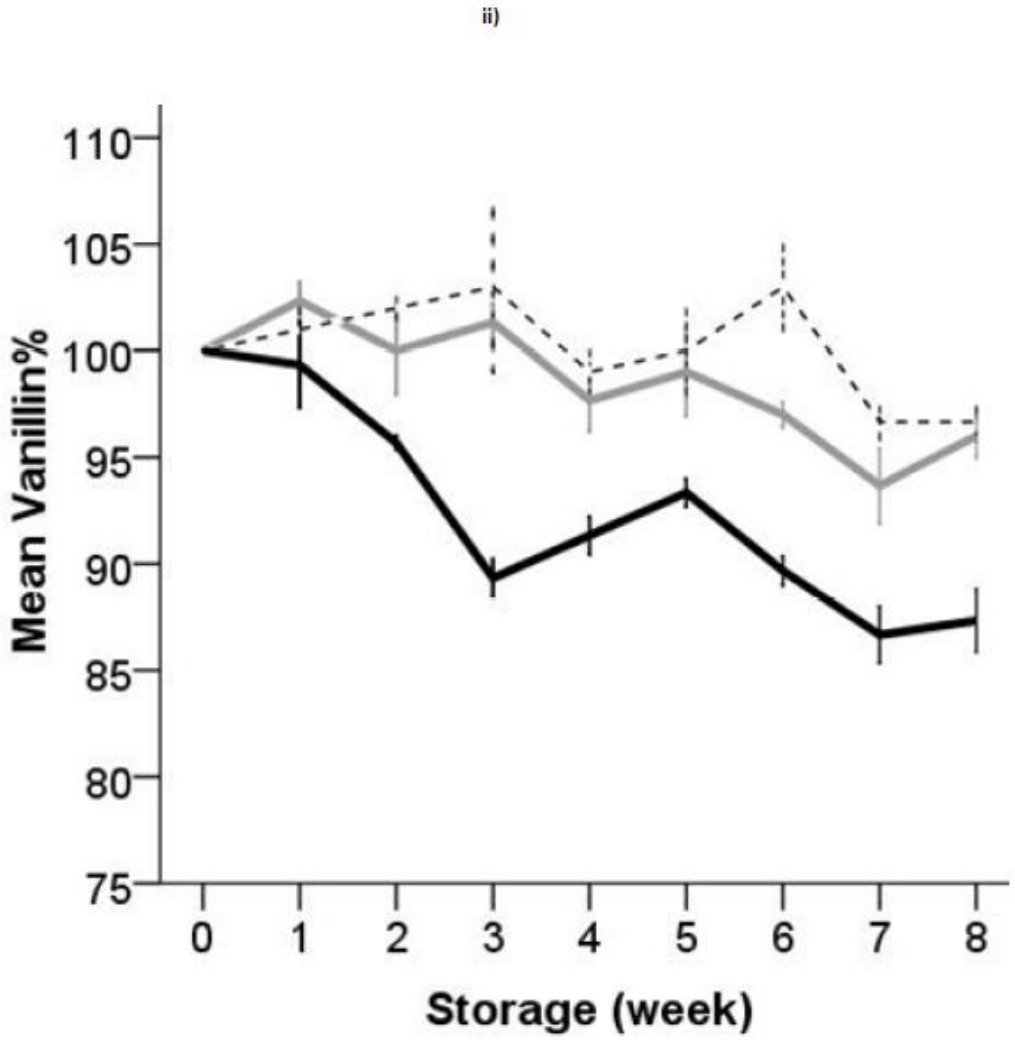
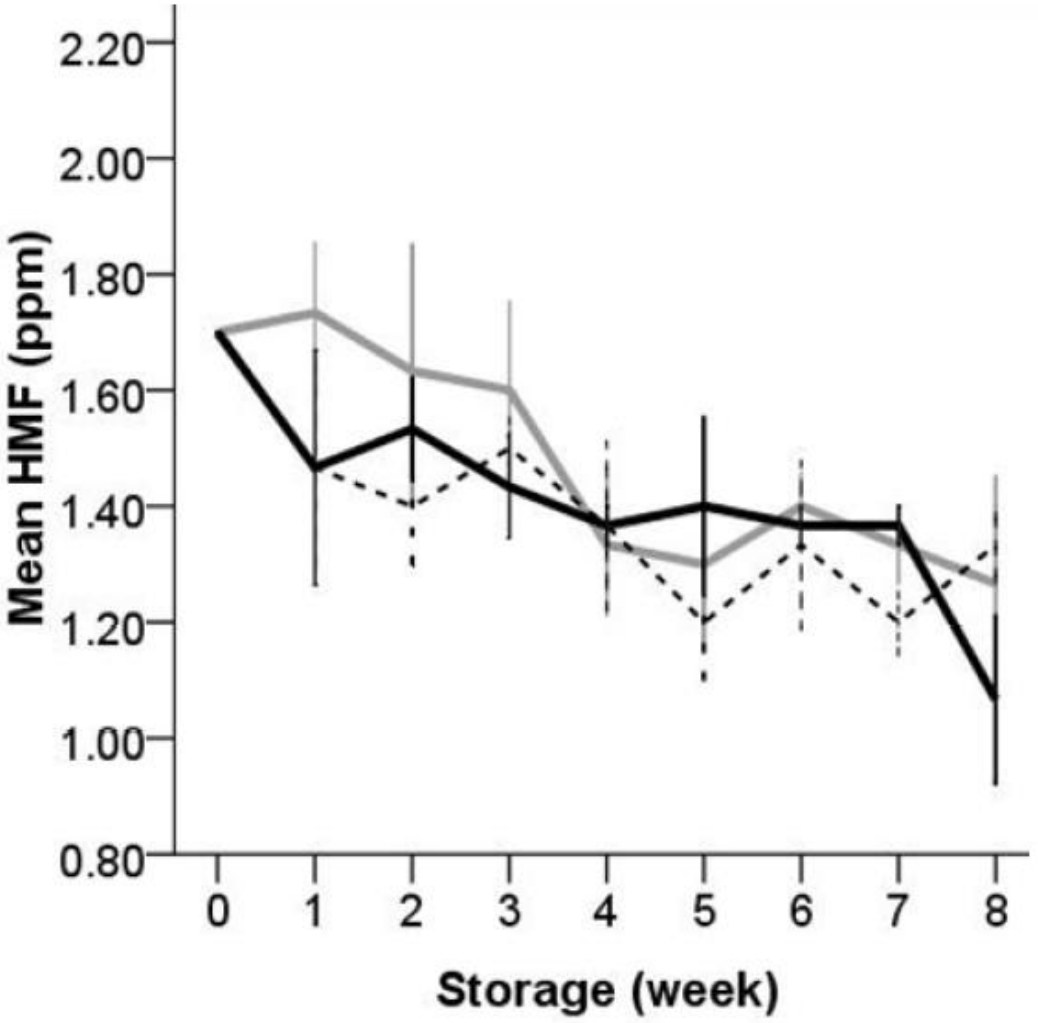
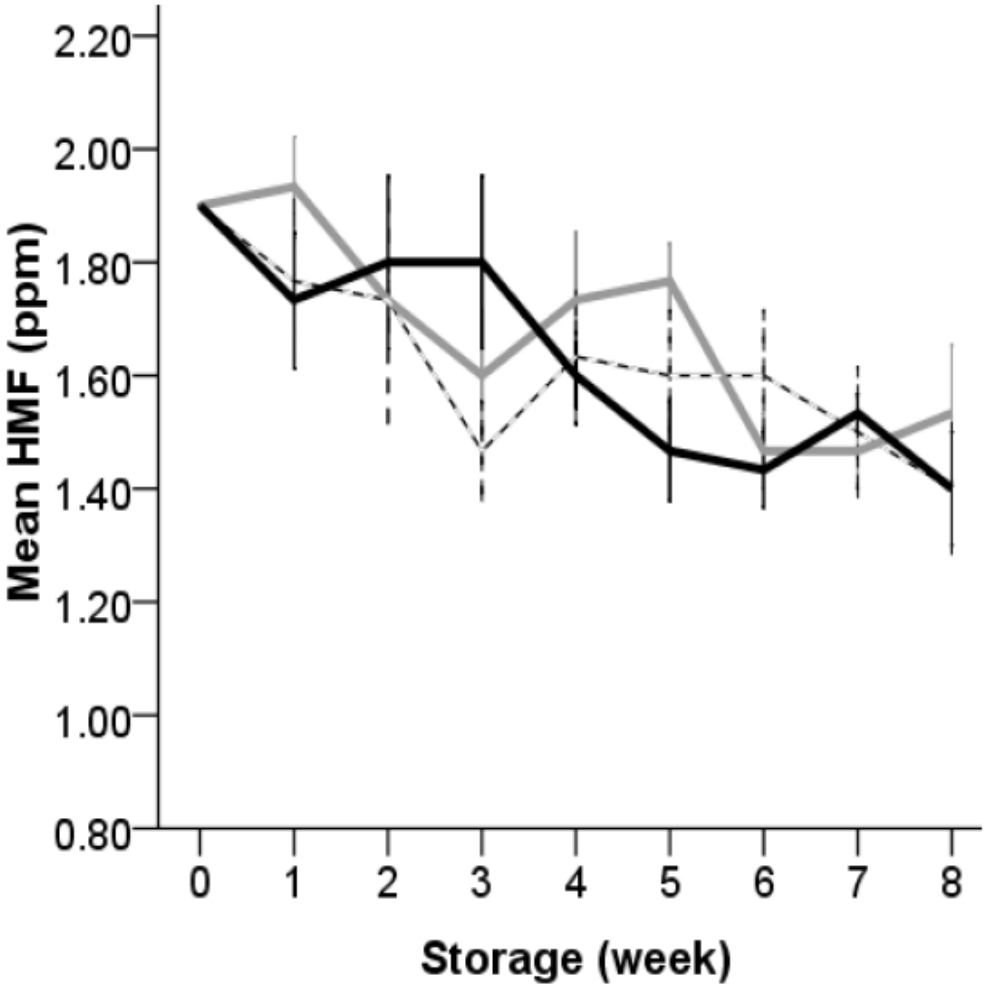


Figure 2: HMF concentration (µg/g biscuit, ppm) in biscuits prepared with a) PG and b) TA then stored for 8 weeks at 20 °C (grey line), 32.5 °C (dotted line) and 45 °C (black line). Data points are displayed ± standard error, n=3.


Mean HMF (ppm)
2.20
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0
1
2
3
4
5
6
7
8
Storage (week)

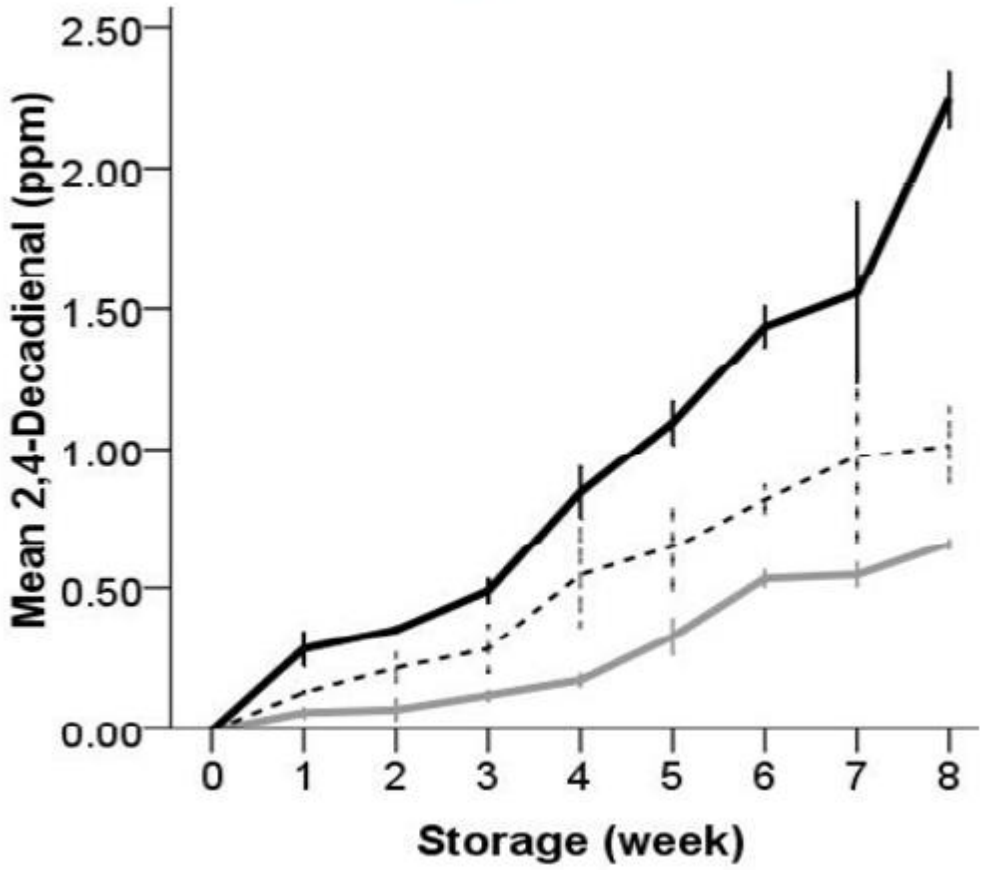
285

ii)



286
287 Figure 3: 2,4-Decadienal concentration (µg/g biscuit, ppm) in biscuits prepared with a)
288 PG and b) TA then stored for 8 weeks at 20 °C (grey line), 32.5 °C (dotted line)
289 and 45 °C (black line). Data points are displayed ± standard error, n=3.

i)


Mean 2,4-Decadienal (ppm)
2.50
2.00
1.50
1.00
0.50
0.00
0
1
2
3
4
5
6
7
8
Storage (week)

290

ii)

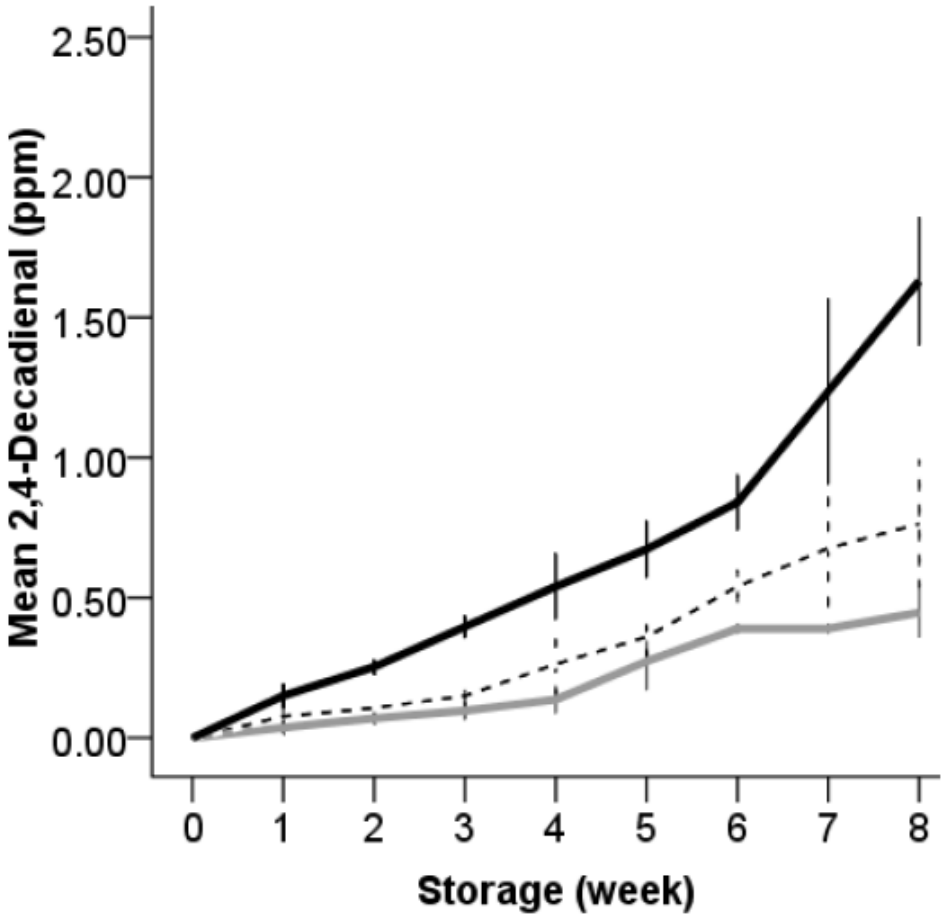
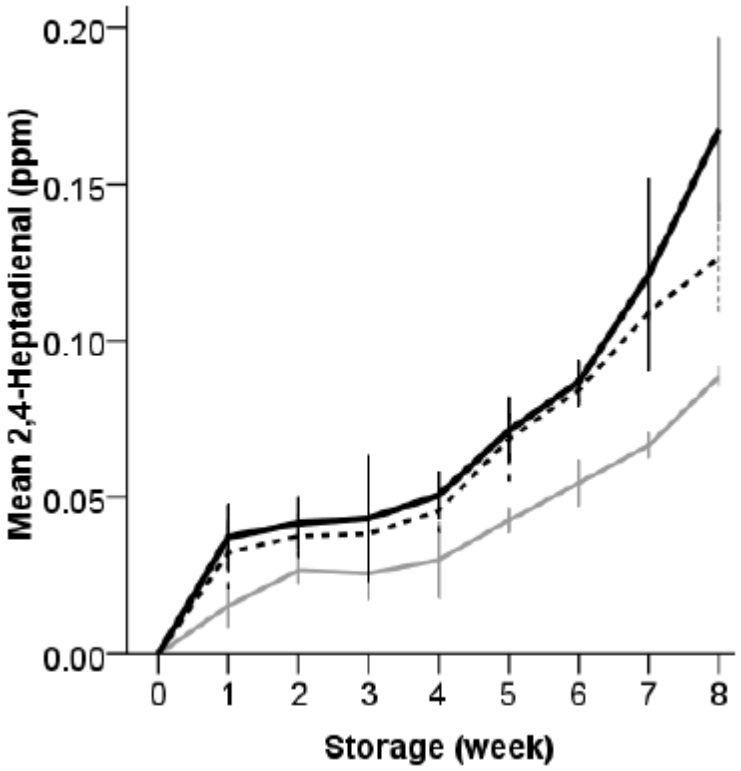
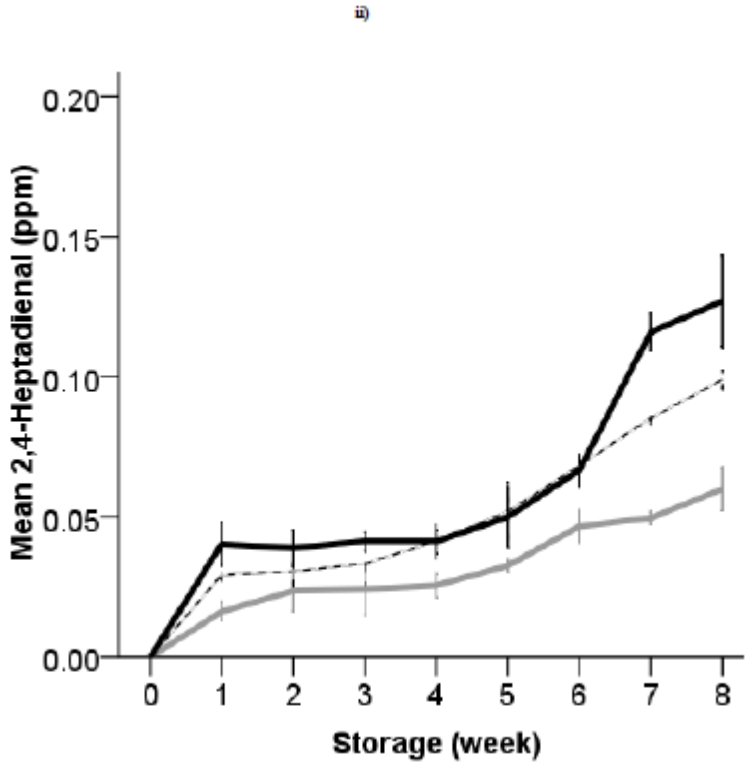


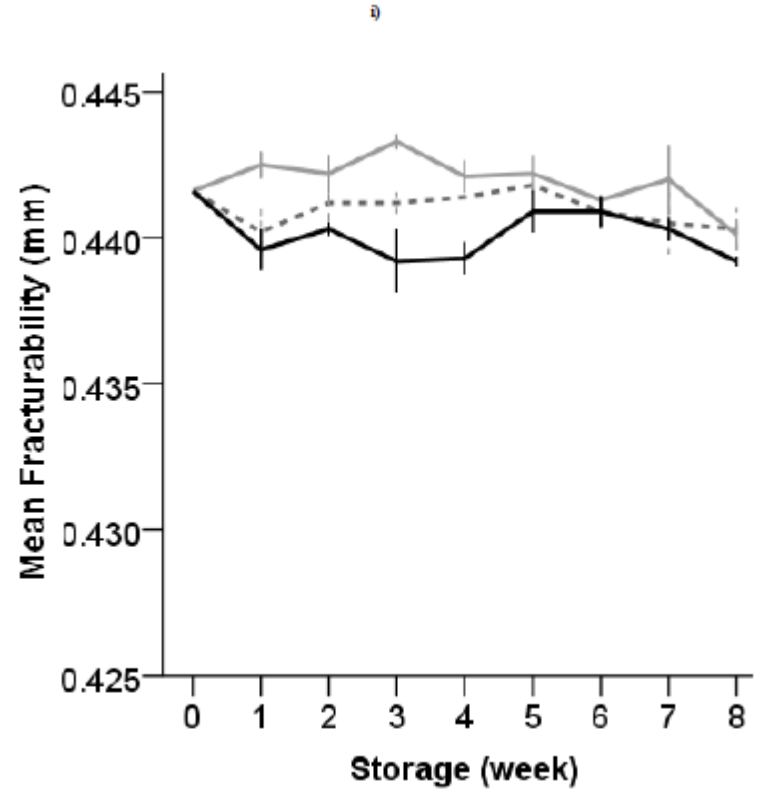
Figure 4: 2,4-Heptadienal concentration (µg/g biscuit, ppm) in biscuits prepared with i) PG and ii) TA then stored for 8 weeks at 20 °C (grey line), 32.5 °C (dotted line) and 45 °C (black line). Data points are displayed ± standard error, n=3.


Mean 2,4-Heptadienal (ppm)
0.20
0.15
0.10
0.05
0.00
0
1
2
3
4
5
6
7
8
Storage (week)

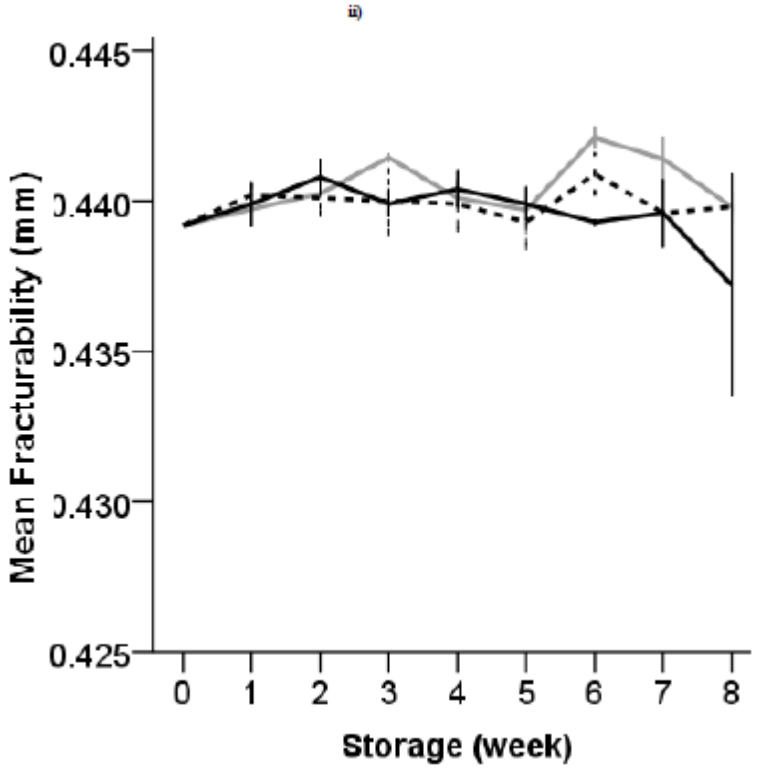
295



296
297 Figure 5: Fracturability (mm) of biscuits prepared with i) PG and ii) TA then stored
298 for 8 weeks at 20 °C (grey line), 32.5 °C (dotted line) and 45 °C (black line).
299 Data points are displayed ± standard error, n=3.



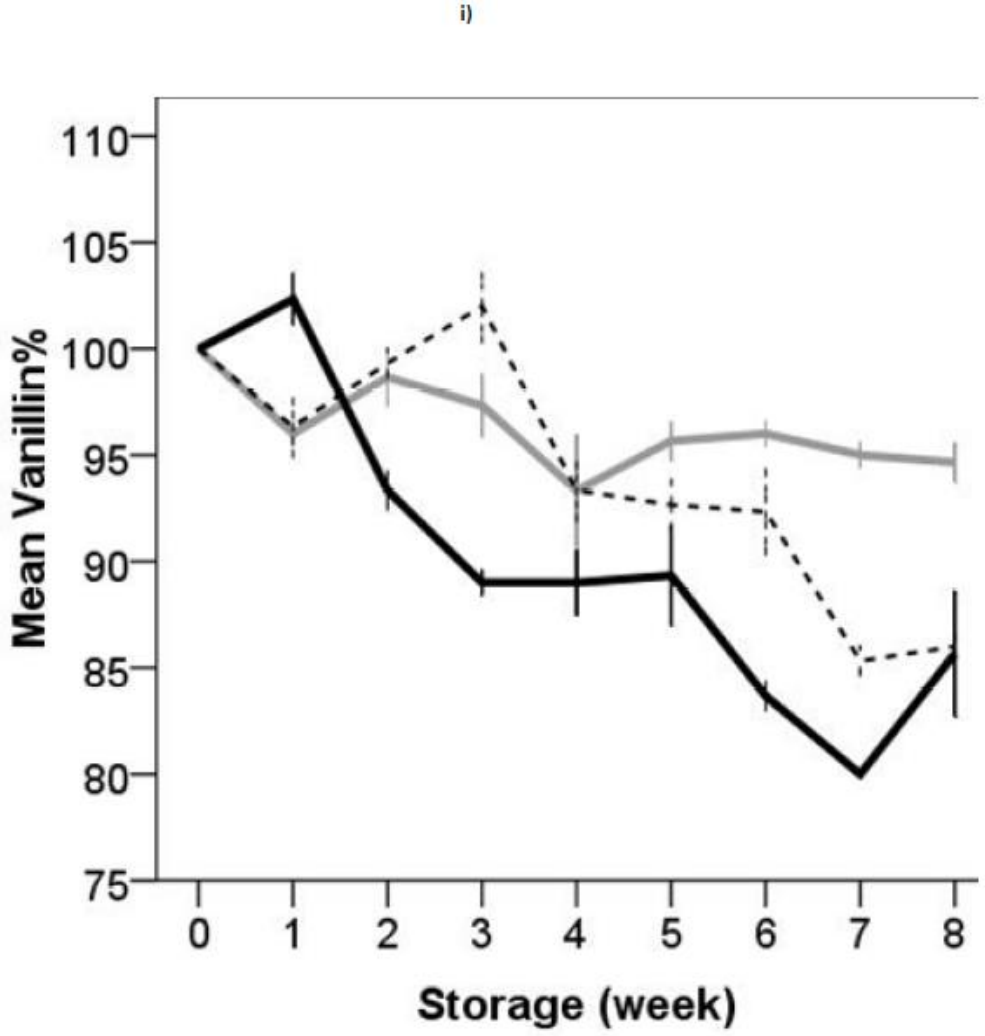
300



301
302 Figure 6: Hardness (g) of biscuits prepared with i) PG or ii) TA then stored for 8

303 weeks at 20 °C (grey line), 32.5 °C (dotted line) and 45 °C (black line). Data points
304 are displayed with ± standard error, n= 2). HMF concentration in all biscuits reduced
305 with storage time ($p < 0.05$), as shown in Fi
306

Figure 1: Vanillin concentration normalized to week 0 (%) in biscuits prepared with a) PG and b) TA then stored for 8 weeks at 20 °C (grey line), 32.5 °C (dotted line) and 45 °C (black line). Data points are displayed ± standard error, n=3.



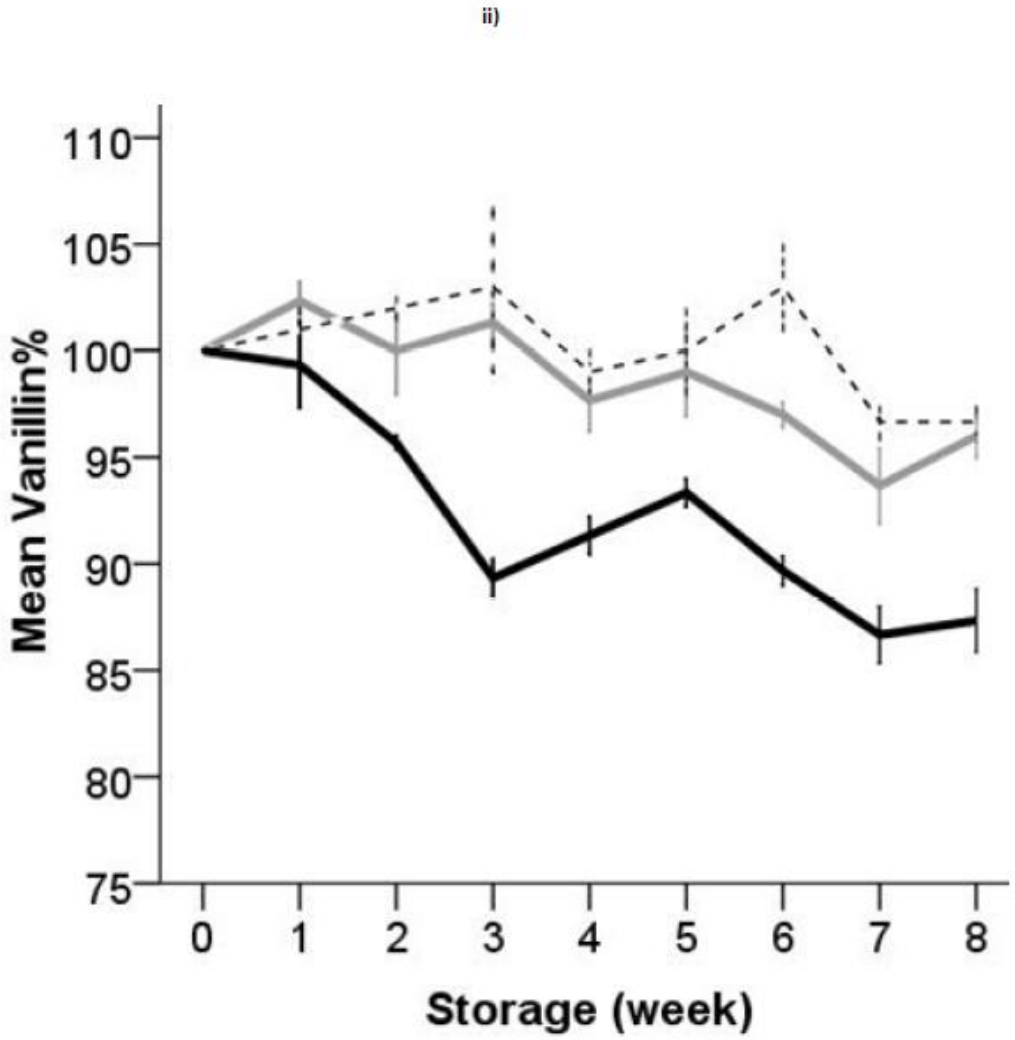
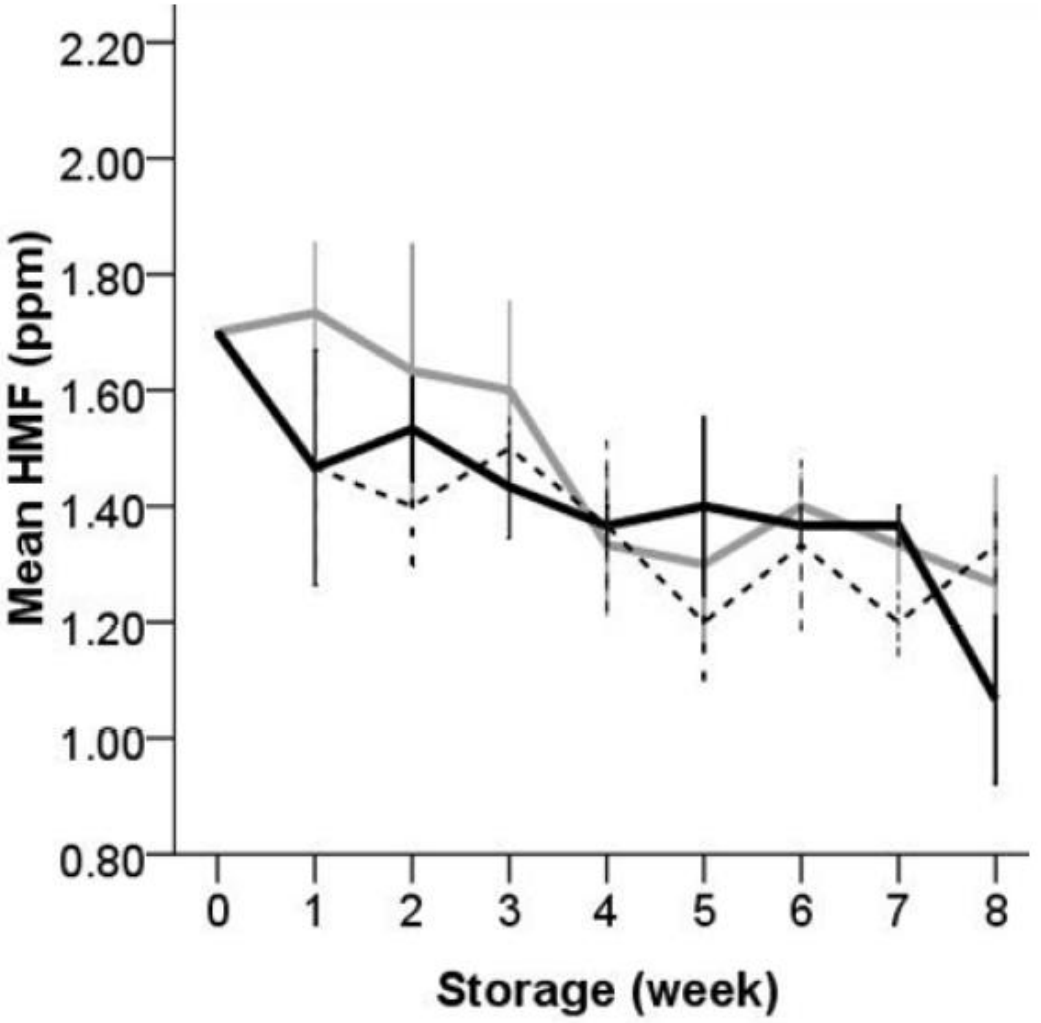
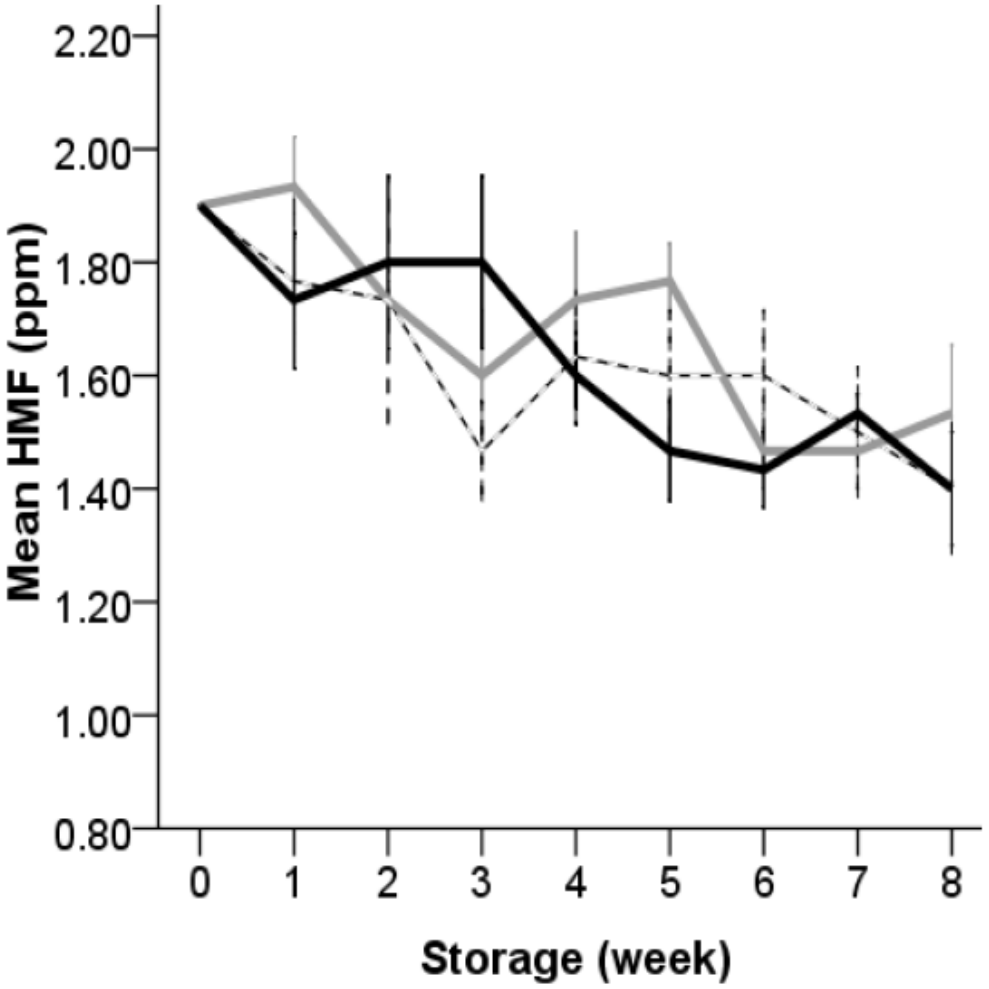


Figure 2: HMF concentration (µg/g biscuit, ppm) in biscuits prepared with a) PG and b) TA then stored for 8 weeks at 20 °C (grey line), 32.5 °C (dotted line) and 45 °C (black line). Data points are displayed ± standard error, n=3.


Mean HMF (ppm)
2.20
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0
1
2
3
4
5
6
7
8
Storage (week)

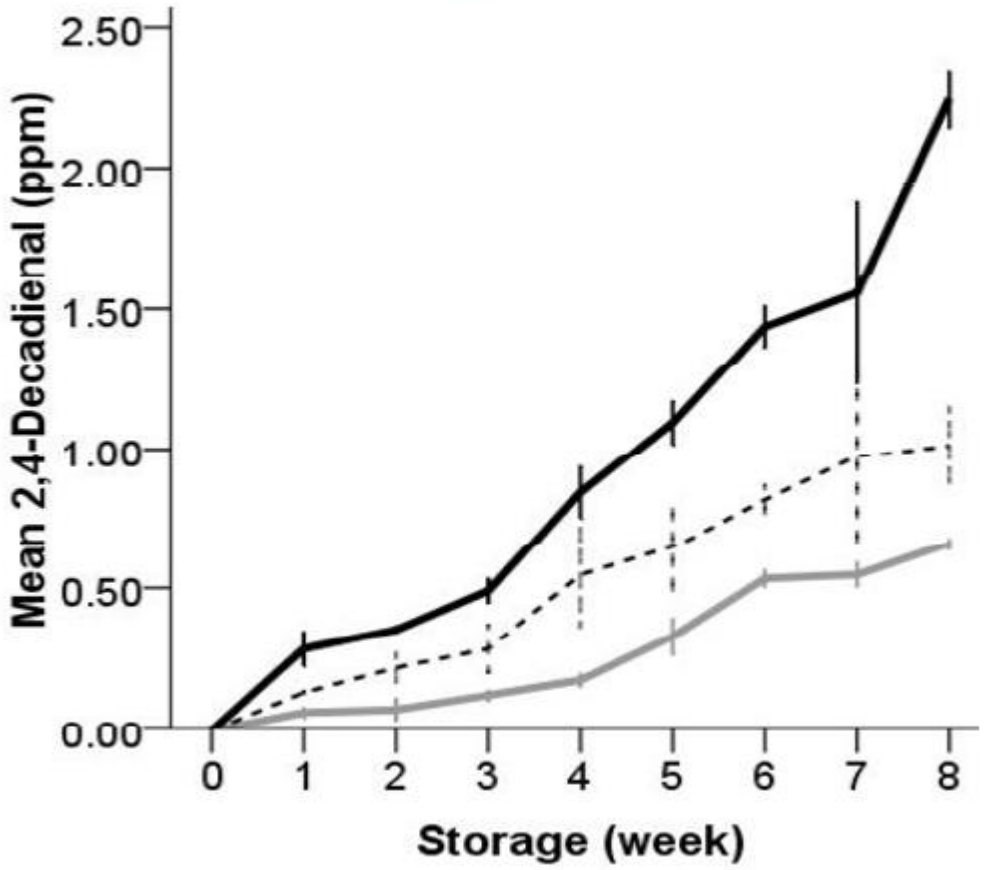
317

ii)



318
319 Figure 3: 2,4-Decadienal concentration (µg/g biscuit, ppm) in biscuits prepared with a)
320 PG and b) TA then stored for 8 weeks at 20 °C (grey line), 32.5 °C (dotted line)
321 and 45 °C (black line). Data points are displayed ± standard error, n=3.

i)


Mean 2,4-Decadienal (ppm)
2.50
2.00
1.50
1.00
0.50
0.00
0
1
2
3
4
5
6
7
8
Storage (week)

322

ii)

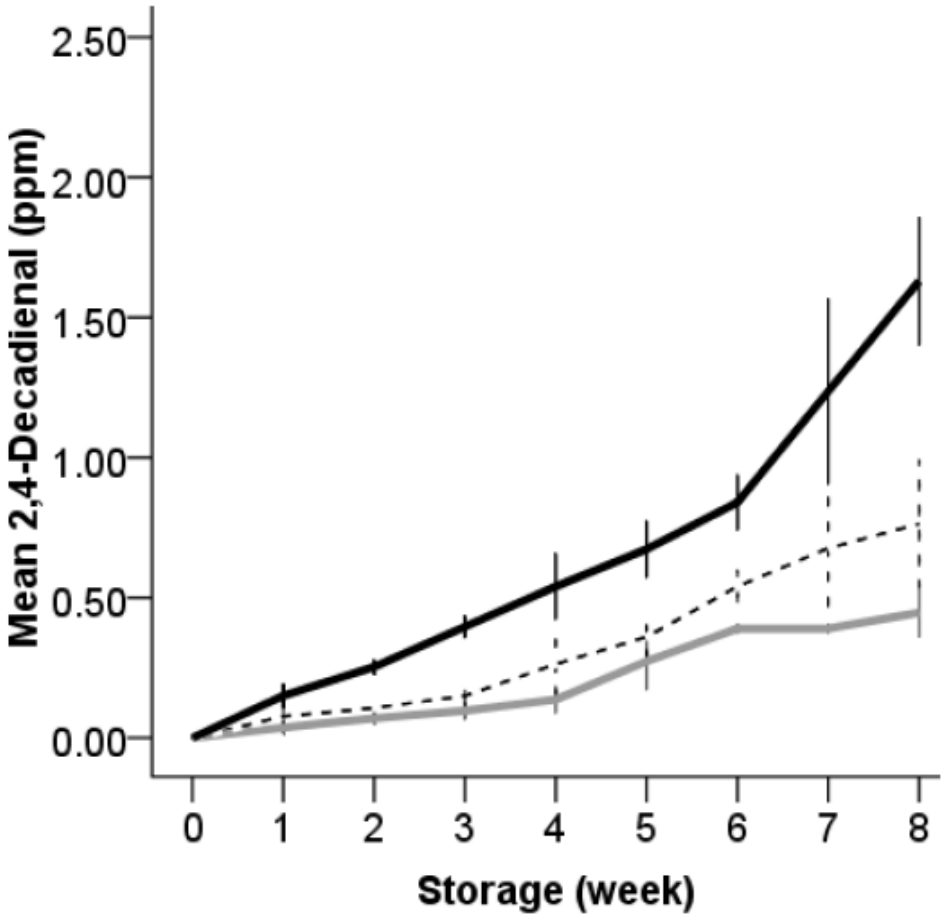
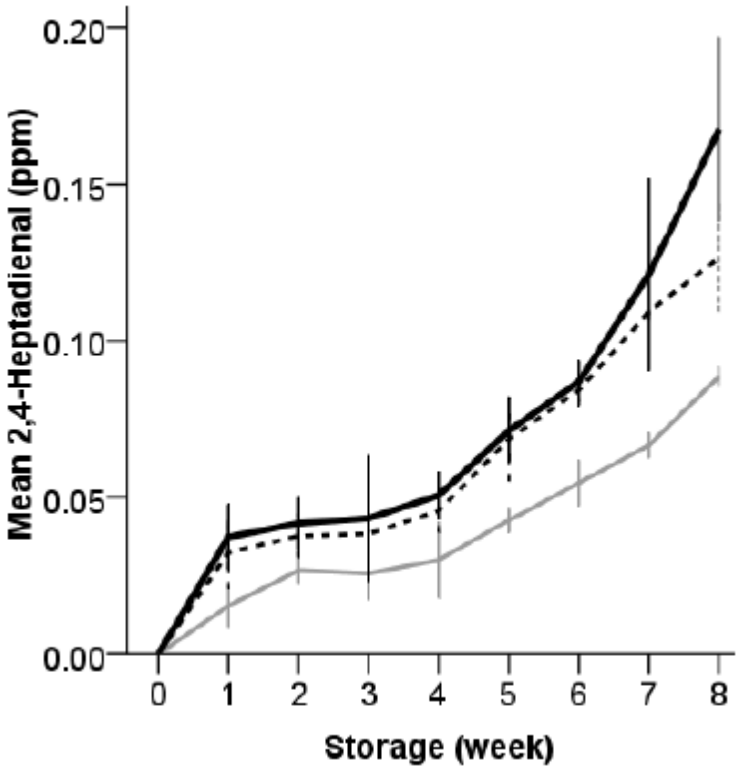
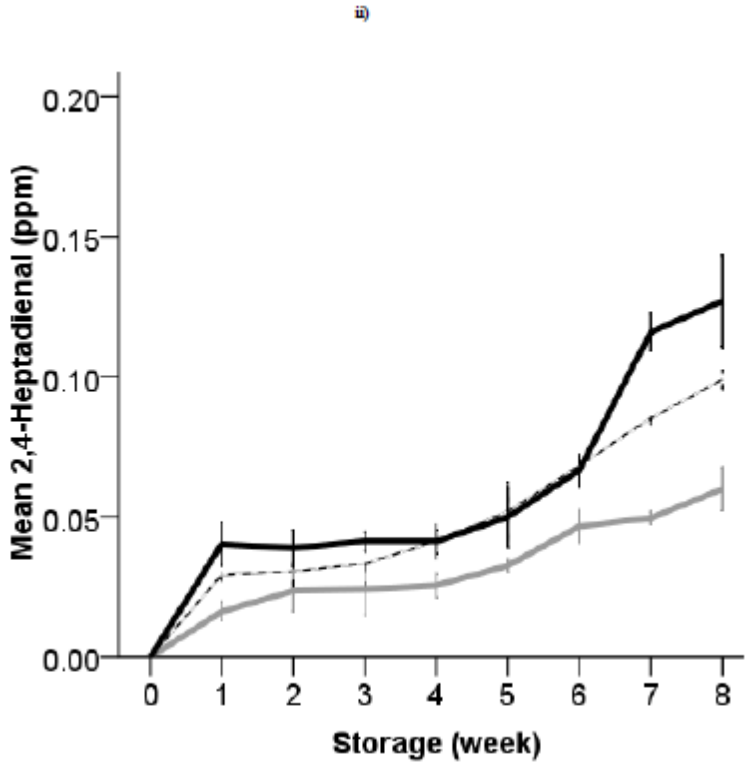


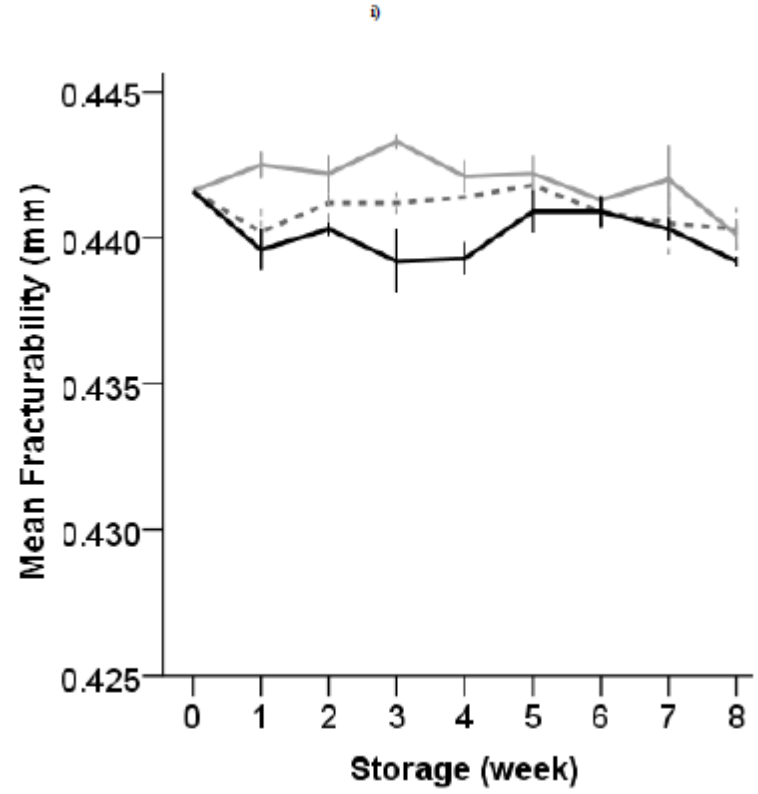
Figure 4: 2,4-Heptadienal concentration (µg/g biscuit, ppm) in biscuits prepared with i) PG and ii) TA then stored for 8 weeks at 20 °C (grey line), 32.5 °C (dotted line) and 45 °C (black line). Data points are displayed ± standard error, n=3.


Mean 2,4-Heptadienal (ppm)
0.20
0.15
0.10
0.05
0.00
0
1
2
3
4
5
6
7
8
Storage (week)

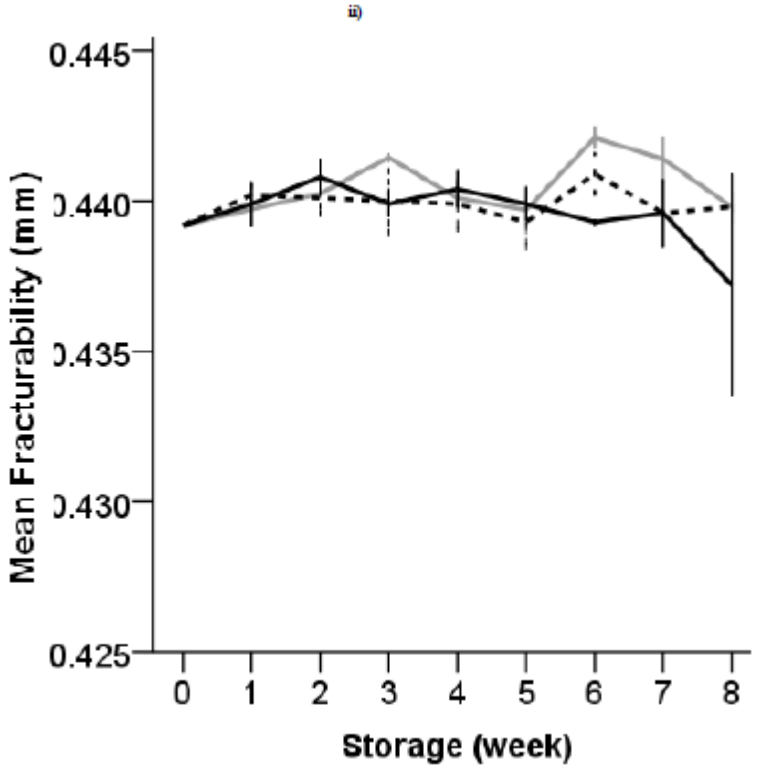
327



328
329 Figure 5: Fracturability (mm) of biscuits prepared with i) PG and ii) TA then stored
330 for 8 weeks at 20 °C (grey line), 32.5 °C (dotted line) and 45 °C (black line).
331 Data points are displayed ± standard error, n=3.



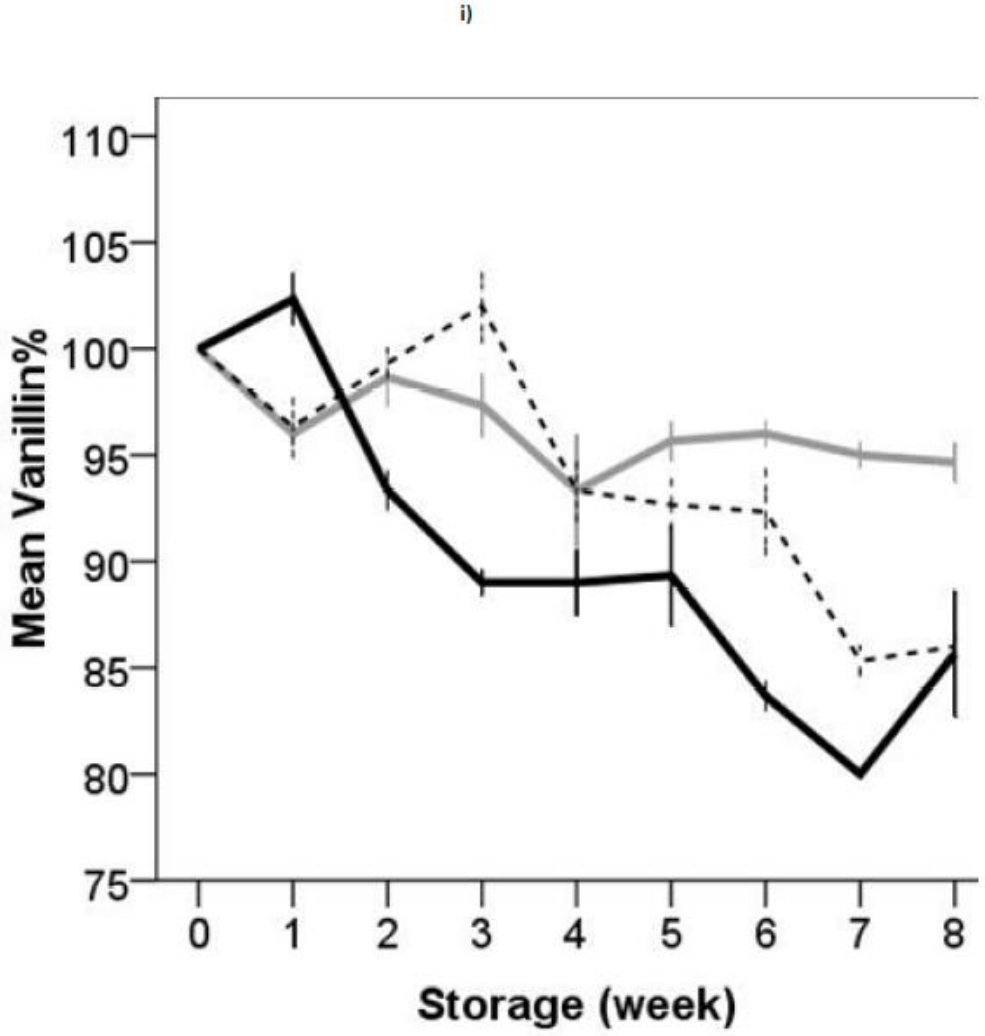
332



333
334 Figure 6: Hardness (g) of biscuits prepared with i) PG or ii) TA then stored for 8

335 weeks at 20 °C (grey line), 32.5 °C (dotted line) and 45 °C (black line). Data points
336 are displayed with ± standard error, n= 2, and storage temperature had no significant
337 impact. As HMF is generated and lost during high temperature baking, the small
338 elevations of temperatures experienced during the accelerated shelf life test are
339 presumed to have negligible impact on the stability or generation of HMF during the
340 shelf life test.
341 There is a significant impact of solvent choice on HMF concentration (Fi
342

Figure 1: Vanillin concentration normalized to week 0 (%) in biscuits prepared with a) PG and b) TA then stored for 8 weeks at 20 °C (grey line), 32.5 °C (dotted line) and 45 °C (black line). Data points are displayed ± standard error, n=3.



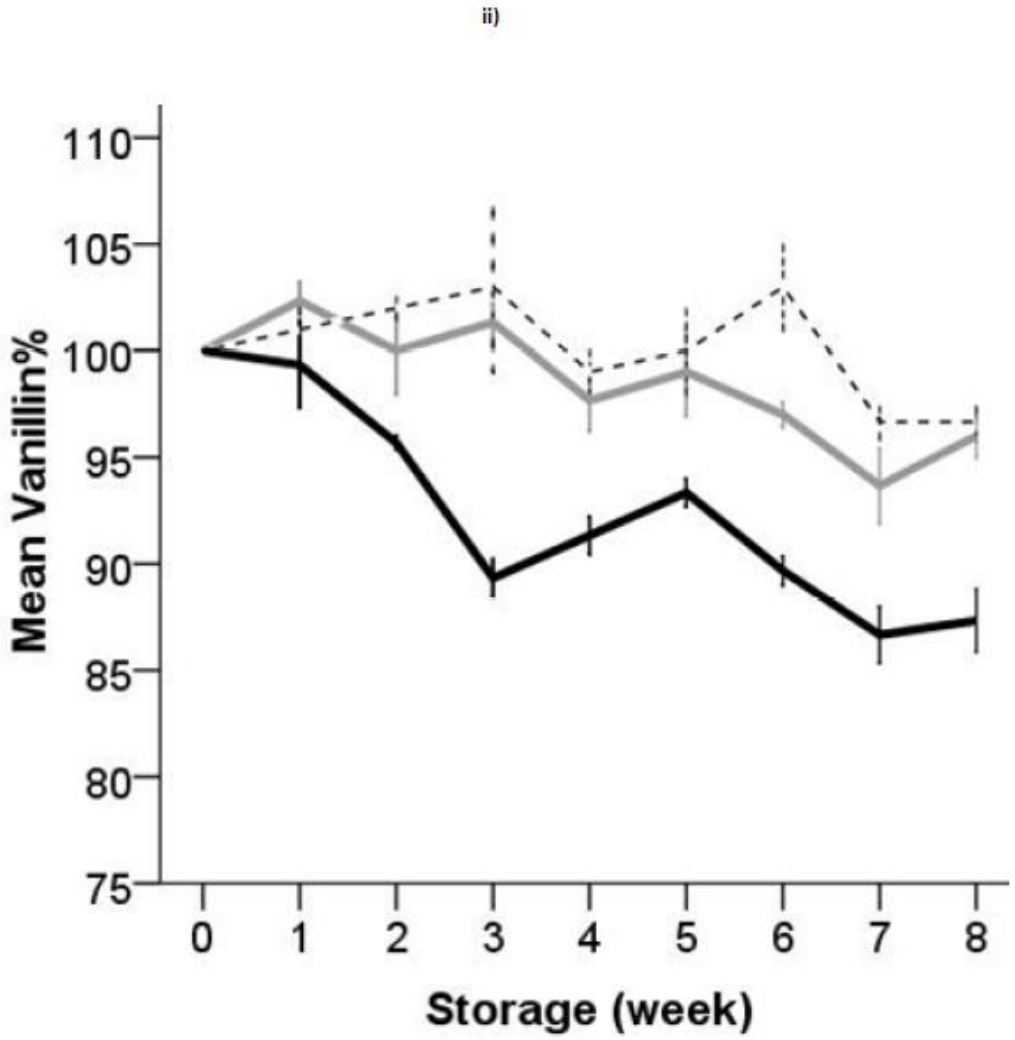
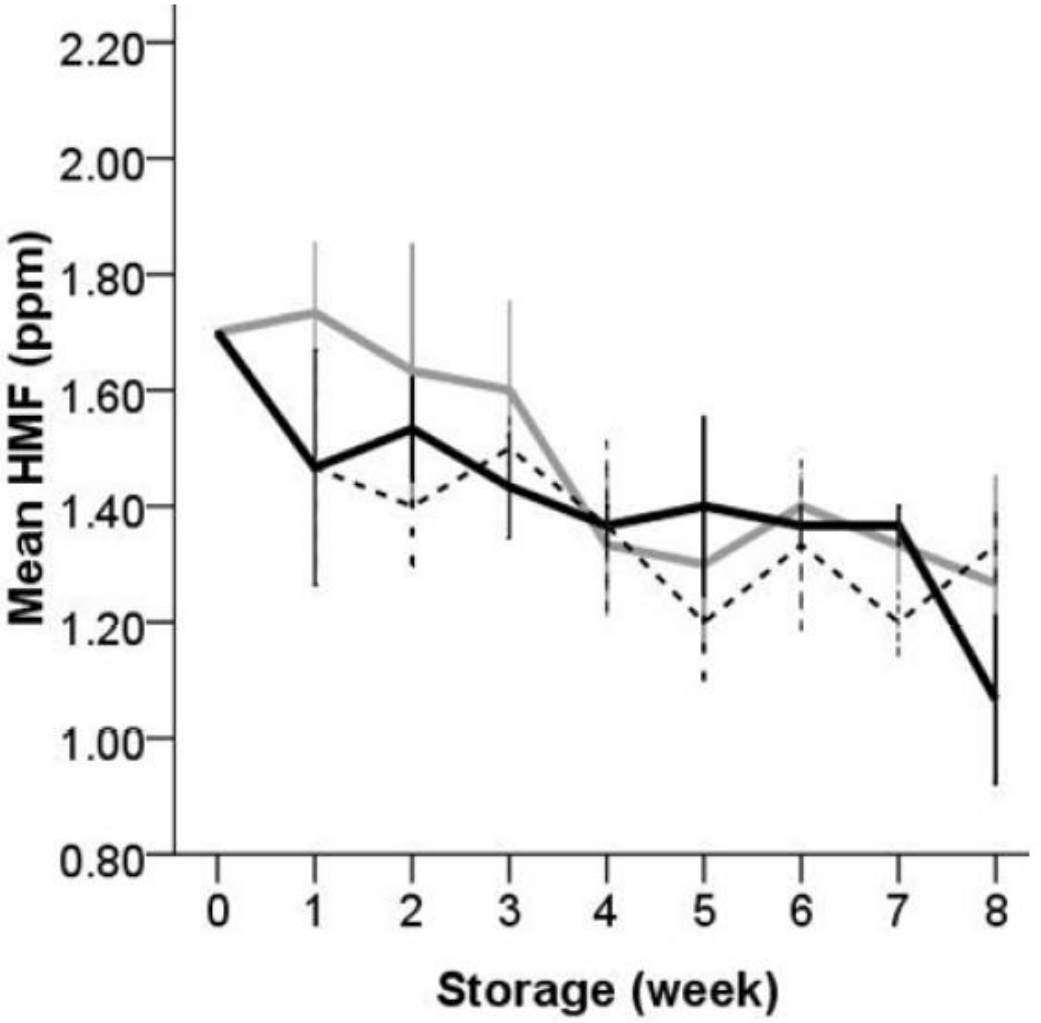
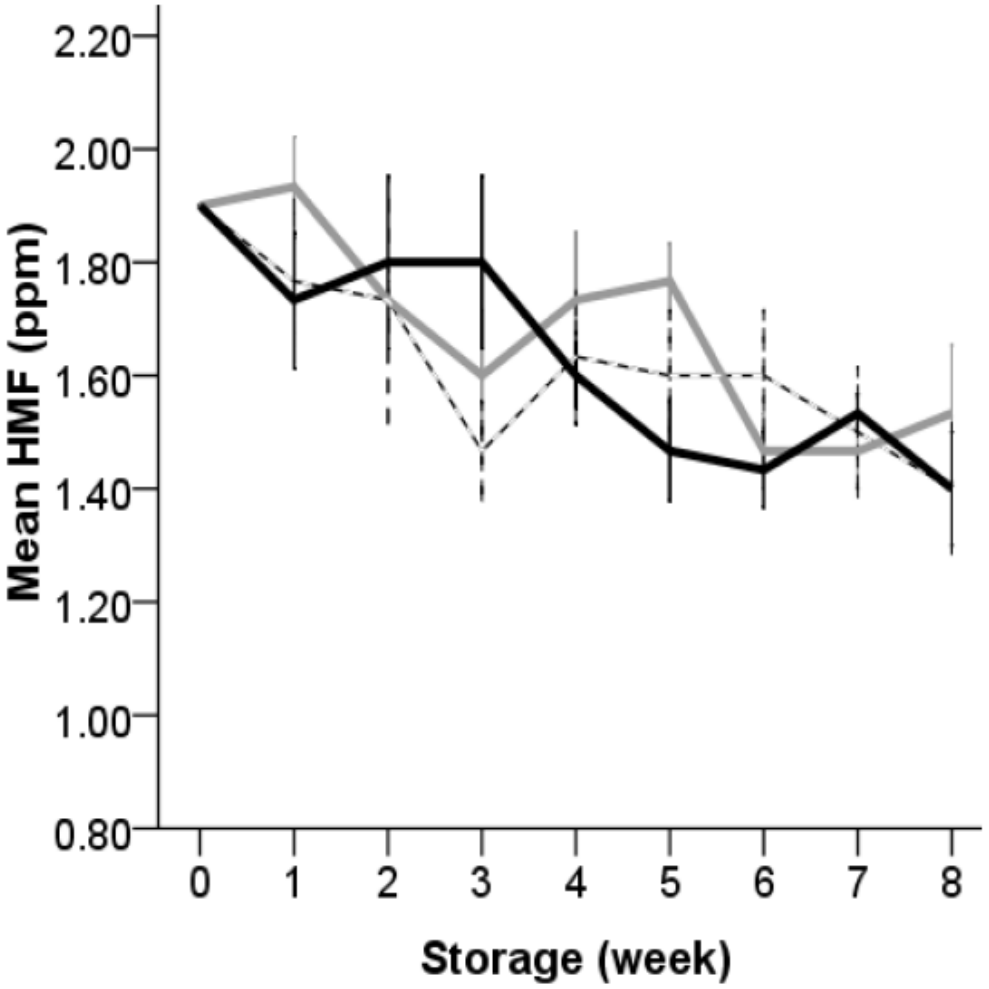


Figure 2: HMF concentration (µg/g biscuit, ppm) in biscuits prepared with a) PG and b) TA then stored for 8 weeks at 20 °C (grey line), 32.5 °C (dotted line) and 45 °C (black line). Data points are displayed ± standard error, n=3.


Mean HMF (ppm)
2.20
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0
1
2
3
4
5
6
7
8
Storage (week)

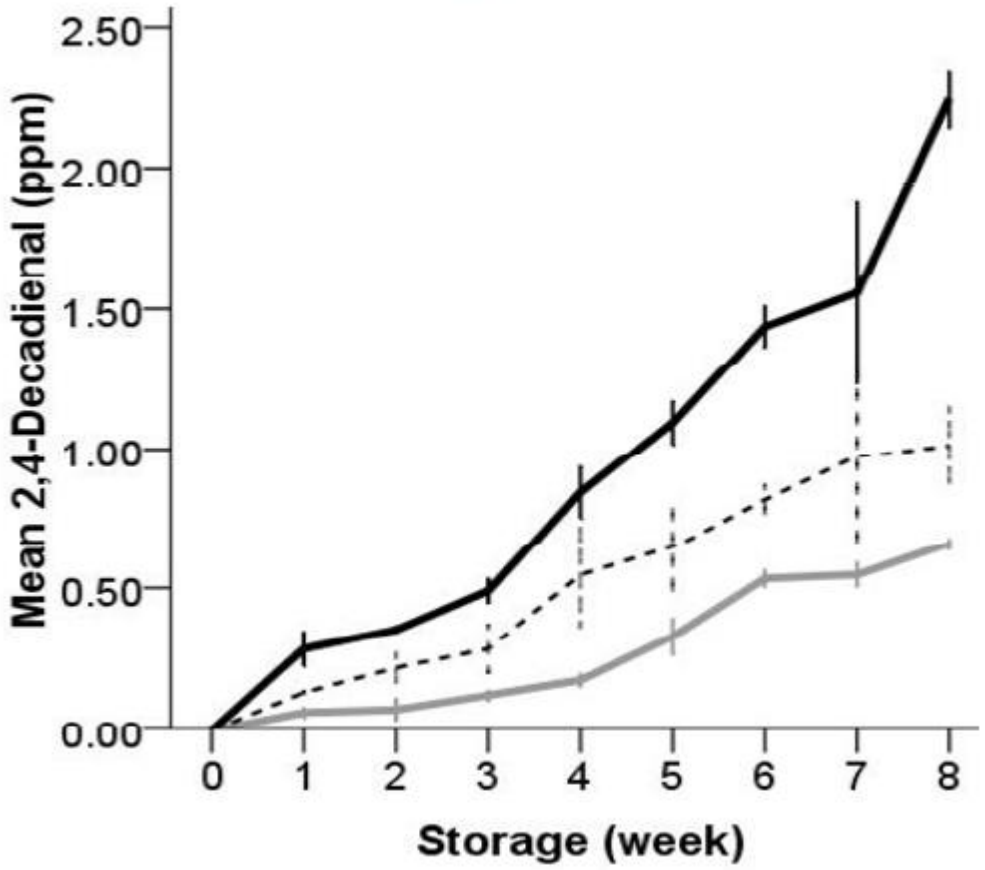
353

ii)



354
355 Figure 3: 2,4-Decadienal concentration (µg/g biscuit, ppm) in biscuits prepared with a)
356 PG and b) TA then stored for 8 weeks at 20 °C (grey line), 32.5 °C (dotted line)
357 and 45 °C (black line). Data points are displayed ± standard error, n=3.

i)


Mean 2,4-Decadienal (ppm)
2.50
2.00
1.50
1.00
0.50
0.00
0
1
2
3
4
5
6
7
8
Storage (week)

358

ii)

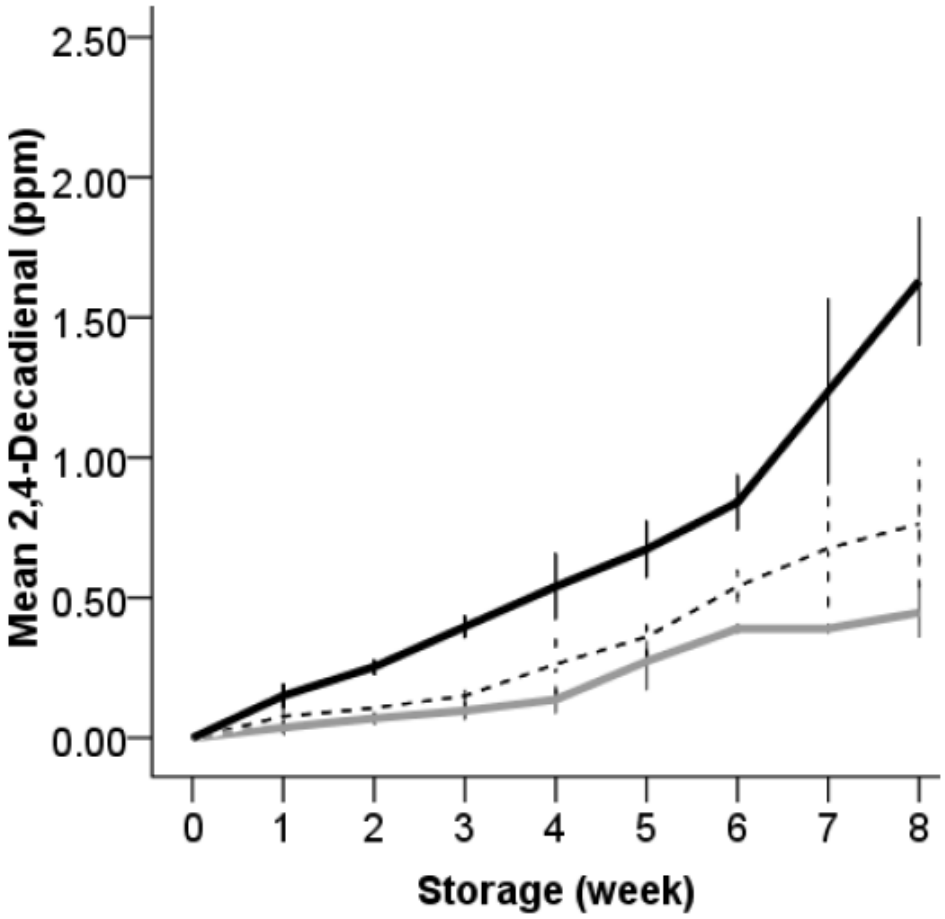
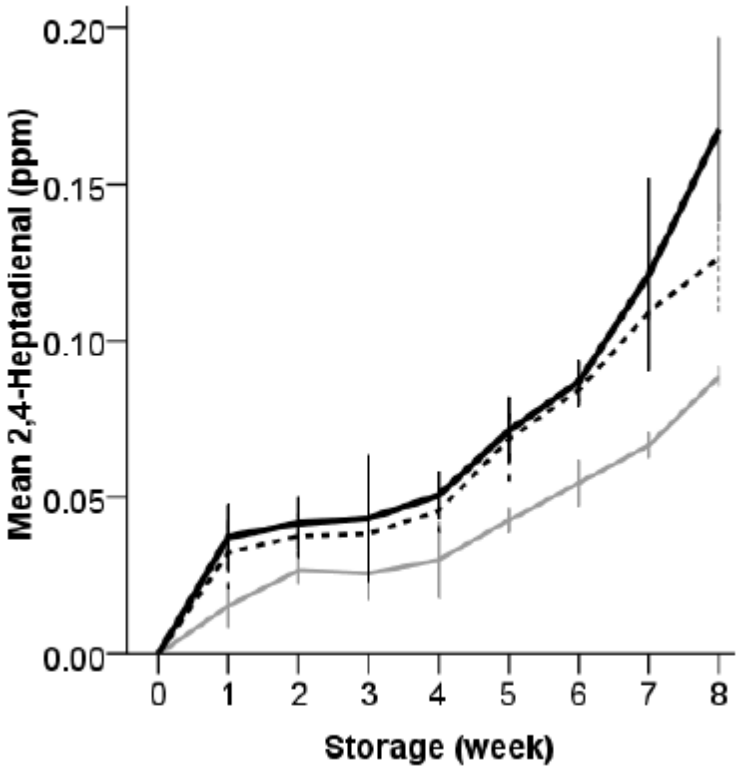
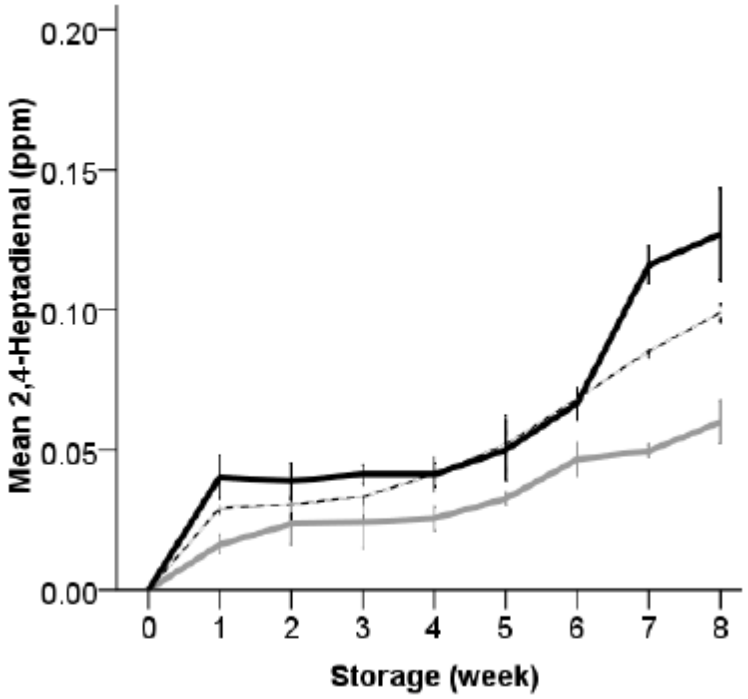


Figure 4: 2,4-Heptadienal concentration (µg/g biscuit, ppm) in biscuits prepared with i) PG and ii) TA then stored for 8 weeks at 20 °C (grey line), 32.5 °C (dotted line) and 45 °C (black line). Data points are displayed ± standard error, n=3.

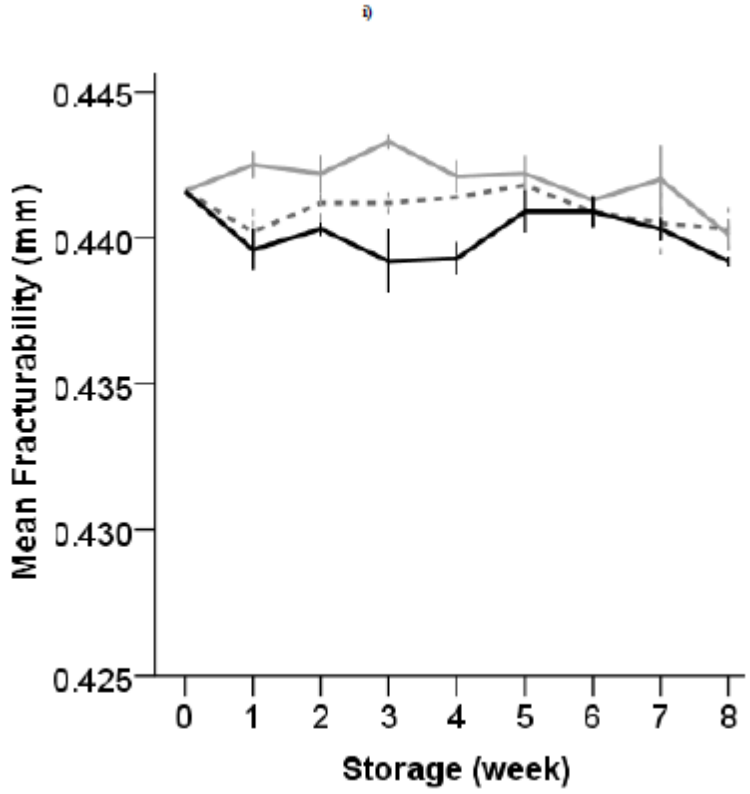

Mean 2,4-Heptadienal (ppm)
0.20
0.15
0.10
0.05
0.00
0
1
2
3
4
5
6
7
8
Storage (week)

363

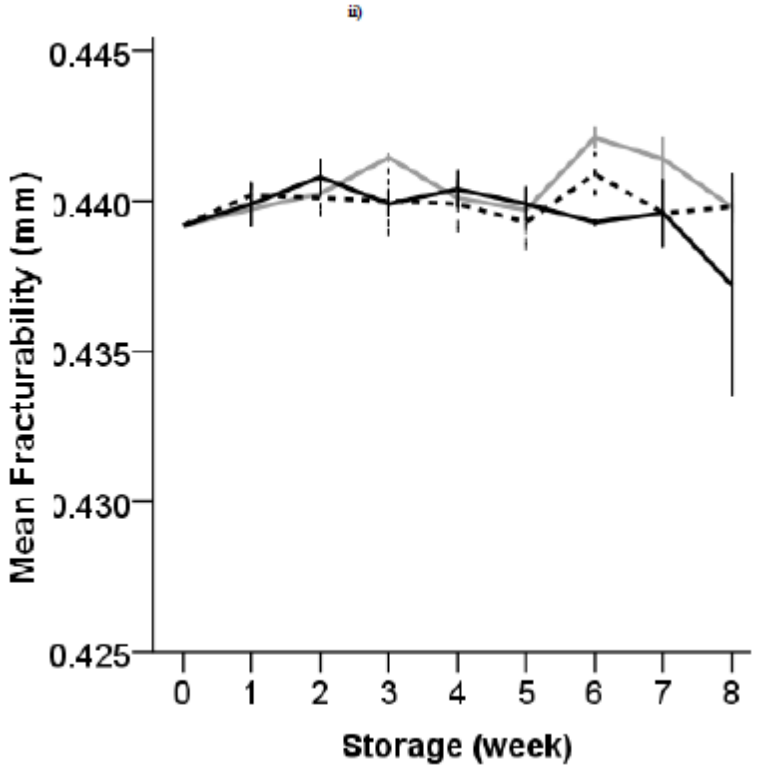
ii)



364
365 Figure 5: Fracturability (mm) of biscuits prepared with i) PG and ii) TA then stored
366 for 8 weeks at 20 °C (grey line), 32.5 °C (dotted line) and 45 °C (black line).
367 Data points are displayed ± standard error, n=3.



368



369
370 Figure 6: Hardness (g) of biscuits prepared with i) PG or ii) TA then stored for 8

371 weeks at 20 °C (grey line), 32.5 °C (dotted line) and 45 °C (black line). Data points
372 are displayed with ± standard error, n= 2). TA biscuits lost significantly less HMF
373 over storage than PG biscuits ($p < 0.05$). This may be due to potential differences in
374 the biscuit micro-structure as a consequence of flavour solvent choice.

375 **Stability of Bi-products of Lipid Oxidation – 2,4-Decadienal and**
376 **2,4-Heptadienal**

377 During the 8 week storage trial both 2,4-decadienal and 2,4-heptadienal increased
378 in concentration with time ($p < 0.001$) for both PG and TA biscuits (Figure 3 and
379 Figure 4). Additionally, storage temperature had a significant impact on the
380 concentrations ($p < 0.001$) in both the PG and TA biscuits, with biscuits stored at
381 higher temperatures having higher levels of both compounds. Biscuits made with TA
382 as the flavour carrier had significantly lower concentrations of both 2,4-decadienal
383 and 2,4-heptadienal than biscuits prepared with PG when stored over 8 weeks ($p <$
384 0.001).

385 **Stability of Biscuit Texture – Fracturability and Hardness**

386 Fresh TA biscuits were significantly more brittle (lower fracturability) than PG
387 biscuit (analysed by TA XT texture analyser). PG biscuits stored at the highest
388 temperatures (45 °C) were significantly more brittle than those stored at 20 °C ($p <$
389 0.05) but only at specific time points during the time course of the study (Figure).
390 There is no effect of temperature on the fracturability of TA biscuits over the eight
391 week storage.

392 There is no significant solvent impact on the biscuit hardness and no significant
393 changes were observed in the biscuit hardness over the eight week storage time as

illustrated in Figure .

Stability of Sensory Quality Factors – Brittleness, Vanilla Flavour, Oily Off-Note

Consumers could not discriminate between the biscuits stored at 45ºC after 3, 6 and 8 weeks for brittleness and vanilla attributes ($P > 0.05$).

Despite the increase in concentration of lipid oxidation markers during biscuit storage, most panellists could not differentiate the oily off-note between the stored and control biscuits, differences were noticed when TA biscuits were stored for six weeks ($p < 0.05$), although this is probably due to sample variation, rather than indicating the start of a perceptual change, as no differences were shown at later time points. This may indicate that the complex matrix and nature of the aroma profile of the samples is limiting the discriminating ability of the test, or that the concentration difference is close to or below the detection limit of the panellists, nonetheless the analytical chemistry results do indicate clear trends towards differences in chemical stability that are anticipated to be perceivable in shelf life studies of longer duration.

General Discussion

The observed differences between PG and TA on initial brittleness (TA > PG), stability of vanillin (TA > PG), resistance to the progression of oxidation (TA > PG), HMF formation (TA > PG) and HMF stability (TA > PG) may be explained by changes in the biscuit microstructure. It has previously been shown by X-ray µ-Computed Tomography that biscuits formulated with PG have smaller pores and higher porosity than those prepared with TA (Yang, et al., 2012). The lower porosity of TA may restrict the migration of vanillin and HMF therefore reducing the rate of

417 loss through volatilisation during storage; furthermore it may also slow the
418 progression of oxidation through restricting the mobility of free radicals or reactive
419 oxygen species across the biscuit matrix.

420 During the formation of the cellular solid (biscuit) from the dough during baking
421 structure is created by the expansion of the dough whilst being subjected to the
422 vaporisation of water and gases from the leavening powders (Chevallier, Colonna,
423 Buleon, & Della Valle, 2000). Although only a tiny amount of flavour solvent is
424 generally added during food preparation, differences in structure have previously been
425 shown, for example chewing gums made with PG and TA solvents have distinctive
426 structural differences (Potineni, 2008). In the case of shortcake biscuits, the
427 differences in volatility and hydrophobicity of the two solvents may interact with
428 dough ingredients in different ways to produce different structures: PG as more
429 volatile and hydrophilic solvent may support the aqueous phase to be more
430 homogeneously distributed within the dough creating larger numbers of smaller pores
431 (Yang, et al., 2012). Additionally, fats were reported to have a delaying effect on the
432 release of carbon dioxide during baking (Chevallier, Colonna, & Lourdin, 2000), and
433 TA as a more heat-stable and more oily-like solvent may therefore generate larger
434 pores at slower rate. The different microstructures formed are therefore proposed to
435 explain the differences in chemical stability.

436 In summary, this paper details for the first time, that choice of flavour delivery
437 solvent in shortcake biscuits can have a significant impact on measured fracturability,
438 the loss of aroma compounds, concentration of HMF and the progression of lipid
439 oxidation in fresh products and during ageing. This interaction effect is an important
440 finding for product developers to consider, either as a positive tool for flavour

441 enhancement over ageing or as a warning that small changes in unperceivable
442 ingredients can have significant impact on stability markers such as lipid oxidation.

443 **Acknowledgments**

444 This work was supported by a Knowledge Transfer Partnership awarded to Aromco
445 Ltd and the University of Nottingham by Technology Strategy Board, UK. The help
446 of Professor Andy Taylor, Paul Yeomans, Jackie Harvey and Ray McKee in setting
447 up this project is acknowledged.

LITERATURE CITED

Ait Ameur, L., Rega, B., Giampaoli, P., Trystram, G., & Birlouez-Aragon, I. (2008). The fate of furfurals and other volatile markers during the baking process of a model cookie. *Food Chemistry, 111*(3), 758-763.

Ait Ameur, L., Trystram, G., & Birlouez-Aragon, I. (2006). Accumulation of 5-hydroxymethyl-2-furfural in cookies during the backing process: Validation of an extraction method. *Food Chemistry, 98*(4), 790-796.

Anklam, E., Gaglione, S., & Muller, A. (1997). Oxidation behaviour of vanillin in dairy products. *Food Chemistry, 60*(1), 43-51.

Chapman, K. W., Rosenberry, L. C., Bandler, D. K., & Boor, K. J. (1998). Light-oxidized flavor development and vitamin A degradation in chocolate milk. *Journal of Food Science, 63*(5), 930-934.

Chevallier, S., Colonna, P., Buleon, A., & Della Valle, G. (2000). Physicochemical behaviors of sugars, lipids, and gluten in short dough and biscuit. *Journal of Agricultural and Food Chemistry, 48*(4), 1322-1326.

Chevallier, S., Colonna, P., & Lourdin, D. (2000). Contribution of major ingredients during baking of biscuit dough systems. *Journal of Cereal Science, 31*(3), 241-252.

Chevallier, S., Della Valle, G., Colonna, P., Broyart, B., & Trystram, G. (2002). Structural and chemical modifications of short dough during baking. *Journal of Cereal Science, 35*(1), 1-10.

Coleman, W. M. (2006). Analysis of the optical and geometrical isomer distributions in selected propylene glycol Acetals. *Journal of Chromatographic Science, 44*(3), 167-173.

de Roos, K. B. (2006). Understanding and controlling the behaviour of aroma compounds in thermally processed foods. *Trends in Food Science & Technology, 17*(5), 236-243.

Dixon, M. D., & Hammond, E. G. (1984). The flavor intensity of some carbonyl compounds important in oxidized fats. *Journal of the American Oil Chemists' Society, 61*(9), 1452-1456.

Elmore, J. S., Dodson, A. T., & Mottram, D. S. (2011). Reactions of propylene glycol with the constituents of food flavourings. In *The Thirteenth Weurman Flavour Research Symposium*, (pp. 137). Zaragoza, Spain: Book of Abstracts.

Fernández-Vázquez, R., Linforth, R., Hort, J., Hewson, L., Vila, D. H., Heredia Mira, F. J., Vicario, I. M., & Fisk, I. (2013). Headspace delivery of limonene from the serum and non-serum fractions of orange juice in-vitro and in-vivo. *LWT - Food Science and Technology, 51*(1), 65-72.

Fisk, I. D., Boyer, M., & Linforth, R. S. T. (2012). Impact of protein, lipid and carbohydrate on the headspace delivery of volatile compounds from hydrating powders. *European Food Research and Technology, 235*(3), 517-525.

Fisk, I. D., Kettle, A., Hofmeister, S., Virdie, A., & Silanes Kenny, J. (2012). Discrimination of Roast and Ground Coffee Aroma. *Flavour, 1*(14).

Fisk, I. D., Linforth, R. S. T., Taylor, A. J., & Gray, D. A. (2011). Aroma encapsulation and aroma delivery by oil body suspensions derived from sunflower seeds (Helianthus annus). *European Food Research and Technology*, *232*(5), 905-910.

Frankel, E. N. (1985). Chemistry of autoxidation: mechanism, products and flavor significance. In D. B. Min & T. H. Smouse (Eds.), *Flavor Chemistry of Fats and Oils*, (pp. 1-37). Champaign, IL, USA: American Oil Chemists's Society.

Gassenmeier, K. (2003). Vanillin and xanthineoxidase - key factors for the generation of a cardboard off-note in vanilla ice cream. *LWT - Food Science and Technology.*, *36*(1), 99-103.

Given, P. S. (2009). Encapsulation of flavors in emulsions for beverages. *Current Opinion in Colloid & Interface Science*, *14*(1), 43-47.

Graf, E., & De Roos, K. (1996). Performance of vanilla flavor in low-fat ice cream. In R. J. McGorrin & J. V. Leland (Eds.), *Flavor-food Interactions*, (pp. 24-35). Washington DC: American Chemical Society.

Heenan, S. P., Hamid, N., Dufour, J.-P., Harvey, W., & Delahunty, C. M. (2009). Consumer freshness perceptions of breads, biscuits and cakes. *Food Quality and Preference*, *20*(5), 380-390.

Josephson, D. B., & Lindsay, R. C. (1987). Retro-aldol related degradations of 2,4-decadienal in the development of staling flavors in fried foods. *Journal of Food Science*, *52*, 1186.

Kant, A., Linforth, R. S. T., Hort, J., & Taylor, A. J. (2004). Effect of beta cyclodextrin on aroma release and flavor perception. *Journal of Agricultural and Food Chemistry*, *52*, 2028-2035.

Kilcast, D., & Subramaniam, P. (2000). Introduction. In D. Kilcast & P. Subramaniam (Eds.), *Stability and Shelf-Life of Food*, (pp. 1-22). Cambridge, UK: Woodhead Publishing.

Leclercq, S., Reineccius, G. A., & Milo, C. (2006). Model studies on the influence of matrix type and storage environment on the stability of a model aroma mixture during storage. *Journal of Agricultural and Food Chemistry*, *55*(2), 421-425.

Potineni, R. P., D. (2008). Influence of flavor solvent on flavor release and perception in sugar-free chewing gum. *Journal of Agricultural and Food Chemistry*, *56*(9), 3254–3259.

Saison, D., De Schutter, D. P., Uyttenhove, B., Delvaux, F., & Delvaux, F. R. (2009). Contribution of staling compounds to the aged flavour of lager beer by studying their flavour thresholds. *Food Chemistry*, *114*(4), 1206-1215.

Sinha, A. K., Sharma, U. K., & Sharma, N. (2008). A comprehensive review on vanilla flavor: Extraction, isolation and quantification of vanillin and others constituents. *International Journal of Food Sciences and Nutrition*, *59*(4), 299-326.

Tietz, M., Buettner, A., & Conde-Petit, B. (2008). Interaction between starch and aroma compounds as measured by proton transfer reaction mass spectrometry (PTR-MS). *Food Chemistry*, *108*(4), 1192-1199.

Ullrich, F., & Grosch, W. (1987). Identification of the most intense volatile flavor compounds formed during autoxidation of linoleic-acid. *Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung*, *184*(4), 277-282.

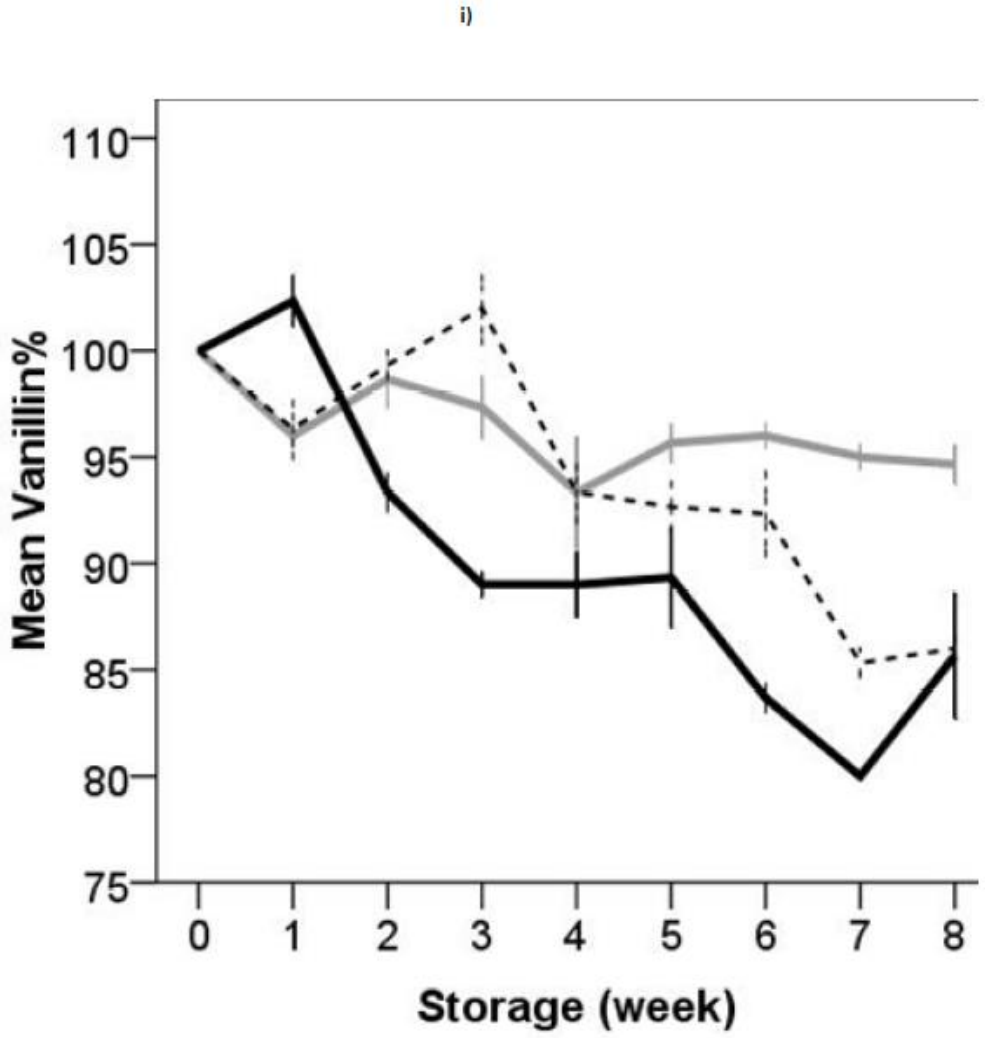
Yang, N. (2012). *Flavour Reformulation and Flavour Stability.* University of Nottingham.

Yang, N., Fisk, I. D., Linforth, R., Brown, K., Walsh, S., Mooney, S., Sturrock, C., & Hort, J. (2012). Impact of flavour solvent on biscuit micro-structure as measured by X-ray micro-Computed Tomography and the distribution of vanillin and HMF (HPLC). *European Food Research and Technology, 235*(6), 1083-1091.

Yang, N., Hort, J., Linforth, R., Taylor, A., Brown, K., Walsh, S., & Fisk, I. (2011). Impact of flavour carrier solvent on vanillin stability and the aroma of short-cake biscuits. In *The Thirteenth Weurman Flavour Research Symposium*, (pp. 280). Zaragoza, Spain: Book of Abstracts.

Yu, T., Macnaughtan, B., Boyer, M., Linforth, R., Dinsdale, K., & Fisk, I. D. (2012). Aroma delivery from spray dried coffee containing pressurised internalised gas. *Food Research International, 49*(2), 702-709.

Figure 1: Vanillin concentration normalized to week 0 (%) in biscuits prepared with a) PG and b) TA then stored for 8 weeks at 20 °C (grey line), 32.5 °C (dotted line) and 45 °C (black line). Data points are displayed ± standard error, n=3.



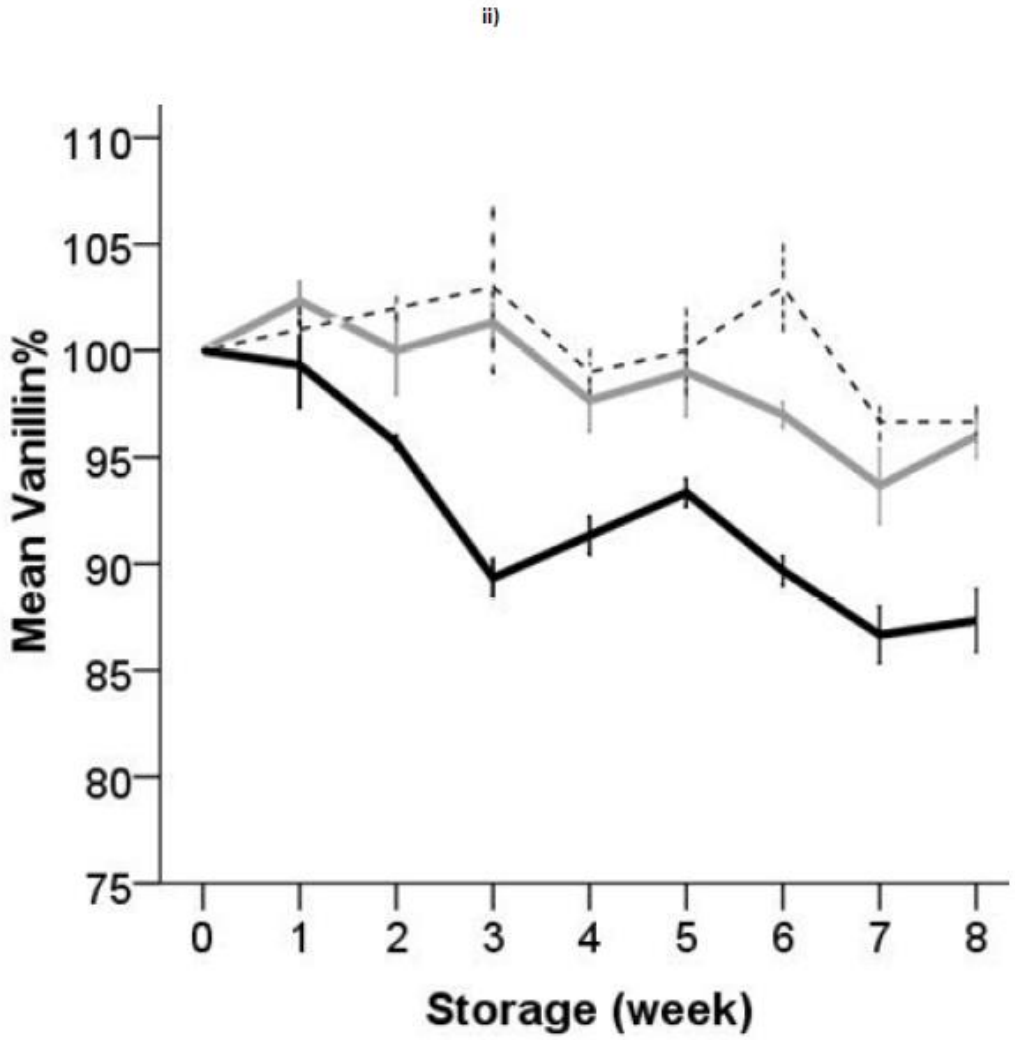
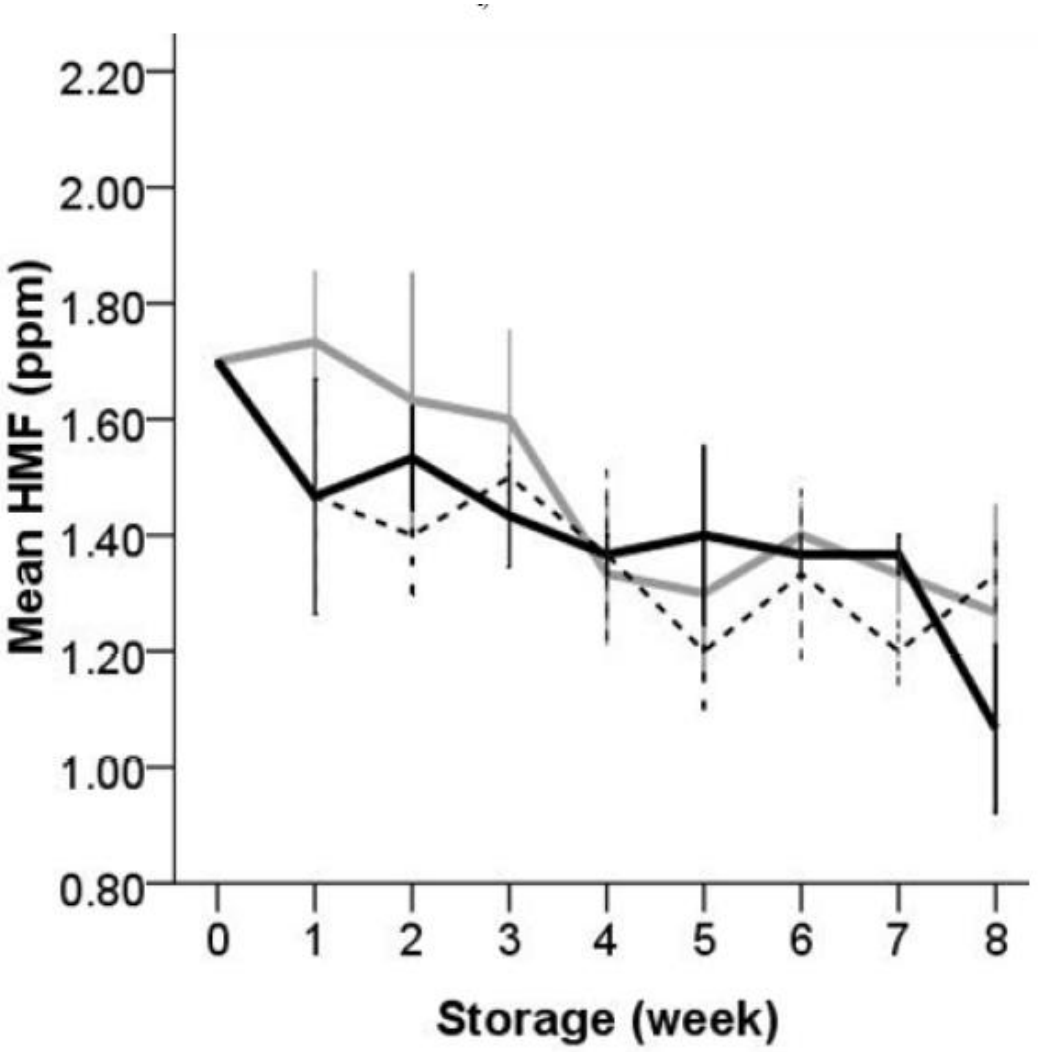


Figure 2: HMF concentration (µg/g biscuit, ppm) in biscuits prepared with a) PG and b) TA then stored for 8 weeks at 20 °C (grey line), 32.5 °C (dotted line) and 45 °C (black line). Data points are displayed ± standard error, n=3.


Mean HMF (ppm)
2.20
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0
1
2
3
4
5
6
7
8
Storage (week)

566

ii)

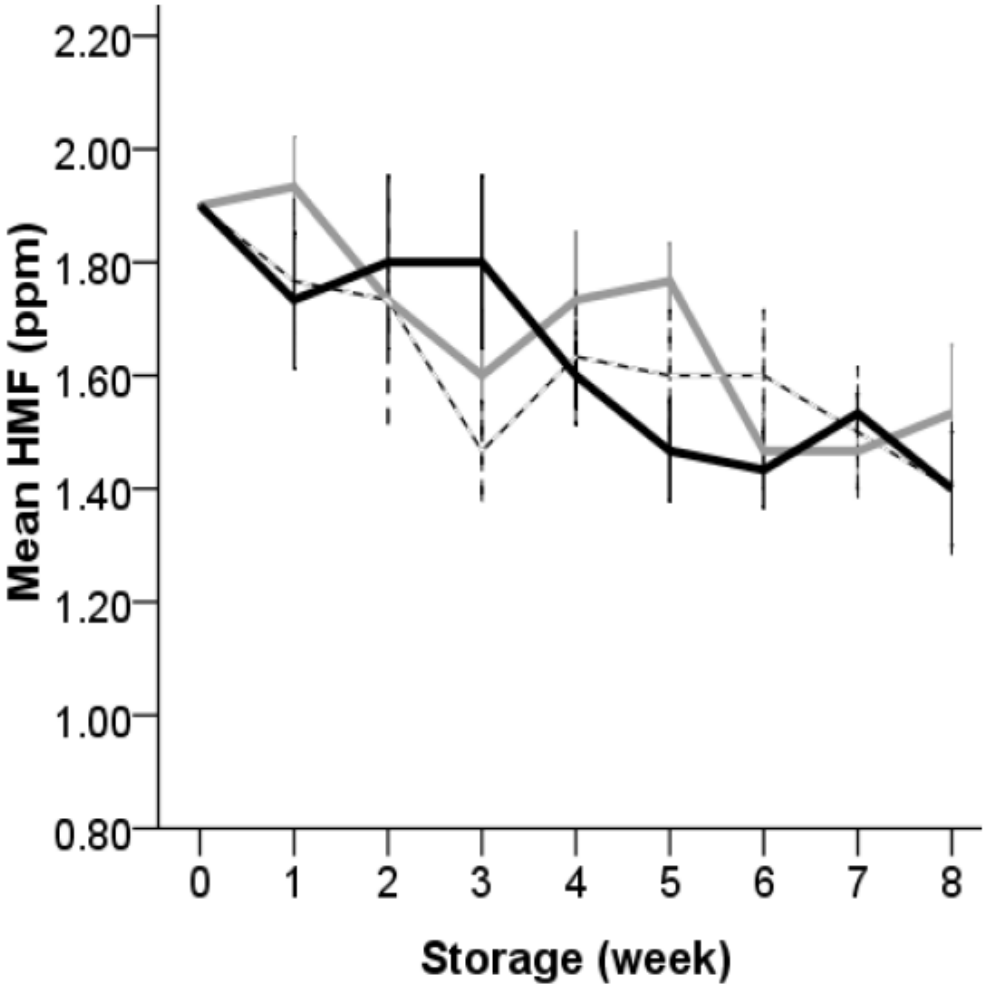
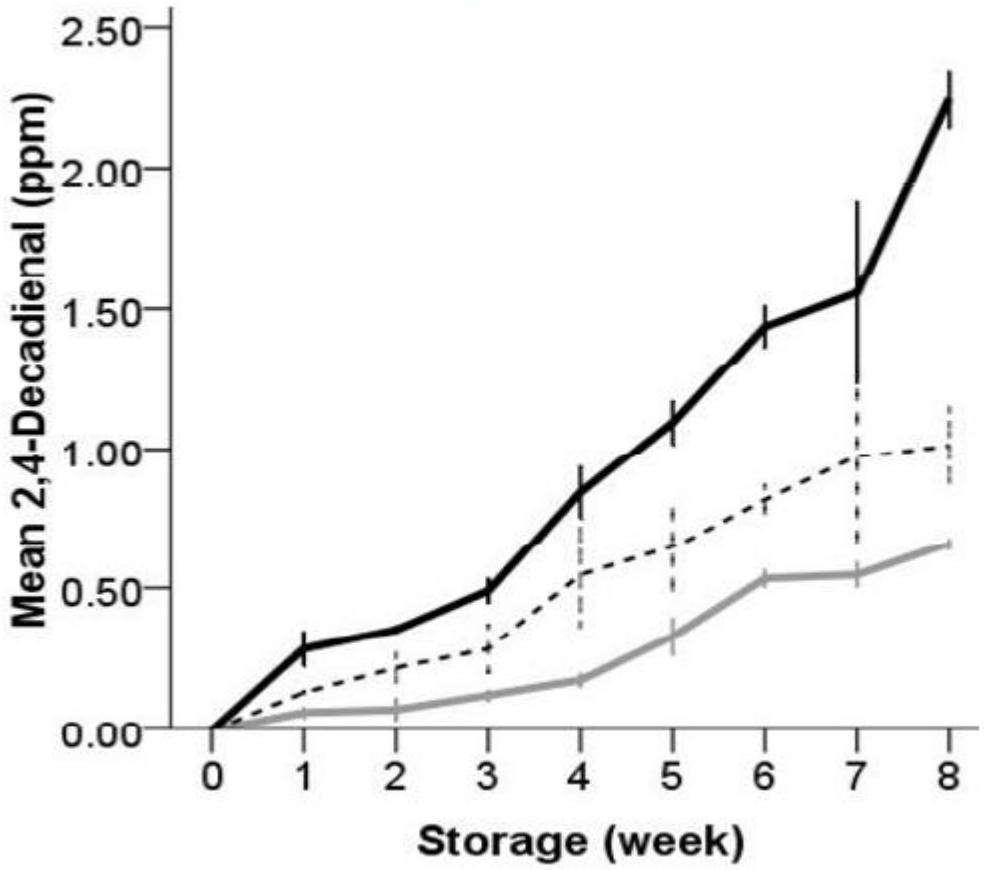


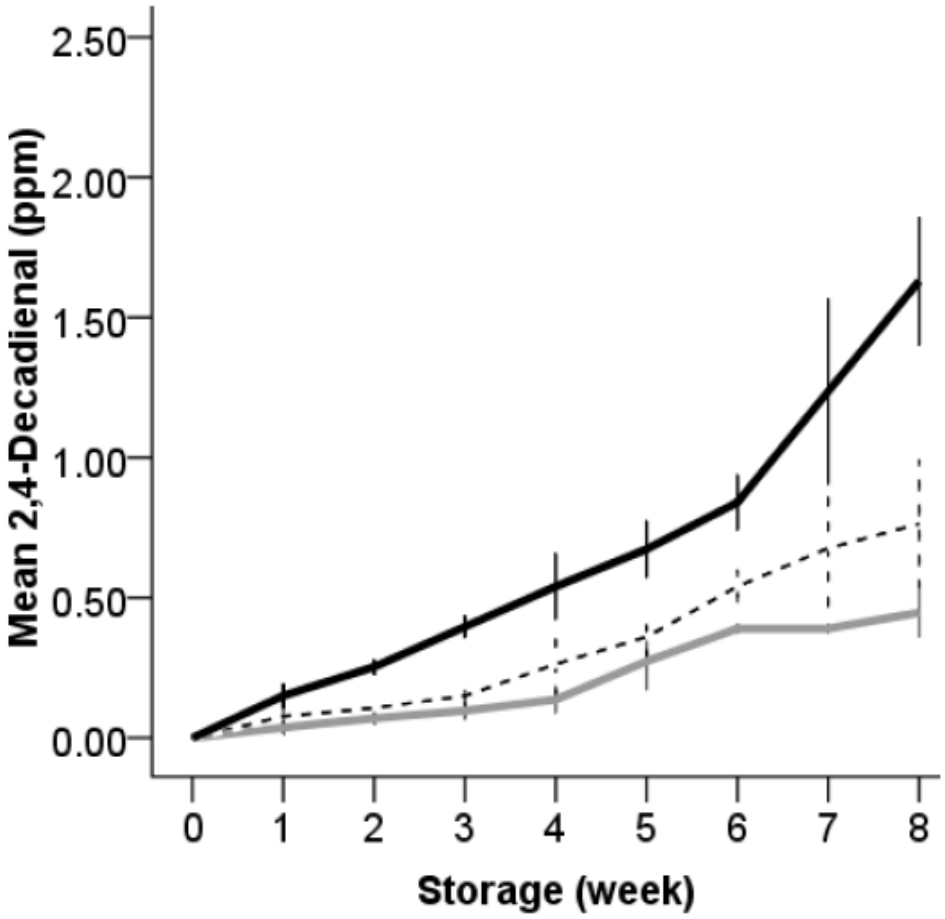
Figure 3: 2,4-Decadienal concentration (µg/g biscuit, ppm) in biscuits prepared with a) PG and b) TA then stored for 8 weeks at 20 °C (grey line), 32.5 °C (dotted line) and 45 °C (black line). Data points are displayed ± standard error, n=3.

i)

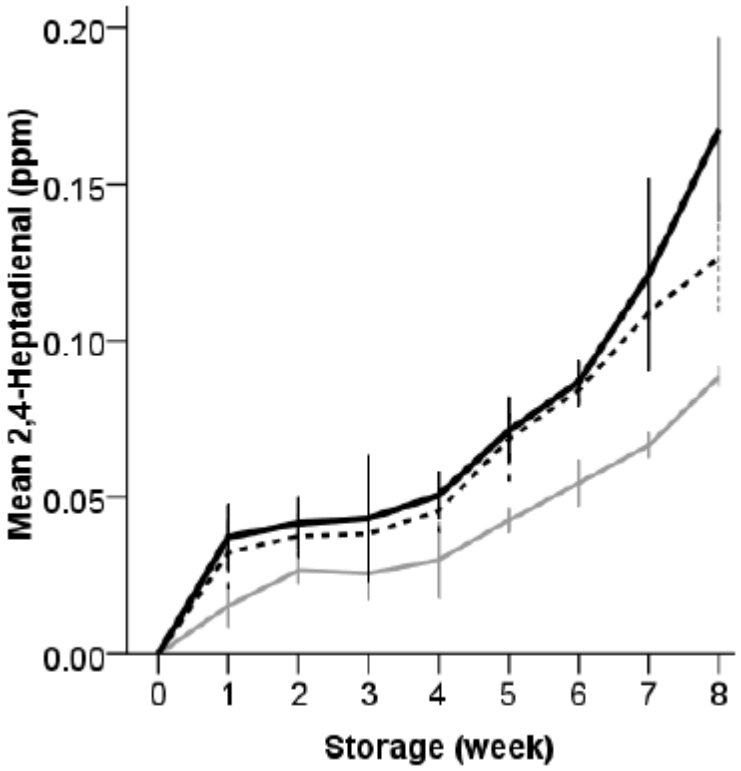

Mean 2,4-Decadienal (ppm)
2.50
2.00
1.50
1.00
0.50
0.00
0
1
2
3
4
5
6
7
8
Storage (week)

571

ii)

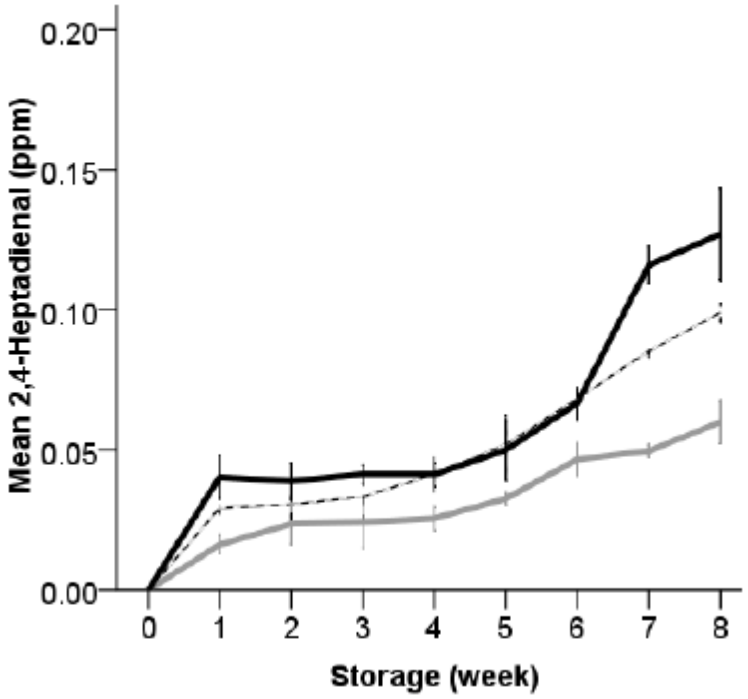


572
573 Figure 4: 2,4-Heptadienal concentration (µg/g biscuit, ppm) in biscuits prepared with i)
574 PG and ii) TA then stored for 8 weeks at 20 °C (grey line), 32.5 °C (dotted
575 line) and 45 °C (black line). Data points are displayed ± standard error, n=3.

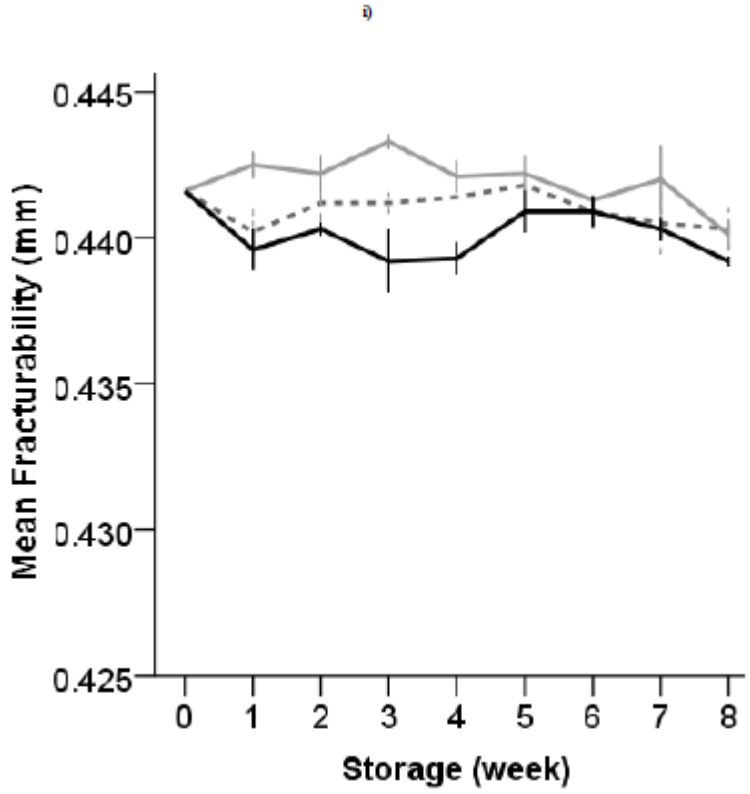

Mean 2,4-Heptadienal (ppm)
0.20
0.15
0.10
0.05
0.00
0
1
2
3
4
5
6
7
8
Storage (week)

576

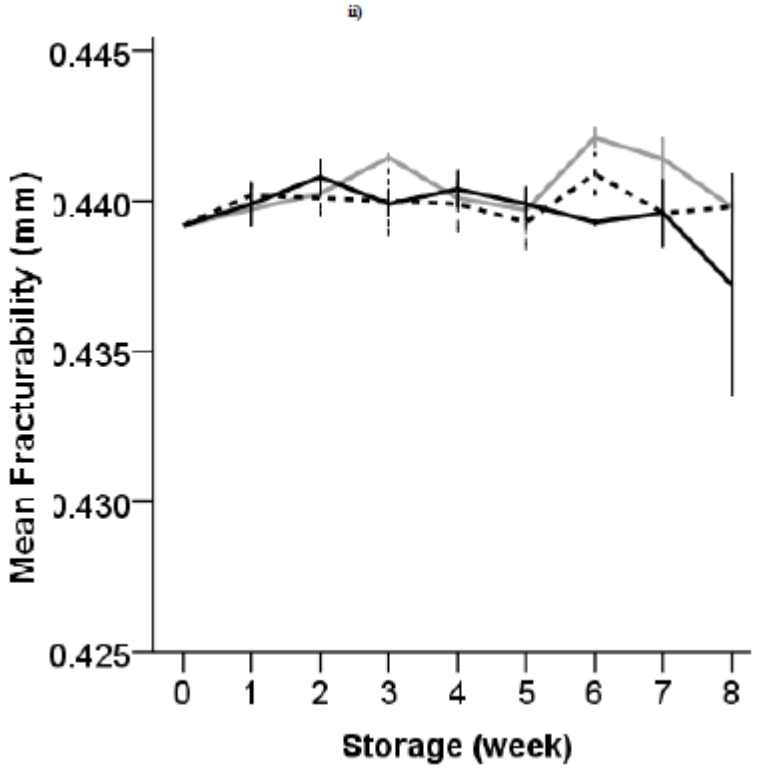
ii)



577
578 Figure 5: Fracturability (mm) of biscuits prepared with i) PG and ii) TA then stored
579 for 8 weeks at 20 °C (grey line), 32.5 °C (dotted line) and 45 °C (black line).
580 Data points are displayed ± standard error, n=3.



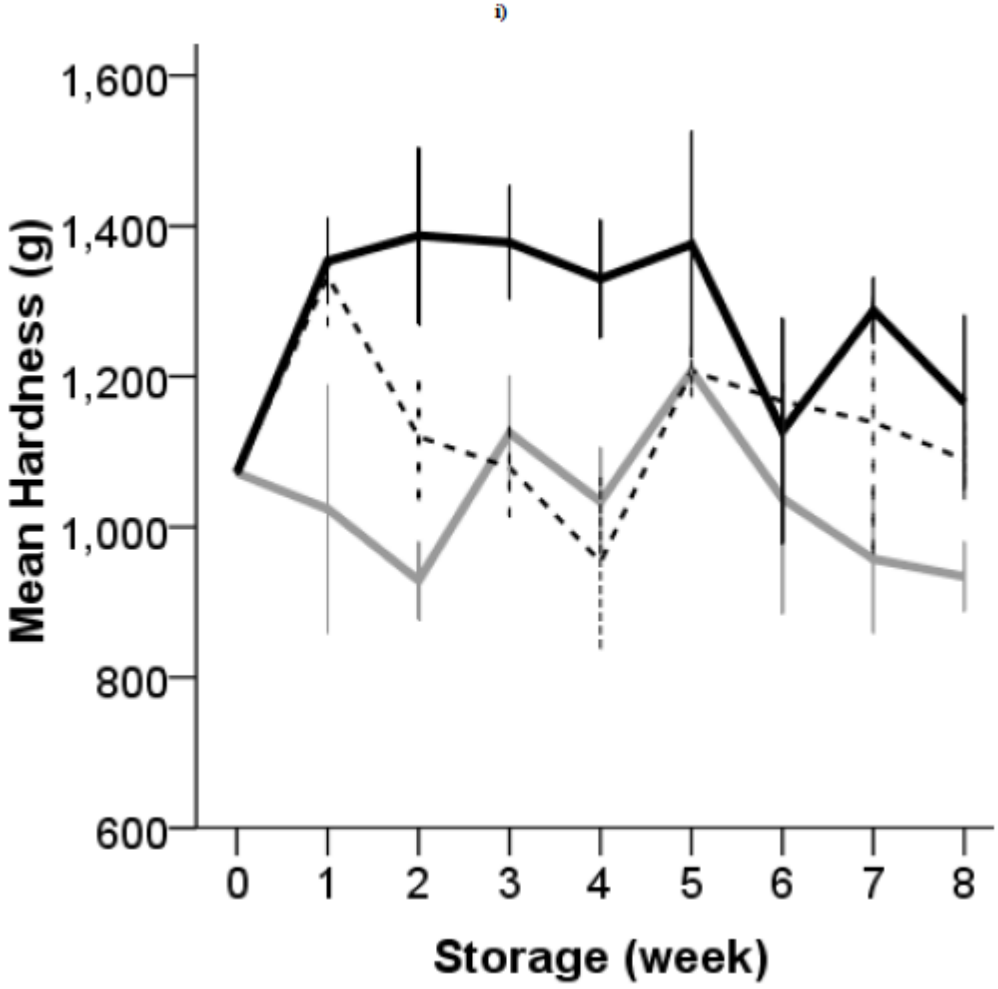
581



582

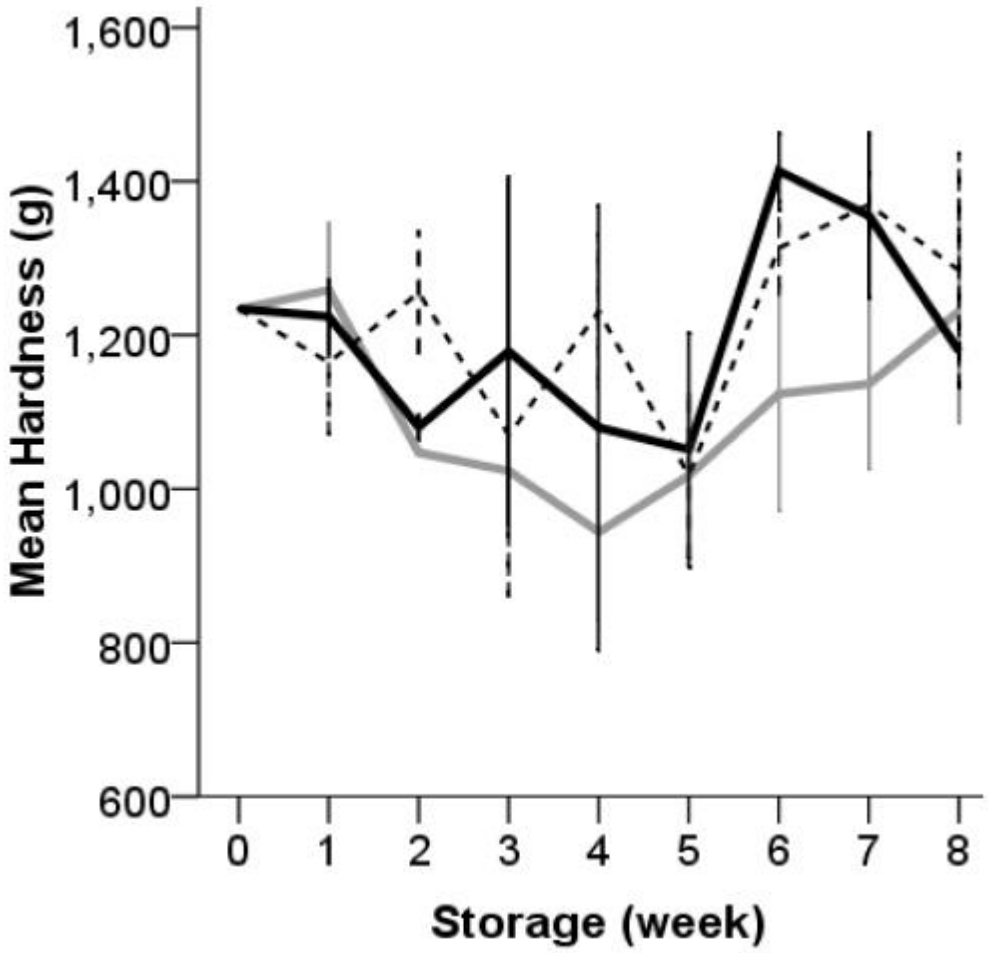
583 Figure 6: Hardness (g) of biscuits prepared with i) PG or ii) TA then stored for 8

584 weeks at 20 °C (grey line), 32.5 °C (dotted line) and 45 °C (black line). Data
585 points are displayed with ± standard error, n= 2: Figure 3: Figure 4:
586 Figure



587

ii)


1,600
1,400
1,200
1,000
800
600
Mean Hardness (g)
0
1
2
3
4
5
6
7
8
Storage (week)

588