# (Bio)chemical reactions associated with ageing of red kidney beans (*Phaseolus vulgaris*) during storage probed by volatile profiling: the role of glass transition temperature.

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**Abstract**

During storage, common beans are susceptible to ageing leading to quality changes, in particular their cooking quality. In this study, kinetics of evolution of volatile compounds was assessed in order to gain insight into possible reactions occurring during ageing of beans. The evolution of volatile compounds of red kidney beans stored at varying conditions of temperature and moisture content relative to their glass transition temperature ($T_g$) were evaluated. Storage conditions highly influenced the evolution of volatile compounds whereby more volatile compounds and higher concentrations were detected in beans stored at higher temperature and moisture content. The volatile marker compounds identified are typical for protein degradation and lipid oxidation reactions, although for beans stored at the highest moisture contents (12.8 and 14.5%) the compounds obtained do not allow to exclude microbial activity. The rate of evolution of selected volatile marker compounds was highly correlated (benzaldehyde (r=0.58), acetic acid (r=0.75), 1-propanol,2-methyl (r=0.84 ) and 2-butanone (r=0.89)) with storage above $T_g$ signifying that the rate and extent of these (bio)chemical reactions can be largely controlled by storing the beans at temperatures not exceeding 20 °C above their $T_g$. Volatile profiling ~~was shown~~showed to be an important approach to monitor quality changes of beans during storage by assessing the nature, rate and extent of (bio)chemical reactions occurring.

**Keywords**: Volatile compounds, cooking quality, kinetics, state diagrams

## 1. Introduction

Once harvested, common beans are usually dried and stored for long periods to bridge the gap between consecutive harvesting seasons. Dried bean seeds are, from a polymer science point of view, viscoelastic materials with limited molecular mobility and can therefore exhibit great quality stability during storage (Walters et al., 2010). However, long term storage of beans at high relative humidity >65% (increased moisture content) and/or temperature (>25 °C) is suggested to promote molecular mobility that facilitates (bio)chemical reactions and consequently deterioration of bean quality (Walters et al., 2005; Peleg, 2019; Chhabra et al., 2019).

Among the quality changes occurring in beans during storage, development of the hard-to-cook (HTC) defect that promotes delayed softening of beans during subsequent cooking (El-Tabey Shehata, 1992; Reyes-Moreno et al., 1993), is of principle concern. Although HTC has been extensively studied, mechanisms leading to its development are not yet fully understood. Current literature suggests involvement of (bio)chemical reactions associated with various cellular components particularly cell membranes, pectin, phenolic acids, cations such as calcium, phytate and associated enzymes such as lipoxygenase, pectin methylesterase, peroxidase and phytases (Chen et al., 2021; Wainaina, Lugumira et al., 2022). In recent studies, membrane degradation has been suggested to be the first step in the ageing induced HTC development during seed storage (Ratajczak et al., 2019; Ebone et al., 2019). Impaired cell membrane integrity and functionality, at high storage temperature and moisture conditions where molecular mobility is enhanced, facilitates enzyme substrate interactions and displacement/diffusion of cations creating access to binding sites. It can be deduced that ageing of beans during storage, that results in quality deterioration, is mainly initiated by particular (bio)chemical reactions that lead to disruption of cellular membranes (Murthy et al., 2003; Chhabra et al., 2019). Therefore, in order to fully understand the mechanisms leading to development of the HTC defect, an evaluation of the nature and kinetics of the associated (bio)chemical reactions is vital.

Volatile compounds are naturally occurring as a result of normal plant metabolism but can also be degradation products of major chemical reactions such as those occurring during post-harvest storage, processing and/or post processing storage and include compounds like aldehydes, ketones, alcohols and heterocyclic compounds (Ma et al., 2016). Given that volatile compounds are major by-products of these (bio)chemical reactions, the extent and rate of

production of these compounds can serve as indicators of what is happening during processing and/or storage (Kebede et al., 2015; Zhang et al., 2022). Until now, very few studies have focused on evolution of volatile compounds during postharvest storage of beans in relation to evaluation of quality deterioration with most of the studies mainly focusing on flavour evolution of beans during cooking and/or how HTC development during storage influences the flavour compounds of beans after cooking (Ma et al., 2016; Mishra et al., 2017; Chigwedere et al., 2019). By only looking at the cooking kinetics of aged beans, possible direct identification of degradation pathways during storage-induced ageing becomes difficult and what happens during storage might be concealed since cooking can accelerate some of these reactions and/or lead to formation of similar degradation products and/or degrade some of these compounds. Given that multiple factors and mechanisms are involved in the ageing process during storage (Fu et al., 2015), an understanding of the sequence of events and cause/effect relationship between events is imperative in underpinning the development of this defect during storage.

In this study, volatile profiling of red kidney beans stored at varying conditions of temperature, moisture content for varying duration of time was carried out. The goal was to gain insight into the evolution of volatile compounds during storage of red kidney beans at varying conditions relative to their glass transition temperature ($T_g$) and to identify possible (bio)chemical reactions associated with ageing of beans during storage. As a first step, volatile compounds (markers) whose amounts were changing the most during storage were selected and their possible reaction pathways proposed. Secondly, kinetic modelling of the selected markers was done to evaluate the reaction kinetics as function of storage temperature and moisture content. Lastly, the estimated rate constants were evaluated against storage above $T_g$ in order to investigate the suitability of state diagrams in controlling the rate of reactions linked to ageing of beans during storage.

## 2. Materials and methods

### 2.1 Raw materials and sample preparation

All chemicals used were of analytical grade, unless otherwise mentioned. As this study is a continuation of our previous work (Wainaina, Kyomugasho, et al., 2022; Wainaina, Wafula, et al., 2022), the same materials (red kidney beans) and sample preparation procedures (glass transition temperature evaluation and storage experiment) were used. Briefly, red kidney beans grown and harvested in Belgium (2019) were sorted to remove dirt and defective/damaged

beans before use. Cleaned beans were equilibrated at 4 °C to different moisture contents using the following saturated salt solutions; sodium nitrate (aw 0.78), sodium bromide (aw 0.63), magnesium chloride (aw 0.33) and potassium hydroxide (aw 0.14) and lithium bromide (aw 0.07). The obtained moisture contents expressed as percent weight loss after oven drying at 105 °C for 20 h were 6.9%, 8.7%, 11.1%, 12.8% and 14.5%. After equilibration, a part of the beans were used for glass transition temperature evaluation and the rest was used for the storage experiment (*section 2.2*).

## 2.2 Storage experiment

Storage of the equilibrated beans was carried out as described in detail in our previous work (Wainaina, Kyomugasho, et al., 2022) and (Wainaina, Wafula, et al., 2022). Briefly, for each moisture content (obtained in *section 2.1*) a part of the equilibrated beans were stored at -40 °C until further use and are here referred to as 'non-stored' beans and the rest were stored at different temperatures (25 °C, 30 °C, 35 °C and 42 °C) up to 6 months with periodic sampling. For each temperature-moisture content combination, separate airtight glass jars were used per sampling time moment and this was done in duplicate. For this study at least 5 sampling time moments (in weeks) were conducted per temperature-moisture content combination. Storage conditions were selected to reflect points at and above the glass transition line of the red kidney bean (Wainaina, Kyomugasho, et al., 2022). The sampled beans were also stored at -40 °C until further analyses.

## 2.3 Volatiles extraction and analysis

Volatile extraction and analysis was conducted using an untargeted fingerprinting approach following the procedure described by Kebede et al., (2015) and Chigwedere et al., (2019) with minor modifications. Frozen whole red kidney beans (obtained from *section 2.3*) were milled into powder (>125 µm) and 2 g was weighed into a 10 mL amber glass vial in an ice bath to which chilled 2.5 mL of saturated sodium chloride solution was added before tightly closing using caps with silicon septum seals. The vials were vortexed to ensure proper mixing before being randomly placed on cooling tray of the autosampler for volatiles profiling. Six replicates were made for each sample. Volatiles profiling was conducted using a headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC–MS) system (Keysight Technologies, Diegem, Belgium) equipped with a CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland) set at 10 °C. Before volatile extraction, each vial was incubated at 40 °C for 20 min, with agitation at 500 rpm after which a pre-conditioned fibre

with a 30/50 µm divinylbenzene/ carboxen/polydimethylsiloxane (DVB/CAR/PDMS) sorptive coating (Stableflex, Supelco, Bellefonte, Pennsylvania) was used to extract volatile compounds from the headspace of the vial for 10 min. Extracted volatiles were desorbed ~~on to~~onto the GC injection port at 230 °C for 2 min, then in splitless mode, ~~they~~ these were injected onto a 30m×0.25mm×0.25 µm polar HP INNOWax capillary column (Agilent Technologies, Santa Clara, California) for separation with helium as the carrier gas at 1.5 mL/min. To facilitate separation and elution of the injected volatile compounds, the GC oven was maintained at 40 °C for 2 min before the temperature was ramped up to 80 °C at a rate of 5 °C/min and maintained for 1 min after which it was ramped again to 220 °C at a rate of 6 °C/min. After a holding time of 2 min, a final ramp at 50 °C/min to 250 °C was made and immediately, the oven was cooled back to 40 °C. The mass spectra were obtained by electron ionization (EI) at 70 eV, and the ion source and quadrupole temperatures were 150 °C and 230 °C, respectively while a mass-to-charge ratio scanning range of 35–550 at 3.8 scans/s was employed. A new fibre was used for each moisture content in order to minimize fibre degradation. In order to obtain a retention index (calibration data), a standard mixture of n-alkanes ($C_8$-$C_{22}$) was used. A 10 µL volume of the alkane standard was mixed with 5 mL of demineralized water in a 10 mL vial and analyzed as described above. A representation of the total ion chromatograms obtained after analysis is shown in Fig. 1, highlighting some volatile compounds with high abundance.

### 2.4 Data analysis and modelling

#### 2.4.1 Multivariate data analysis and markers selection

Multivariate data analysis (MVDA) and markers selection was done following the procedure described by Kebede et al., (2015) and Chigwedere et al., (2019). Due to the complex nature of the chromatograms obtained after GC-MS analysis, a pre-processing step is necessary in order to extract 'pure' component spectra from co-eluted compounds in the chromatograms obtained. To perform this, an automated mass spectral deconvolution and identification system (AMDIS) (Version 2.72, 2014, National Institute of Standards and Technology, Gaithersburg, MD) in which a retention index calibration file was also build was used as the first pre-processing step. The deconvoluted spectra were further analysed with Mass Profiler Professional (MPP) (Version 12.0, 2012, Keysight Technologies, Diegem, Belgium) for filtering and peak alignment from which a spreadsheet containing peak areas and samples was obtained and was used as an input for the multivariate statistical data analysis. MVDA was

carried out using Solo (Version 8.6.2, 2018, Eigenvector Research, Wenatchee, WA). All data were mean-centred and the variables were weighted by their standard deviation to give them equal variance. In a first step, principal component analysis (PCA) was conducted as an exploratory technique to evaluate each data set and to detect potential outliers. To study the evolution during storage, per storage temperature and moisture content, partial least squares (PLS) regression was performed, with the volatiles as X-variables and the storage time as Y-variable. For determining the complexity of the PLS model, the lowest number of latent variables (LVs) that maximally describe the change during storage was used. The selected PLS models were comprised of latent variables that contributed at least 2% of the Y-variance explained by the models. To investigate the change in the volatile fraction as a function of time, qualitative graphical presentations of scores (a projection of objects in a variable space) and loadings (a projection of variables on a coordinate system) was employed by construction of bi-plots using OriginPro 2020 (Origin Lab Corporation, Northampton, MA, USA). Following this, volatile compounds that were clearly changing during storage were selected through variable identification (VID) coefficients. These values range between -1 and +1 and correspond to the correlation coefficient between each original X-variable and predicted (by the selected PLS-model) Y-variable. In this work, variables with an absolute VID value higher than 0.80 were considered to be important. These variables were identified and linked to possible reaction pathways. Identification of the compounds was performed by comparing the deconvoluted mass spectrum with the reference mass spectra from National Institute of Standards and Technology (NIST) spectral library (NIST14, version 2.2, Gaithersburg, MD). For identification, a threshold match of 90% was taken into account. A visual inspection of spectral matching between the detected compound and the match from the library as well as comparison of the retention index were also performed.

2.4.2 Kinetic modelling of selected markers

The evolution of selected markers during storage was modelled following a zero order model (**Equation 1**) using the SAS Proc NLIN procedure (SAS version 15.1, SAS Institute, Inc., Cary, NC, USA).

$$A = A_0 - Kt \qquad \textbf{Equation 1}$$

A is peak area of marker compound at a particular storage time (t, weeks), $A_0$ is peak area at time zero of storage and K ($week^{-1}$) is marker evolution rate constant.

2.4.3 Modelling influence of storage above $T_g$ ($T-T_g$) on evolution rate of markers

To determine the effect of storing beans at temperatures above their $T_g$ on marker evolution rate, an empirical exponential equation (**Equation 2**) (Kyomugasho et al., 2020) was used.

$$K = K_0 \times \exp(a*(T-T_g)) \qquad \textbf{Equation 2}$$

K is marker evolution rate, $K_0$ is a pre-exponential factor, a is an empirical constant, T is the storage temperature and $T_g$ is the glass transition temperature. Parameters a and $K_0$ were estimated by non-linear regression using the SAS Proc NLIN procedure (SAS version 15.1, SAS Institute, Inc., Cary, NC, USA).

## 3. Results and discussion

### 3.1 Qualitative evaluation of evolution of volatile compounds during storage of red kidney beans

#### 3.1.1 Identification of volatile compounds

The total headspace volatile compounds ~~detected~~ identified in raw red kidney beans stored at varying temperature-moisture content ranged between 53 to 120 after deconvolution. The number of detected compounds increased with increase in storage moisture content and temperature signifying additional reactions at higher storage temperature and moisture content conditions. Fig. 2 is a representation of the bi-plots obtained from a graphical projection of the score and loading plots of 14.5% (the highest moisture content evaluated) at three different temperatures (25, 30 and 35 °C). The selected PLS models had 2, 2 and 3 LV's for beans stored at 25, 30 and 35 °C, respectively. Similar bi-plots were obtained for the other temperature-moisture content conditions and are shown in Supplementary Figs 1-4, but the number of compound markers are different as shown in Table 1.

For each bi-plot, two LVs that explain a considerable part of the Y-variance (effect of storage time) are plotted whereby the first LV (LV1) explains the largest part (>90%) of the variability in the data compared to the subsequent LVs. Volatile compounds are indicated with open circles while the samples are indicated with coloured symbols and a clear trend of increase of

these compounds with storage time from left to right can be observed for each temperature. The arrow shows the direction of the Y-variable (storage time) and volatile compounds positioned in the direction of this arrow are assumed to increase with storage time and vice versa. Volatile compounds whose concentrations change significantly with storage time (marker compounds) were selected by calculating their VID coefficients and are indicated as bold open circles and represent compounds with VID coefficients of ≥0.8.

The marker compounds selected for the different temperature-moisture content conditions are represented in Table 1. Similar to the total volatile compounds evolution, it is evident that there were more marker compounds as storage temperature and moisture content increased which indicates an effect of these factors on the volatiles profile of red kidney beans during storage. It was also observed that most marker compounds significantly increased (formation/production) during storage. The compounds that formed over time during storage belong to the following classes; alcohols (1-propanol,2-methyl, 1-hexanol, 2-butanol, isopropyl alcohol, 2-pentanol, benzyl alcohol, 2-ethyl-1-hexanol, phenylethyl alcohol, 3-furanmethanol), aldehydes (benzaldehyde, 3-furaldehyde, hexanal, 2-heptenal, 2-octenal, 2,4-heptadienal, 2-hexenal, butanal-2-methyl-, propanal), acids (acetic acid, butanoic acid-2-methyl), ketones (2-butanone, acetone, 2-furanonedihydro-4-methyl), esters (ethyl acetate, methyl butoxyacetate), hydrocarbons (pentane, 1,4-hexadiene-3-ethyl, 1-pentene-2-methyl), furan derivatives (furan-2-ethyl) and sulphur containing compounds (dimethyl sulphide). It is evident that alcohols and aldehydes were the most abundant classes of volatiles which is in line with what is reported in literature for raw pulses (Khrisanapant et al., 2019).

3.1.2 Linking identified volatile compounds to possible (bio)chemical pathways

Alcohols and aldehydes are mostly formed from enzymatic and nonenzymatic lipid degradation but some have also been linked to amino acid degradation (Karolkowski et al., 2021). On the one hand, for instance hexanal has been linked to degradation of linoleic acid (n-6) while propanal has been associated with degradation of linolenic acid (n-3) (Colville et al., 2012). On the other hand, some aldehydes could also be formed from amino acids degradation through for instance Strecker degradation which involves oxidative deamination and decarboxylation of α-amino acids and occurs in presence of di-carbonyl compounds formed during Maillard reactions (Colville et al., 2012; Chigwedere et al., 2019). These aldehydes include benzaldehyde and 2-methylbutanal from phenylalanine and isoleucine degradation respectively. Similarly, alcohols can also be formed from lipid degradation as well as amino

acid degradation. Alcohol dehydrogenase can promote interconversions between aldehydes and alcohols and the activity of this enzyme can be facilitated by different storage conditions (Taylor et al., 1999). In this study, it was interesting to observe that 1-propanol,2-methyl, a branched alcohol, was ~~detected~~ identified in all the storage conditions explored. Branched alcohols have been reported to be formed from amino acid degradation (Chigwedere et al., 2019) and in particular 1-propanol,2-methyl has been associated with valine degradation (Karolkowski et al., 2021).

Ketones have been reported to result from predominantly breakdown of unsaturated fatty acid hydroperoxides. In this study, 2-butanone was the most abundant ketone and has also been reported in other non-cooked bean varieties in literature (Ma et al., 2016). In addition, 2-furanonedihydro-4-methyl was also observed especially in beans with the highest moisture content (14.5%). However, it is not clear from literature how this compound is formed. Organic acids such as acetic acid and 2-methyl butanoic acid detected in this study are mainly associated with amino acid degradation (Karolkowski et al., 2021). Presence of acids could also be due to microbial activity/growth such as mould growth especially during storage at higher moisture contents and temperatures. Other minor classes of volatiles detected in raw beans are esters, furan derivatives and sulphur containing compounds. Esters are derived from esterification of acids and alcohols formed during lipid oxidation, furan compounds are formed through several pathways such as lipid oxidation and thermal degradation of amino acids with carbohydrates whereas sulphur compounds such as dimethyl sulphide can be formed from oxidation of methionine (Chigwedere et al., 2019).

It can be noted that most of the volatile compounds ~~detected~~ identified are mainly linked to oxidation of unsaturated free fatty acids and amino acid degradation which are also suggested to be the main (bio)chemical reactions linked to ageing of seeds (Murthy et al., 2003; Colville et al., 2012; Chhabra et al., 2019). As such, unsaturated fatty acids such as linoleic acid and linolenic acids are substrates for enzymatic (lipoxygenase) and nonenzymatic (autooxidation) lipid oxidation whereas degradation of amino acids such as valine, phenylalanine, isoleucine and methionine have been linked to production of branched volatile compounds (Karolkowski et al., 2021). Therefore, the composition of the beans plays an important role in influencing the volatile profiles and the type of (bio)chemical reactions occurring are governed by storage conditions employed. Given that red kidney beans are mainly composed of starch (53%) and proteins (26%) with limited lipids (2%) (Hayat et al., 2014a) (and there are generally minor variations in composition among other different common bean varieties (Hayat et al., 2014b)),

it can be hypothesized that amino acid degradation is the predominant source of volatile compounds with additional lipid oxidation, sugar hydrolysis and potentially microbial fermentation leading to formation of volatile compounds especially at higher moisture contents and temperatures. These additional reactions could also explain why more volatile compounds were ~~detected~~ identified in beans stored at higher temperatures and moisture contents.

### 3.2 Kinetics of evolution of marker compounds during storage of red kidney beans

Characterizing the kinetics of reactions is important in gaining insight into nature and rate of (bio)chemical reactions occurring during storage of beans. Firstly, the main marker compounds (occurring in at least 6 storage temperature-moisture content conditions) from the most abundant volatiles classes being alcohols, aldehydes, ketones and acids were selected respectively 1-propanol,2-methyl, benzaldehyde, 2-butanone and acetic acid. Secondly, the evolution of these selected marker compounds was evaluated as a function of storage time and the results are depicted in Figs 3-6. Looking into these graphs (Figs 3-6), it was evident that the compounds increased with storage time and their evolution was best fit by a zero order model according to which the rate of evolution of a compound is independent of reactants concentration (Van Boekel, 2008).

The kinetic parameters obtained after modelling are shown in Table 2 and visualized in 3D plots shown in Supplementary Fig. 5. While the rate of evolution of 1-propanol,2-methyl is significant with increasing storage temperature as well as moisture content (at all the explored conditions), the case of the other compounds is different. Acetic acid evolution was for instance only relevant during storage at higher moisture contents (and was accelerated by temp) which could be an indication of possible microbial activity that can be facilitated at these high moisture conditions as discussed in *section 3.1.2*. On the other hand, the kinetics of evolution of 2-butanone were relevant at higher temperatures at low moisture content conditions whereas the kinetics of benzaldehyde were relevant at moderate temperature and moisture content conditions. Given that these compounds are primary products of either lipid oxidation and amino acid degradation as discussed in *section 3.1.2*, it could be that they are further degraded or react with other degradation products during adverse (high temperature and moisture content) storage conditions. The rate of these reactions can be enhanced at these conditions, particularly, lipid oxidation has been shown to be enhanced as temperature (and moisture content to a certain level) increases whereas amino acid degradation has been shown to be

311 facilitated by both temperature and moisture content increase (Murthy et al., 2003; Colville et
312 al., 2012).

313 These results are an indication that (bio)chemical reactions occurring during storage of beans
314 are greatly facilitated by storage temperature and moisture content conditions, with the
315 extent/influence being dependent on the nature of the reaction. In other words, the primary
316 mechanisms that initiate ageing of beans during storage differs depending on the storage
317 conditions. Furthermore, since kinetics of 1-propanol,2-methyl and 2-butanone evolution were
318 relevant even at the lowest moisture content (6.9%) albeit at a very low rate ($0.49\pm0.06 \times 10^5$
319 and $0.64\pm0.09 \times 10^5$ $week^{-1}$ respectively) as can be seen in Table 2, it can be concluded that
320 grains produce some volatile compounds even when stored at low moisture content conditions
321 especially in combination with high temperatures (generally above 35 °C), signifying some
322 metabolic processes are still occurring. Therefore, the goal of postharvest storage should
323 mainly be to slow down this ageing process as much as possible to ensure quality preservation
324 during long term storage.

325 3.3 Influence of storage above $T_g$ on evolution of marker compounds

326 The theory of $T_g$ is widely used to relate storage of foods and deterioration kinetics (Walters et
327 al., 2005). Therefore, it was important to evaluate the link between the reaction kinetics of the
328 selected marker compounds and storage above $T_g$. The obtained $T_g$ values and corresponding
329 T-$T_g$ values for the storage conditions evaluated are represented in Supplementary Table 1. The
330 reader is directed to our previous study (Wainaina, Kyomugasho, et al., 2022) for a detailed
331 discussion on the $T_g$ results as the red kidney beans samples used for this study are the same as
332 in our previous study. The graphs of the correlations of the evolution kinetics of marker
333 compounds and how far above the $T_g$ the beans were stored (temperature difference between
334 storage temperature and the $T_g$ of the beans) are shown in Fig. 7 from which it can be observed
335 that the rate constants of evolution of these marker compounds increased when the storage
336 temperature was further above the $T_g$ of the beans. The correlation coefficients (r) were 0.88,
337 0.84, 0.75 and 0.58 for 2-butanone, 1-propanol,2-methyl, acetic acid and benzaldehyde,
338 respectively. Therefore, the further above $T_g$ the beans were stored, the faster the rate at which
339 these volatile compounds evolved. This can be attributed to enhanced molecular mobility
340 particularly well above $T_g$ and therefore promotion of underlying (bio)chemical reactions that
341 are otherwise retarded/inhibited below $T_g$ (in glassy state) (Murthy et al., 2003). The results
342 clearly show the plasticizing effect of temperature and moisture influencing the reactions

343 occurring. Therefore, the concept of $T_g$ can be used to control the rate of biochemical reactions
344 linked to ageing of beans thereby maintaining quality during prolonged storage. The authors
345 propose storage of beans at temperature-moisture content conditions that ensure the beans are
346 below their $T_g$ or at storage temperatures that do not exceed 20 °C above their $T_g$ (i.e. $T\text{-}T_g \leq$
347 20 °C), since at these conditions the (bio)chemical reactions leading to quality deterioration
348 would be limited. Therefore, for beans with a moisture content of 6.9, 8.7, 11.1, 12.8 and
349 14.5%, the storage temperature should not exceed 55, 43, 28, 19 and 10 °C respectively.

350 **Conclusion**

351 The evolution of volatile compounds during storage of red kidney beans was influenced by
352 storage conditions whereby beans stored at high temperatures and moisture content had more
353 volatile compounds and higher concentrations, respectively referring to additional
354 (bio)chemical reactions and increased reaction rates. The classes with the most abundant
355 volatile compounds were alcohols, aldehydes and ketones which are compounds linked to
356 mostly amino acid or protein degradation as well as lipid oxidation reactions associated with
357 deteriorative changes of beans during storage. From the overall volatile compounds
358 ~~detected~~identified, several marker compounds were selected and the rate of evolution of the
359 selected marker compounds during storage increased with increase in storage temperature and
360 moisture content. The volatile marker compounds identified are mostly associated with protein
361 degradation and lipid oxidation reactions. It was also interesting to observe that kinetics of
362 specific volatile marker compounds were relevant for particular storage conditions signifying
363 that storage conditions also influence the nature/type of primary (bio)chemical reactions.
364 Furthermore, the kinetics of marker compounds highly correlated with storage above $T_g$
365 showing the importance of the concept of $T_g$ in controlling the rate of (bio)chemical reactions
366 linked to deteriorative changes during storage of beans. Ageing is a complex phenomenon that
367 involves a myriad of (bio)chemical and metabolic processes and volatile compounds profiling
368 can be useful in identifying the nature, rate and extent of primary reactions taking place to
369 initiate this process.

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## Conflicts of interest

None

## References


Chen, D., Pham, U. T. T., Van Loey, A., Grauwet, T., Hendrickx, M., & Kyomugasho, C. (2021). Microscopic evidence for pectin changes in hard-to-cook development of common beans during storage. *Food Research International*, *141*(January), 110115. https://doi.org/10.1016/j.foodres.2021.110115

Chhabra, R., . S., & Singh, T. (2019). Seed Aging, Storage and Deterioration: An Irresistible Physiological Phenomenon. *Agricultural Reviews*, *40*(03). https://doi.org/10.18805/ag.r-1914

Chigwedere, C. M., Tadele, W. W., Yi, J., Wibowo, S., Kebede, B. T., Loey, A. M. Van, Grauwet, T., & Hendrickx, M. E. (2019). Insight into the evolution of flavor compounds during cooking of common beans utilizing a headspace untargeted fingerprinting approach. *Food Chemistry*, *275*(June 2018), 224–238. https://doi.org/10.1016/j.foodchem.2018.09.080

Colville, L., Bradley, E. L., Lloyd, A. S., Pritchard, H. W., Castle, L., & Kranner, I. (2012). Volatile fingerprints o seeds o four species indicate the involvement of alcoholic fermetation, lipid peroxidation an Maillard reactions in seed deterioration during ageing and dessication stress. *Journal of Experimental Botany*, *63*(18), 6519–6530. https://doi.org/10.1093/jxb/err313

Ebone, L. A., Caverzan, A., & Chavarria, G. (2019). Physiologic alterations in orthodox seeds due to deterioration processes. *Plant Physiology and Biochemistry*, *145*(September), 34–42. https://doi.org/10.1016/j.plaphy.2019.10.028

El-Tabey Shehata, A. M. (1992). Hard to cook phenomenon in legumes. *Food Reviews International*, *8*, 191–221.

Fu, Y. B., Ahmed, Z., & Diederichsen, A. (2015). Towards a better monitoring of seed ageing under ex situ seed conservation. *Conservation Physiology*, *3*(1), 1–16.

https://doi.org/10.1093/conphys/cov026

Hayat, I., Ahmad, A., Ahmed, A., Khalil, S., & Gulfraz, M. (2014). Exploring the potential of red kidney beans (Phaseolus vulgaris L.) to develop protein based product for food applications. *Journal of Animal and Plant Sciences*, *24*(3), 860–868.

Hayat, Imran, Ahmad, A., Masud, T., Ahmed, A., & Bashir, S. (2014). Nutritional and health perspectives of beans (*Phaseolus vulgaris* L.): An Overview. *Critical Reviews in Food Science and Nutrition*, *54*(5), 580–592. https://doi.org/10.1080/10408398.2011.596639

Karolkowski, A., Guichard, E., Briand, L., & Salles, C. (2021). Volatile compounds in pulses: A review. *Foods*, *10*(12), 1–27. https://doi.org/10.3390/foods10123140

Kebede, B. T., Grauwet, T., Magpusao, J., Palmers, S., Michiels, C., Hendrickx, M., & Loey, A. Van. (2015). An integrated fingerprinting and kinetic approach to accelerated shelf-life testing of chemical changes in thermally treated carrot puree. *Food Chemistry*, *179*, 94–102. https://doi.org/10.1016/j.foodchem.2015.01.074

Khrisanapant, P., Kebede, B., & Leong, S. Y. (2019). A comprehensive characterisation of volatile and fatty acid profiles of legume seeds. *Foods*, 6–9.

Kyomugasho, C., Kamau, P. G., Aravindakshan, S., & Hendrickx, M. E. (2020). Evaluation of storage stability of low moisture whole common beans and their fractions through the use of state diagrams. *Food Research International*, *July*, 109794. https://doi.org/10.1016/j.foodres.2020.109794

Ma, Z., Boye, J. I., Azarnia, S., & Simpson, B. K. (2016). Volatile flavor profile of Saskatchewan grown pulses as affected by different thermal processing treatments. *International Journal of Food Properties*, *19*(10), 2251–2271. https://doi.org/10.1080/10942912.2015.1121494

Mishra, P. K., Tripathi, J., Gupta, S., & Variyar, P. S. (2017). Effect of cooking on aroma profile of red kidney beans (*Phaseolus vulgaris*) and correlation with sensory quality. *Food Chemistry*, *215*(November 2017), 401–409. https://doi.org/10.1016/j.foodchem.2016.07.149

Murthy, U. M. N., Kumar, P. P., & Sun, W. Q. (2003). Mechanisms of seed ageing under different storage conditions for *Vigna radiata* (L.) Wilczek: Lipid peroxidation, sugar hydrolysis, Maillard reactions and their relationship to glass state transition. *Journal of*

*Experimental Botany*, *54*(384), 1057–1067. https://doi.org/10.1093/jxb/erg092

Peleg, M. (2019). Modeling Degradation Kinetics in Dry Foods Storage under Varying Temperature and Moisture Content—Theoretical Evaluation. *Food Engineering Reviews*, *11*(1), 1–13. https://doi.org/10.1007/s12393-018-9185-y

Ratajczak, E., Małecka, A., Ciereszko, I., & Staszak, A. M. (2019). Mitochondria are important determinants of the aging of seeds. *International Journal of Molecular Sciences*, *20*(7). https://doi.org/10.3390/ijms20071568

Reyes-Moreno, C., Paredes-López, O., & Gonzalez, D. E. (1993). Hard-to-cook phenomenon in common beans—A review. *Critical Reviews in Food Science & Nutrition*, *33*(3), 227–286.

Taylor, A. G., Lee, P. C., & Zhang, M. (1999). Volatile Compounds as Indicators of Seed Quality and Their Influence on Seed Aging. *Seed Technology*, *21*(1), 57–65.

Van Boekel, M. A. J. . (2008). Kinetic modelling of reactions in foods. In *CRC press*.

Wainaina, I., Kyomugasho, C., Wafula, E., Sila, D., & Hendrickx, M. (2022). An integrated kinetic and polymer science approach to investigate the textural stability of red kidney beans during post-harvest storage and subsequent cooking. *Food Research International*, *154*(January), 110988. https://doi.org/10.1016/j.foodres.2022.110988

Wainaina, I., Lugumira, R., Wafula, E., Kyomugasho, C., Sila, D., & Hendrickx, M. (2022). Insight into pectin-cation-phytate theory of hardening in common bean varieties with different sensitivities to hard-to-cook. *Food Research International*, *151*(October 2021), 110862. https://doi.org/10.1016/j.foodres.2021.110862

Wainaina, I., Wafula, E., Van Loey, A., Sila, D., Hendrickx, M., & Kyomugasho, C. (2022). Kinetics of phytate hydrolysis during storage of red kidney beans and the implication in hard-to-cook development. *Food Research International*, *159*(111581).

Walters, C., Ballesteros, D., & Vertucci, V. A. (2010). Structural mechanics of seed deterioration: Standing the test of time. *Plant Science*, *179*(6), 565–573. https://doi.org/10.1016/j.plantsci.2010.06.016

Walters, C., Hill, L. M., & Wheeler, L. J. (2005). Dying while dry: Kinetics and mechanisms of deterioration in desiccated organisms. *Integrative and Comparative Biology*, *45*(5), 751–758. https://doi.org/10.1093/icb/45.5.751

Zhang, T., Ayed, C., Fisk, I. D., Pan, T., Wang, J., Yang, N., & Sun, Q. (2022). Evaluation of volatile metabolites as potential markers to predict naturally-aged seed vigour by coupling rapid analytical profiling techniques with chemometrics. *Food Chemistry*, *367*. https://doi.org/10.1016/j.foodchem.2021.130760

## Tables

**Table 1**. Volatile compounds obtained in red kidney beans at varying storage temperatures and moisture contents. Compounds are ranked based on their increasing VID coefficients per moisture content-temperature condition. RI is the retention index on the column.

| 14.5% Moisture content | | | | | |
|---|---|---|---|---|---|
| ***25 °C*** | | | ***30 °C*** | | |
| VID | Marker | RI | VID | Marker | RI |
| 0.806 | 2-Furanone, dihydro-3-methyl- | 1605 | 0.827 | 2-Hexenal | 1228 |
| 0.814 | 2-Furanone, dihydro-4-methyl- | 1631 | 0.835 | Acetic acid | 1484 |
| 0.829 | 1-Hexanol | 1367 | 0.849 | 3-Furaldehyde | 1448 |
| 0.853 | 1-Butanol, 3-methyl- | 1223 | 0.849 | Furan,2-ethyl | 958 |
| 0.890 | Acetic acid | 1484 | 0.850 | 2-Furanone, dihydro-3-methyl- | 1605 |
| 0.919 | 2-Butanol | 1035 | 0.868 | 1-Hexanol, 2-ethyl- | 1502 |
| 0.936 | 3-Furanmethanol | 1703 | 0.899 | 2-Furanone, dihydro-4-methyl- | 1632 |
| 0.941 | Butanal, 2-methyl- | 918 | 0.900 | 1-Hexanol | 1367 |
| 0.943 | Benzaldehyde | 1536 | 0.910 | 2-Pentanol | 1139 |
| 0.953 | Benzyl alcohol | 1901 | 0.918 | Benzaldehyde | 1536 |
| 0.970 | 2-Pentanol | 1139 | 0.921 | 1-Ethyl-5-methylcyclopentene | 1477 |
| 0.981 | 3-Furaldehyde | 1448 | 0.930 | Butanal, 2-methyl- | 919 |
| 0.985 | Phenylethyl Alcohol | 1934 | 0.963 | Benzyl alcohol | 1901 |
| 0.987 | 1-Propanol, 2-methyl- | 1115 | 0.985 | 1-Propanol, 2-methyl- | 1115 |
| | | | 0.993 | Phenylethyl alcohol | 1934 |
| ***35 °C*** | | | | | |
| VID | Marker | RI | | | |
| -0.855 | 3-Hexanol | 1213 | | | |
| 0.814 | 2-Heptenal | 1332 | | | |
| 0.820 | 2,3-Pentanedione | 1074 | | | |
| 0.829 | 3,5-Octadien-2-one | 1531 | | | |
| 0.834 | Hexanal | 1093 | | | |
| 0.834 | 1-Hexanol | 1367 | | | |
| 0.842 | 2-Furanone, dihydro-4-methyl | 1632 | | | |
| 0.851 | 1-Penten-3-ol | 1179 | | | |
| 0.858 | 2-Penten-1-ol | 1337 | | | |
| 0.859 | Acetic acid | 1485 | | | |
| 0.861 | Maltol | 1987 | | | |
| 0.863 | Furan, 2-ethyl | 957 | | | |
| 0.865 | Propanal | 849 | | | |
| 0.873 | 1-Penten-3-one | 1029 | | | |
| 0.879 | Oxalic acid, diallyl ester | 1503 | | | |
| 0.891 | 2-Pentenal | 1142 | | | |
| 0.899 | 2-Octenal | 1437 | | | |

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|---|---|---|
| 0.902 | 2,4-Heptadienal | 1504 |
| 0.905 | 2-Butanol | 1036 |
| 0.907 | 2-Pentanol | 1139 |
| 0.931 | Isopropyl alcohol | 936 |
| 0.935 | Ethyl acetate | 899 |
| 0.942 | 1-Propanol, 2-methyl | 1115 |
| 0.953 | 2,4-Heptadienal | 1477 |
| 0.960 | Phenylethyl alcohol | 1934 |

**12.8% Moisture content**

***25 °C***

| VID | Marker | RI |
|---|---|---|
| 0.818 | Benzyl alcohol | 1900 |
| 0.824 | Acetic acid | 1483 |
| 0.831 | 2-Butanone | 910 |
| 0.831 | Butanal, 2-methyl | 919 |
| 0.900 | Benzaldehyde | 1536 |
| 0.962 | 1-Propanol, 2-Methyl | 1114 |
| 0.972 | 3-Furaldehyde | 1448 |

***30 °C***

| VID | Marker | RI |
|---|---|---|
| -0.811 | Didocyl phosphate | 1439 |
| 0.811 | 3,4-Dimethyldihydrofuran-2,5-dione | 1605 |
| 0.819 | Acetone | 859 |
| 0.826 | Methyl butoxyacetate | 1698 |
| 0.828 | 1-Butanol | 1163 |
| 0.839 | 2-Butanone | 910 |
| 0.843 | 1-Ethyl-1-hexanol | 1502 |
| 0.847 | 1-Butanol, 3-methyl | 1223 |
| 0.904 | Benzaldehyde | 1536 |
| 0.907 | Phenylethylalcohol | 1933 |
| 0.910 | Acetic acid | 1483 |
| 0.913 | 3-Furanmethanol | 1702 |
| 0.959 | 3-Furaldehyde | 1448 |
| 0.965 | 1-Propanol, 2-methyl | 1115 |
| 0.981 | Benzyl alcohol | 1901 |

***35 °C***

| VID | Marker | RI |
|---|---|---|
| 0.809 | 5-Hepten-2-one, 6-methyl | 1348 |
| 0.814 | Butanoic acid, 4-hydroxy | 1647 |
| 0.817 | 1,4-Hexadiene, 3-ethyl | 1477 |
| 0.823 | Acetophenone | 1665 |
| 0.827 | 2-Ethyl-1-hexanol | 1502 |
| 0.830 | 1-Butanol, 2-methyl | 1223 |
| 0.832 | Furan, 2-ethyl | 958 |
| 0.840 | 1-Butanol, 3-methyl | 1223 |
| 0.875 | Benzyl alcohol | 1901 |
| 0.881 | 2-Furanone, dihydro-4-methyl | 1631 |
| 0.896 | 2-Butanone | 909 |
| 0.902 | 2-Butanol | 1035 |
| 0.903 | Acetic acid | 1483 |

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|---|---|---|
| | Butanoic acid, 3- | |
| 0.913 | methyl | 1698 |
| 0.916 | Acetone | 859 |
| 0.962 | 1-Butanol | 1163 |
| 0.962 | Phenylethyl alcohol | 1934 |
| 0.963 | 1-Propanol, 2-methyl | 1114 |

**11.1% Moisture content**

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| ***25 °C*** | | | ***30 °C*** | | |
|---|---|---|---|---|---|
| VID | Marker | RI | VID | Marker | RI |
| 0.923 | 1-Propanol, 2-methyl | 1115 | 0.943 | 1-Propanol, 2-methyl | 1115 |
| ***35 °C*** | | | ***42 °C*** | | |
| VID | Marker | RI | VID | Marker | RI |
| 0.849 | Benzaldehyde | 1537 | 0.819 | Ethyl acetate | 900 |
| 0.874 | 2-Ethyl-1-hexanol | 1502 | 0.824 | 1-Butanol, 2-methyl | 1223 |
| 0.883 | 3-Furanmethanol | 1701 | 0.848 | Butanoic acid, 2-methyl | 1698 |
| 0.911 | Acetone | 859 | 0.886 | Benzaldehyde | 1536 |
| 0.927 | 3-Furaldehyde | 1448 | 0.887 | 3-Furanmethanol | 1702 |
| 0.944 | 2-Butanone | 910 | 0.925 | Dimethyl sulphide | 836 |
| 0.965 | 1-Propanol, 2-methyl | 1115 | 0.928 | Isopropyl alcohol | 934 |
| | | | 0.937 | Furan, 2-ethyl | 959 |
| | | | 0.952 | 2-Ethyl-1-hexanol | 1502 |
| | | | 0.952 | 3-Furaldehyde | 1448 |
| | | | 0.959 | 2-Butanone | 910 |
| | | | 0.961 | 2-Butanol | 1035 |
| | | | 0.967 | Phenylethyl alcohol | 1933 |
| | | | 0.970 | Benzyl alcohol | 1900 |
| | | | 0.976 | Acetone | 859 |
| | | | 0.979 | 1-Butanol | 1163 |
| | | | 0.994 | 1-Propanol, 2-methyl | 1115 |

**8.7% Moisture content**

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| ***25 °C*** | | | ***30 °C*** | | |
|---|---|---|---|---|---|
| VID | Marker | RI | VID | Marker | RI |
| 0.858 | Butanal, 2-methyl | 919 | 0.864 | 1-Hexanol, 2-ethyl | 1502 |
| | | | 0.889 | 1-Propanol, 2-methyl | 1114 |
| | | | 0.917 | Dimethyl sulphide | 836 |
| | | | 0.932 | Butanal, 2-methyl | 919 |
| ***35 °C*** | | | ***42 °C*** | | |
| VID | Marker | RI | VID | Marker | RI |
| 0.814 | Butanal, 2-methyl | 919 | 0.822 | Acetic acid, methyl ester | 865 |
| 0.823 | 2-Ethyl, 1-hexanol | 1501 | 0.884 | 1-Hexanol, 2-ethyl | 1502 |
| 0.830 | Pentane | 807 | 0.895 | 2-Butanone | 910 |
| 0.856 | 1-Pentene, 2-methyl | 817 | 0.907 | Acetone | 859 |
| 0.871 | 2-Butanone | 910 | 0.921 | Dimethyl sulphide | 836 |
| 0.961 | 1-Propanol, 2-methyl | 1113 | 0.978 | 1-Propanol, 2-methyl | 1113 |

**6.9% Moisture content**

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| *35 °C* | | | *42 °C* | | |
|---|---|---|---|---|---|
| VID | Marker | RI | VID | Marker | RI |
| 0.809 | 2-Butanone | 910 | 0.814 | Phenylethyl alcohol | 1932 |
| 0.814 | 1-Propanol, 2-methyl | 1114 | 0.882 | 2-Butanone | 910 |
| | | | 0.943 | Dimethyl sulphide | 836 |
| | | | 0.975 | 1-Propanol, 2-methyl | 1114 |

**Table 2.** Parameter estimates ± standard errors for kinetics of evolution of selected marker compounds modelled using zero order model (Equation 1). K is the rate of evolution of marker compound (week$^{-1}$). Different superscript letters in a row (moisture content) represent significant differences in rate constants. Abbreviation "n.d." stands for "not determined" while "<dl" stands for "below detection limit".

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **1-propanol, 2-methyl** | | | | | | | | |
| | *25 °C* | | *30 °C* | | *35 °C* | | *42 °C* | |
| %Moisture content | K (week$^{-1}$) | $(\times10^5)R^2$ adj | K (week$^{-1}$) | $(\times10^5)R^2$ adj | K (week$^{-1}$) | $(\times10^5)R^2$ adj | K (week$^{-1}$) | $(\times10^5)R^2$ adj |
| 6.9 | n.d. | | n.d. | | 0.49±0.06$^{b}$ | 0.72 | 1.26±0.05$^{a}$ | 0.96 |
| 8.7 | <dl | | 0.10±0.02$^{c}$ | 0.56 | 0.45±0.03$^{b}$ | 0.93 | 1.28±0.03$^{a}$ | 0.99 |
| 11.1 | 0.56±0.06$^{c}$ | 0.79 | 0.89±0.10$^{c}$ | 0.78 | 2.36±0.19$^{b}$ | 0.87 | 5.15±0.18$^{a}$ | 0.97 |
| 12.8 | 1.27±0.07$^{b}$ | 0.94 | 1.97±0.12$^{ab}$ | 0.92 | 3.32±0.46$^{a}$ | 0.69 | n.d. | |
| 14.5 | 3.37±0.05$^{b}$ | 0.99 | 6.74±0.09$^{a}$ | 0.99 | 8.14±0.61$^{a}$ | 0.86 | n.d. | |
| **Acetic acid** | | | | | | | | |
| | *25 °C* | | *30 °C* | | *35 °C* | | *42 °C* | |
| %Moisture content | K (week$^{-1}$) | $(\times10^5)R^2$ adj | K (week$^{-1}$) | $(\times10^5)R^2$ adj | K (week$^{-1}$) | $(\times10^5)R^2$ adj | K (week$^{-1}$) | $(\times10^5)R^2$ adj |
| 6.9 | n.d. | | n.d. | | <dl | | <dl | |
| 8.7 | <dl | | <dl | | <dl | | <dl | |
| 11.1 | <dl | | <dl | | <dl | | <dl | |
| 12.8 | 0.89±0.19$^{b}$ | 0.48 | 1.95±0.16$^{b}$ | 0.86 | 3.68±0.36$^{a}$ | 0.82 | n.d. | |
| 14.5 | 1.59±0.17$^{b}$ | 0.75 | 3.02±0.35$^{ab}$ | 0.72 | 5.29±0.65$^{a}$ | 0.69 | n.d. | |
| **2-butanone** | | | | | | | | |
| | 25 °C | | 30 °C | | 35 °C | | 42 °C | |
| %Moisture content | K (week$^{-1}$) | $(\times10^5)R^2$ adj | K (week$^{-1}$) | $(\times10^5)R^2$ adj | K (week$^{-1}$) | $(\times10^5)R^2$ adj | K (week$^{-1}$) | $(\times10^5)R^2$ adj |
| 6.9 | n.d. | | n.d. | | 0.64±0.09$^{a}$ | 0.67 | 1.20±0.19$^{a}$ | 0.62 |
| 8.7 | <dl | | <dl | | 0.46±0.08$^{b}$ | 0.57 | 1.35±0.13$^{a}$ | 0.83 |
| 11.1 | <dl | | <dl | | 2.16±0.16$^{a}$ | 0.86 | 3.15±0.37$^{a}$ | 0.76 |
| 12.8 | 0.96±0.13$^{b}$ | 0.71 | 0.52±0.15$^{ab}$ | 0.34 | 2.10±0.29$^{a}$ | 0.71 | n.d. | |
| 14.5 | <dl | | <dl | | <dl | | n.d. | |
| **Benzaldehyde** | | | | | | | | |
| | *25 °C* | | *30 °C* | | *35 °C* | | *42 °C* | |
| %Moisture content | K (week$^{-1}$) | $(\times10^5)R^2$ adj | K (week$^{-1}$) | $(\times10^5)R^2$ adj | K (week$^{-1}$) | $(\times10^5)R^2$ adj | K (week$^{-1}$) | $(\times10^5)R^2$ adj |
| 6.9 | n.d. | | n.d. | | <dl | | <dl | |
| 8.7 | <dl | | <dl | | <dl | | <dl | |
| 11.1 | <dl | | <dl | | 0.58±0.10$^{a}$ | 0.59 | 1.25±0.15$^{a}$ | 0.76 |
| 12.8 | 0.81±0.09$^{a}$ | 0.76 | 1.38±0.12$^{a}$ | 0.84 | <dl | | n.d. | |
| 14.5 | 1.24±0.08$^{a}$ | 0.89 | 1.57±0.14$^{a}$ | 0.82 | <dl | | n.d. | |

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## Figures

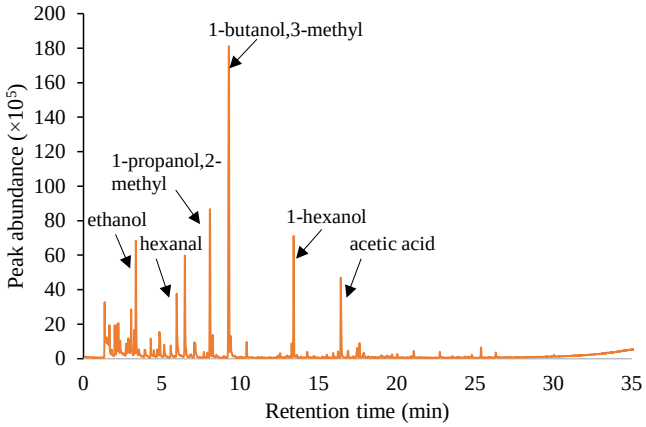


**Figure 1**. Representative total ion chromatogram of the headspace fraction of red kidney beans stored at 14.5% moisture content and 35 °C.

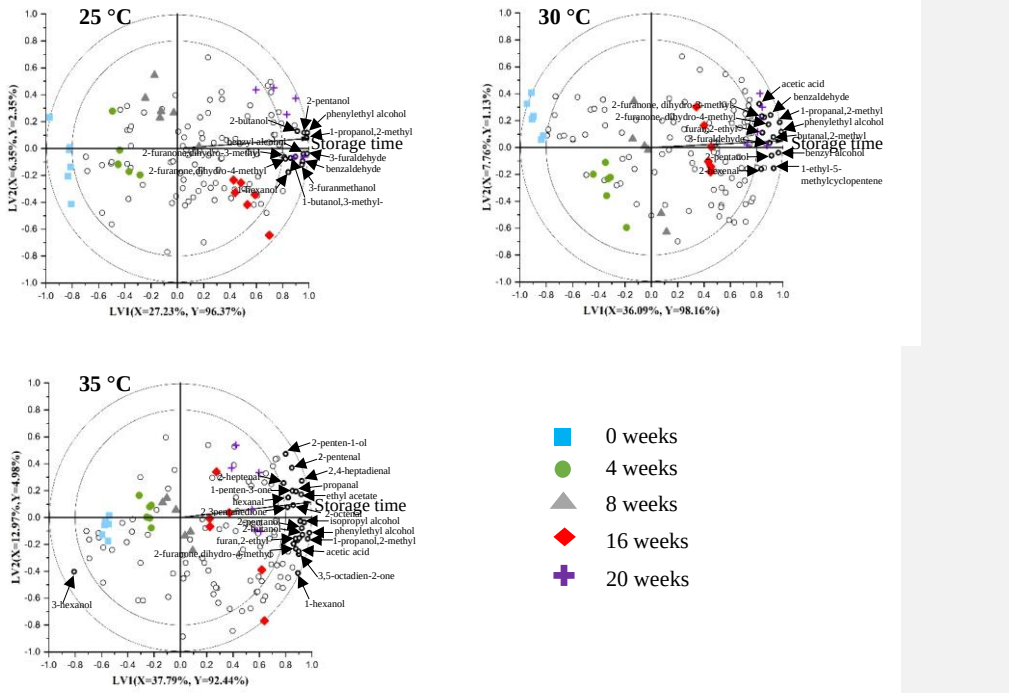


**Figure 2**. Bi-plots showing the evolution of volatile compounds during storage of red kidney beans at 14.5% moisture content and 25, 30 and 35 °C. The selected models had 2, 2 and 3 LVs for 25, 30 and 35 °C respectively. Colored symbols represent red kidney bean samples stored from 0 to 20 weeks. Open circles represent volatile compounds while open bold circles represent marker compounds with VID≥0.8.

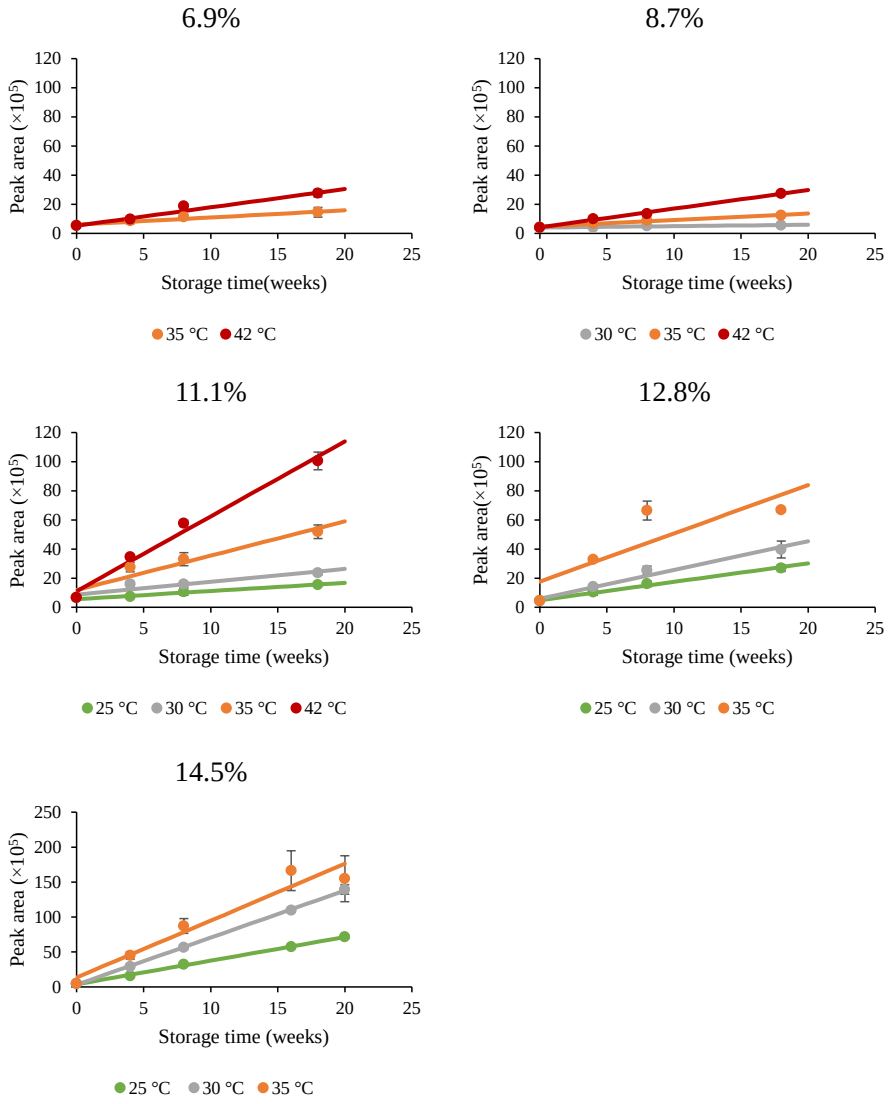


**Figure 3.** Kinetics of evolution of 1-propanol,2-methyl during storage. The markers represent experimental values and the solid lines represent modelled data using zero order model (Equation 1).

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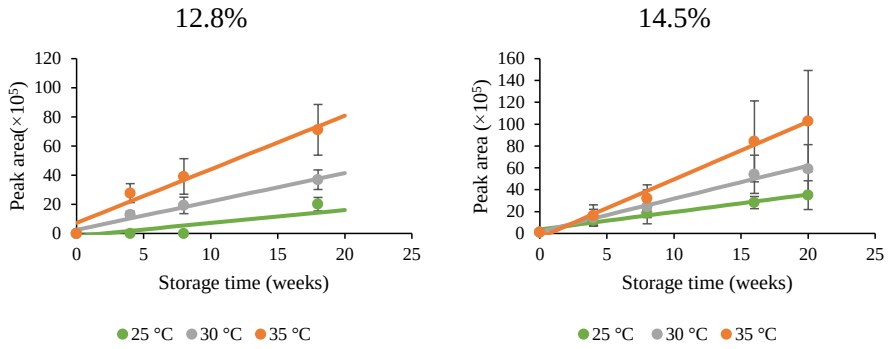


**Figure 4.** Kinetics of evolution of acetic acid during storage. The markers represent experimental values and the solid lines represent modelled data using zero order model (Equation 1).

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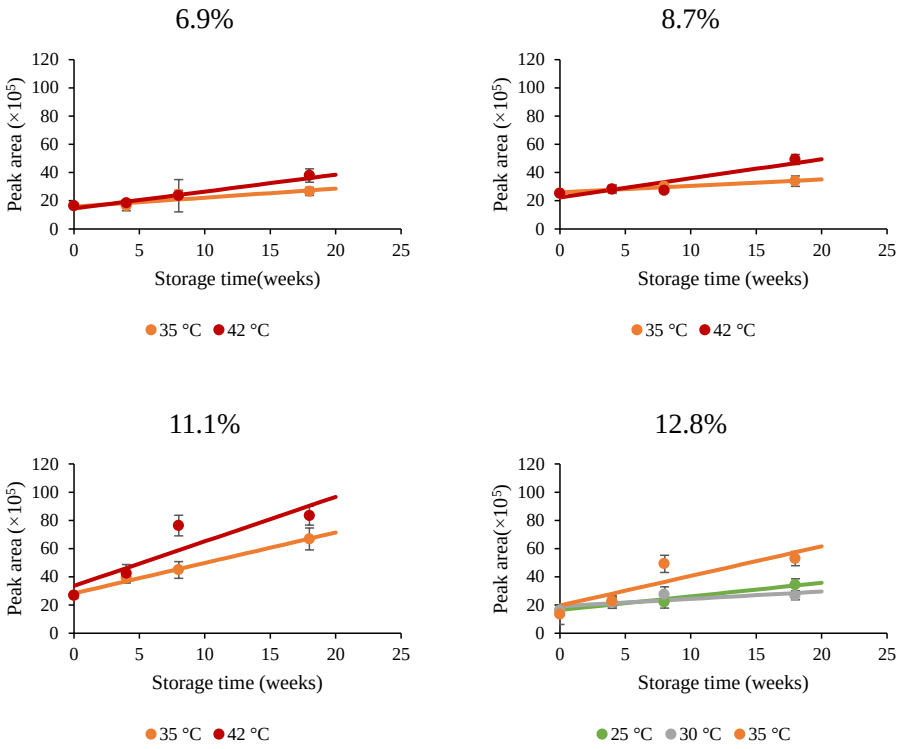


**Figure 5.** Kinetics of evolution of 2-butanone during storage. The markers represent experimental values and the solid lines represent modelled data using zero order model (Equation 1).

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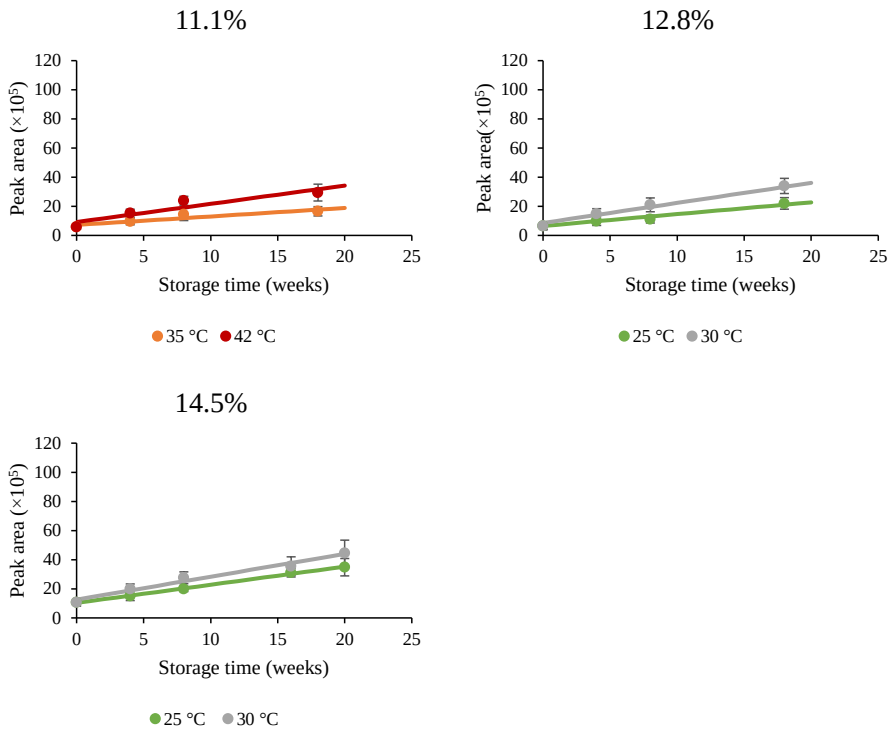


**Figure 6.** Kinetics of evolution of benzaldehyde during storage. The markers represent experimental values and the solid lines represent modelled data using zero order model (Equation 1).

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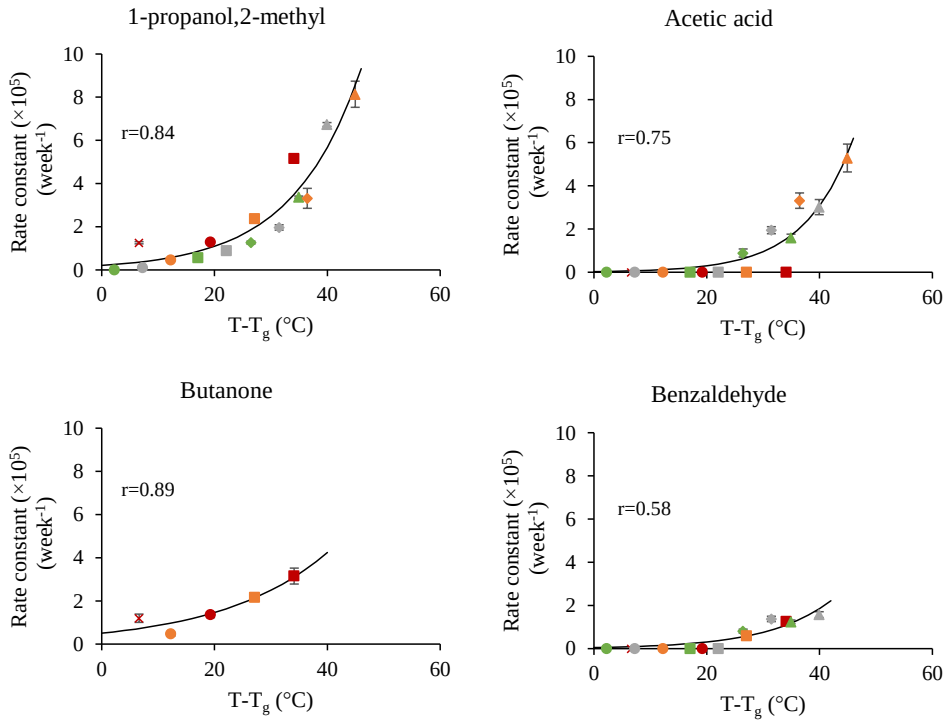


**Figure 7.** The change in kinetics of evolution of selected marker compounds as a function of storage above $T_g$ ($T-T_g$). Markers represent samples stored at specific moisture content and temperature combinations. Different colors represent different storage temperatures (green: 25 °C, grey: 30 °C, orange: 35 °C and red: 42 °C) whereas different markers represent different storage moisture contents (×: 6.9%, ○: 8.7%, □: 11.1%, ◊: 12.8% and Δ: 14.5%). The solid lines represent modelled values using empirical exponential equation (Equation 2).

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