1 **Instability of common beans during storage causes hardening: the role of glass** 2 **transition phenomena**

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11 Chemical compounds studied in this article

12 Phosphorus pentoxide _ PubChem CID 14812
13 Potassium hydroxide_ PubChem CID 14797
14 Sodium iodide_PubChem CID 5238
15 Potassium iodide_PubChem CID 4875
16 Sodium bromide_ PubChem CID 253881
17 Ammonium sulphate_ PubChem CID 6097028
18 Potassium sulphate_ PubChem CID 24507
19 Potassium chloride_PubChem CID 4873
20 Natamycin_PubChem CID 5284447
21 Microcrystalline cellulose_PubChem CID 62698
22

23 **Abstract**

24 Long-term storage of common beans leads to loss of cooking quality and an ill-defined solution,
25 appropriate storage, is recommended. Therefore, the polymer science theory of glasses that
26 hypothesizes stability of a system below its glass transition temperature (T_g) was applied to
27 determine bean stability during storage in relation to cooking behavior. Since composition
28 influences T_g , powders of cotyledons and seed coats in addition to whole beans were
29 equilibrated above different saturated salt solutions in order to generate materials with different
30 moisture contents. A thermal mechanical compression test which measures compressibility
31 changes in a system upon reaching its glass-rubber transition temperature region was
32 conducted to obtain the T_g . A T_g -moisture relation was established, whose relevance was
33 confirmed by storage and cooking experiments which showed development of hard-to-cook in
34 beans stored above T_g but not below it. Therefore, this relation constitutes a stability map for
35 storage of common beans.

Keywords

Common beans; Cotyledons; Seed coats; Stability; Glass transition temperature; Moisture content

1 Introduction

The postharvest state of seeds that is characterized by a time-dependent deterioration process leading to loss of viability and eventual mortality is termed aging (Walters, 1998). Hydrolytic and oxidative reactions are both a cause and consequence of aging and include lipid and protein degradation, damage of membranes, loss of enzyme activity, accumulation of mutations in chromosomes and nucleic acids damage (Murthy *et al.*, 2003; Walters, 1998). According to Walters *et al.* (2005), longevity of seeds is hereditary and the rate of degradation and progression towards death is exacerbated by storage conditions to which the seeds are exposed. Legume seeds are no exception to this and it has been known for long that most of them undergo physiological and/or biochemical changes during storage. In particular, common beans (*Phaseolus vulgaris*) may undergo color changes in colored seed coats (Edmister *et al.*, 1990), partial or complete water impermeability of the seed coat (termed hard shell) and hardening of cotyledons (termed hard-to-cook) (El-Tabey Shehata, 1992) among others. Attempts to decipher some of these changes have yielded no more than a list of postulations put forward as mechanisms through which they occur. Factors that influence their occurrence can be grouped into agronomic conditions, such as mineral composition and content of the soil (Paredes-López *et al.*, 1989), intrinsic characteristics of the seeds, such as variety differences, environmental conditions (temperature and relative humidity) under which the seeds are stored, and storage time (Njoroge *et al.*, 2015).

Regardless of composition, food systems can be stored under conditions which greatly delay degradation phenomena thereby prolonging their shelf life. In this context, the glass transition theory can provide a very useful approach (Roos, 2010). When food matrices undergo a fast decrease in temperature, the aqueous phase becomes very viscous, eventually assuming a metastable glassy state wherein mobility is infeasible except for molecular rotations and vibrations. The temperature at which the system reaches this state is the glass transition temperature (T_g). Therefore, at temperatures below T_g , diffusion-limited reactions which are mainly responsible for degradation in foods are blocked thereby ensuring physicochemical stability of the system.

Temperature and moisture content relations that ensure stability of food systems can be established by determination of T_g values of a food system containing different quantities of water at a constant temperature. To the best of our knowledge, the concept of T_g has been studied in the context of loss of viability after storage (Sun and Leopold, 1994; Murthy *et al.*, 2003) but so far not in the context of aging and loss of cooking quality hence it was addressed in this study. In the aforementioned studies germination tests, as well as products of lipid peroxidation, Maillard reactions and Amadori reactions were used to assess loss of viability and monitor deterioration reactions, respectively. In the current study the development of hard-to-cook (HTC) defect was selected as an indicator of the loss of cooking quality of beans. Because composition differences might influence T_g , it was imperative to analyze also bean cotyledons and seed coats separately, besides the whole bean. Whole bean, cotyledon and seed coat powders were equilibrated above different saturated salt solutions in order to generate bean materials having different moisture content. The T_g values of these materials were determined by a thermal mechanical compression test (TMCT), based on the change in compressibility of a material in the T_g region resulting from the transition between the glassy to the rubbery state upon heating. This approach has been used successfully before in the context of mechanical properties and processing (Boonyai *et al.*, 2007; Pelgrom *et al.*, 2013). The current study aims to provide well-defined appropriate conditions (temperature and moisture content) allowing for stability of common beans during storage, specifically in relation to cooking behavior, in order to prevent the development of defects and their associated consequences.

2 Materials and methods

2.1 Raw Materials

Common beans obtained from Thika Station (Kenya Agricultural and Livestock Research Organization) were sorted and cleaned to eliminate defective seeds and dirt, respectively. Since in this study, it was intended to test the hypothesis that composition differences influence T_g , the beans were peeled manually using a knife to yield cotyledons and seed coats. Cotyledons, seed coats and whole beans were milled using an IKA A10Basic mill (Germany) with incorporation of 5 ppm of an anti-mold powder, natamycin (E235), to inhibit mold growth during subsequent storage steps. Subsequently, the powders were sieved utilizing a 500 μm sieve. Milling was done in duplicate hence all subsequent treatments and analyses were conducted following this duplication. The experimental approach that was utilized for this study is illustrated in Fig. 1. In order to determine the water adsorption behavior and T_g values of these materials, they were

conditioned to generate samples differing in moisture content as described in the following section.

2.2 Generation of materials of different moisture content

Since adsorption and desorption behavior is dissimilar, a phenomenon termed hysteresis, it was critical to ensure that only moisture adsorption occurred in the samples. To that end, the powdered whole beans, cotyledons and seed coats were first incubated above phosphorus pentoxide (P_2O_5) for 10 days in order to condition them to a similar, very low moisture content as a starting point. Thereafter, each material was incubated above each saturated salt solution (Table 1) in hermetically sealed containers. Selected targeted a_w values were in the range 0.1 to 0.99. The relative humidities of the enclosed environments corresponded to the a_w values of the different saturated salt solutions thereby equilibrating the samples to different moisture contents. During equilibration, the materials were incubated at 4 °C as required for stability of natamycin, until attainment of equilibrium as determined gravimetrically. Incubation times depended on the a_w of the saturated salt solution, being longer for higher a_w values. In general, it took 3 weeks to attain equilibrium but the materials were stored for a period of at least 4 weeks to a maximum of 6 weeks.

2.3 Characterization of bean materials

2.3.1 Particle size distribution

Particle size distribution (PSD) was determined on bean materials incubated above P_2O_5 only (starting material) using an LS 13 320 laser diffraction analyzer equipped with a Tornado dry powder system module (Beckman Coulter Inc., Miami, Florida, USA). Approximately 2.8 g of material was weighed into the measurement cell. The principle of measurement involved illumination of the sample with 750 nm laser light from a halogen lamp with concomitant scattering of the light by the sample particles. From the scattering patterns and based on the Fraunhofer theory, the LS 13320 software determined the PSD of the analyzed sample.

2.3.2 Determination of moisture content and adsorption isotherms

Moisture content of materials incubated above both P_2O_5 and the different saturated salt solutions was determined gravimetrically using the oven-drying method following a procedure described by Chigwedere *et al.* (2018). Duran borosilicate bottles that were preheated in a Jouan EB 115 (Thermo Electron Corporation, USA) oven at 103 °C for 1 h were cooled in a desiccator before approximately 2 g of bean materials was weighed into them. The materials were dried at 103 °C for 20 h and moisture content was calculated as weight loss from the

samples. Moisture content was expressed on dry basis and thereafter, adsorption isotherms, which describe the water uptake behavior of materials, were constructed (moisture content vs a_w).

2.3.3 Glass transition temperature measurement

The glass transition temperature was measured using the thermal mechanical compression test (TMCT) developed by Boonyai *et al.* (2007). This method involves thermal scanning of a compressed material in its glassy state with change in compressibility upon reaching the T_g range. Implementation and optimization of the TMCT were done following the work of Boonyai *et al.* (2007) with modifications in utilized equipment and measurement specifications. A modular compact rheometer (MCR302, Anton Paar, Austria) equipped with a stainless steel sample holder (cup, $\Phi=27$ mm) and a cylindrical custom-made flat head aluminum probe ($\Phi=25$ mm) with which a normal compression force on the sample was exerted. The dimensions of the cylinder and cup created an annulus space of 1 mm in the geometry, allowing for movement of the probe, and minimizing moisture exchange between the sample and its environment during measurement. Since the principle of measurement involved thermal scanning of a sample under compression, it was imperative to optimize for the thermal scanning rate. To that end, 1 g of skimmed milk powder was equilibrated at 10 °C for 5 min under a 10 N constant normal compression force before it was increased to 30 N followed by thermal scanning up to 100 °C at either 1 or 2 °C/min. The displacement of the probe at constant normal force (30 N) as a function of the temperature was detected and recorded. A Rheo Compass software (Rheo Compass 1.17, Anton Paar, Austria) was used for data collection.

Changes in compressibility of the sample would be detected by displacement of the probe thereby necessitating elimination of any other factors that would potentially induce 'false' probe displacements. Such factors included rearrangement of particles to fill air pockets amongst them and this was prevented by the equilibration phase which facilitated for any possible rearrangements to occur before thermal scanning. Another factor would be degradation of particles due to changes in physical structure under the compression force during measurement. Peleg (1983) showed that 30 N would not lead to particle degradation. Lastly, expansion of the sample holder during thermal scanning would also induce displacement of the probe. Correction for this thermal expansion was explored by thermal scanning of 1 g of oven-dried (103 °C for 1 h) microcrystalline cellulose using the selected thermal scanning rate (1 °C/min) from the optimization mentioned earlier. Microcrystalline cellulose was oven-dried so that it would remain incompressible in the thermal scanning range explored in this study.

Therefore, displacement of the probe during its thermal scanning was attributed solely to expansion of the sample holder and the data generated thereof was subtracted from sample data as a way of correcting for thermal expansion of the sample holder.

After optimization of the method, T_g values of bean materials of different moisture contents were analyzed. One gram of material was equilibrated under a normal force of 10 N at 10 °C for 5 min after which it underwent thermal scanning at 1 °C/min under a normal force of 30 N.

Nonetheless, the thermal scanning range depended on the expected moisture content of the sample. Samples stored at a_w values ≤ 0.42 were expected to have low moisture content and hence high T_g values thus they were thermally scanned from 10 to 100 °C. At higher moisture contents ($0.42 \leq a_w \leq 0.90$), thermal scanning was from 0 to 100 °C and at the highest moisture content explored in this study ($a_w = 0.98$), much lower T_g values were expected hence thermal scanning was from -5 to 80 °C. In order to correct for thermal expansion, the dried microcrystalline cellulose was analyzed using all the different thermal scanning ranges. Analyses were done in duplicate and following data analysis (Section 2.5), plots of T_g vs moisture content (on dry basis) were constructed for each material.

2.4 Determination of relevance of the determined glass transition temperatures

In order to assess the relevance of T_g values obtained, that is, to test the hypothesis of stability of a material below its T_g , whole beans and cotyledons were stored below, around and above their T_g values. Subsequently, through cooking experiments, their cooking behaviors were assessed for HTC development, herein considered as stability indicator. Relevance of the T_g results could be proven only if materials stored below T_g would not exhibit HTC behavior whilst those stored above it would.

2.4.1 Storage of whole beans and cotyledons

Certain combinations of temperatures, relative humidities and storage time have been shown to induce HTC development in common beans (Njoroge *et al.*, 2015; Reyes-Moreno *et al.*, 1994). In this study, whole beans and cotyledons were stored at 35 °C for 3 months above different saturated salt solutions. The salts included sodium iodide, potassium iodide and potassium chloride, which when saturated at 35 °C conferred relative humidities of 35, 67 and 83%, respectively (Greenspan, 1977). It was targeted that whole beans and cotyledons stored under these conditions would be below, around and above their T_g values, respectively.

2.4.2 Determination of cooking profiles

Cooking profiles of the stored whole beans and cotyledons were determined according to a procedure described by Chigwedere *et al.* (2018). The materials were soaked separately in demineralized water at 25 °C for 16 h in a ratio of 1:5 (w/v) followed by sampling of 20 soaked, non-cooked seeds. The rest of the seeds were cooked at 95 °C, with sampling of 20 seeds every 30 min except for beans conditioned at 83% relative humidity which were sampled after 60 min. Hardness was measured on a cotyledon from each sampled seed thereby necessitating peeling of sampled whole beans. Measurements were performed in compression mode using a TA-X2i texture analyzer (Stable Microsystems, Goldaming, England) equipped with a 25 kg force cell and a P25 cylindrical flat head alumina probe ($\Phi = 25$ mm). The pre-test, test and post-test speeds of the probe were 2, 1 and 2 mm/s, respectively. The average of the hardness measurements per sampling time and the associated standard deviations were plotted against cooking time to generate cooking profiles. Cooking profiles of non-stored whole beans and cotyledons were generated as reference profiles for assessment of HTC development. Just after harvest, bean varieties that had 80% of bean seeds cooked within 120 min as assessed by fingerpressing tests were categorized as being easy-to-cook (Kinyanjui *et al.*, 2015). Therefore, non-stored beans are herein considered HTC-free because their cooking satisfied the criterion stated above.

2.5 Data modeling and analysis

Adsorption isotherms

Adsorption isotherms were modeled by the Guggenheim, Anderson, and de Boer (GAB) model (equation 1) using SAS version 9.3 (SAS Institute, Inc., Cary, NC, USA).

$$M_w = M_g C K a_w / (1 - K a_w) (1 + (C - 1) K a_w) \quad (1)$$

The M_w is moisture content (dry basis), a_w is water activity and, M_g , K and C characterize sorption properties of the materials. The M_g is the GAB moisture content corresponding to the monomolecular layer of water molecules and, C and K are energy constants related to the net heat of sorption of the monolayer and multilayers, respectively (Sablani *et al.*, 2007).

Cooking profiles

Cooking profiles obtained from cooking experiments were modeled with modified first order kinetics (equation 2) using SAS version 9.3 (SAS Institute, Inc., Cary, NC, USA).

$$H = H_0 \exp(-kt) \quad (2)$$

In equation 2, H is the hardness of a cotyledon at any time t min during cooking, H_0 is the initial hardness, that is H at $t=0$ min, and k is the softening rate constant. In order to assess the fit of the model to the experimental data and the spread of residuals, the R^2_{adjusted} and root mean square error were calculated, respectively, from the obtained analysis of variance table. Statistical differences among k -values were determined from 95% confidence intervals estimated by the model.

3 Results and discussion

3.1 Particle size distribution

The PSD of powdered materials equilibrated above P_2O_5 for 10 days is shown in Fig. 2. Whilst whole bean and cotyledon powders showed a bimodal PSD, seed coat powder was unimodal. The seed coat powder consisted largely of particles with a diameter between 100 and 1000 μm . Although whole bean and cotyledon powders also contained similar-sized particles, their volume fractions were much lower than those of seed coat particles. This means seed coat powder contained very hard particles that resisted size reduction during milling unlike the other materials with bimodal distributions. During maturation of legume seeds, palisade cell walls in their seed coats contract leading to hardening of the seed coats (Corner, 1951). Despite whole bean and cotyledon powders having bimodal PSDs, the former had a higher volume fraction of larger sized particles and a lower volume fraction for the smaller-sized particles compared to cotyledon powder. This difference can be attributed to presence of seed coat material in whole bean powder. The seed coat constitutes 9-11% of the dry matter content of a bean seed (Moraghan *et al.*, 2006). Therefore, presence of this fraction could be the most likely reason for the slight difference in PSD between whole bean powder and cotyledon powder.

3.2 Moisture content and adsorption isotherms

The equilibrium moisture content of bean materials incubated under different conditions of a_w was used to construct adsorption isotherms (Fig. 3). For all the bean materials, the equilibrium moisture content values increased with increase in a_w . The adsorption isotherms were sigmoidal and according to Vertucci & Leopold (1987), such profiles are characteristic of legume seeds as was also observed for lentil seeds by Menkov (2000). Caurie (1970) cited by Al-Muhtaseb *et al.* (2002) proposed an explanation for the exhibition of sigmoidal sorption isotherms in foods. It was suggested that above approximately 22% moisture content (dry basis), water in the food system can exert and sustain vapor pressure that is close to that of pure water. Changes in a_w due to decrease of moisture content below 22% moisture then give rise to the sigmoidal shape.

In the current study, for each a_w , the equilibrium moisture content values for whole bean, cotyledon and seed coat powders were similar. Therefore, they had similar adsorption isotherms despite their differences in composition. At very low moisture contents, a monolayer of water molecules is adsorbed onto highly hydrophilic biopolymers including proteins and polysaccharides. As such, this layer is strongly bound and is characterized by an enthalpy of vaporization that is higher than that of pure water (Al-Muhtaseb *et al.*, 2002). This layer of water is unavailable for physical, chemical and microbial reactions hence at such moisture content values, a food system is stable. Moisture content corresponding to the monolayer water molecules was estimated by fitting the GAB model to the data and all estimated parameters are shown in Table 2. The moisture contents of the different bean materials corresponding to their monomolecular layers of water molecules ranged from 7.3 to 8.6% and were not significantly different (statistical results not shown).

With increase in moisture content, multilayers of water molecules bound to the matrix by capillary condensation are formed above the monolayer. Unlike for strongly-bound water, these layers exhibit solvent properties and can support some biochemical reactions. In this region (up to $\sim 0.6 a_w$), moisture content is not highly dependent on a_w . However, further increase in moisture content leads to excess water which exhibits properties of free or bulk water in the system. This region of the isotherm is characterized by a high dependency of moisture content on a_w (Al-Muhtaseb *et al.*, 2002).

It is surprising that in this study, at very low moisture content, the water-adsorption behavior was similar for cotyledon and seed coat powders even though they have different compositions, for example, protein content. Starch, which by volume and weight constitutes the majority of dry matter of cotyledons did not seem to play a significant role in water adsorption probably due to its non-gelatinized state.

3.3 Glass transition temperature

3.3.1 Compressibility behavior of skimmed milk powder during optimization

The compressibility curves obtained during optimization of the heating rate using skimmed milk powder are shown in Fig. 4. Fig. 4A shows a linear increase in displacement of the probe with increased temperature up to around 35-40 °C where the slope changed followed by resumption of the linear behavior. This compressibility behavior was observed at both heating rates.

However, 1 °C/min showed better reproducibility compared to 2 °C/min, hence it was selected for all subsequent analyses. Changes in compressibility are clearly demonstrated in Fig. 4B for

the selected heating rate after correction for thermal expansion of the sample holder. This behavior can be explained in three phases; before, during and after change in slope. The first phase involves simultaneous heating of the compressed powder and the sample holder which undergoes thermal expansion, necessitating downward movement of the probe in order to maintain the normal compression force at 30 N. Fig. 4B shows compressibility curves being superimposable with the x-axis during this phase meaning no net displacement. Therefore, in this phase, displacement of the probe was exclusively due to thermal expansion of the sample holder hence the sample was still in the glassy state. With further heating, the curves entered the second phase in which they started deviating from the x-axis giving a net displacement of the probe (Fig. 4B). This is because the skimmed milk powder had reached its glass-rubber transition temperature range thus it became compressible, giving the compressibility curve a convex shape around the transition temperature zone. In this phase, the behavior of the skimmed milk powder can be described as plastic. Beyond this temperature region, the curves showed linearity which is herein considered as the last phase of the compressibility behavior and the powder can be described as exhibiting viscoelastic behavior. The T_g was determined as the intersection point of the tangents to the linear parts of the curve (transition region).

3.3.2 Compressibility behavior of bean materials

Whole bean, cotyledon and seed coat powders that were incubated above P_2O_5 did not exhibit a glass-rubber transition within the temperature range considered for the TMCT, just like the dried microcrystalline cellulose. This is attributed to the low moisture content of the powders (2.4-3.7%, dry matter basis). Compressibility behavior of powdered bean materials of different moisture contents exhibited similar behavior hence compressibility curves for whole bean powders only are shown in Fig. 5A. The compressibility curve for the powder with the lowest moisture content is similar to the one for skimmed milk powder (Fig. 4B) which can be considered as ideal, unlike curves for the powders with higher moisture content which deviated from the x-axis before the powders approached the glass-rubber transition region. This could be possibly due to changes in the ability of the particles to pack, that is, fill voids between them, as their moisture content increased. However, the change in slope of the compressibility curves (giving a convex-nature) was very evident thereby clearly showing the transition region. Therefore, the T_g was approximated from such compressibility curves as illustrated in Fig. 5B. At higher moisture content ($\geq 20\%$), the shapes of the compressibility curves deviated much further from the ideal behavior by showing a concave behavior at the beginning of the analyses. These distorted curves reveal that the samples were most probably above their T_g already when the

analyses commenced at -5 °C. Unfortunately, this was the lowest cold condition achievable with the equipment that was in use, hence all compressibility curves obtained from bean material powders with moisture $\geq 20\%$ were not considered for approximation of T_g . In addition, the sample holder being exposed to ambient temperature, starting the analysis of such samples at very low temperatures would pose a risk of vapor condensation on the inner walls of the cup, eventually increasing the moisture content of the sample. As a result, samples with 7-16% moisture only were considered. Although this moisture content range is limited, it is of practical relevance. In the case that common bean seeds do not dry sufficiently on the plant prior to harvesting, the first postharvest unit operation is drying to around 8-10% moisture in order to extend storability.

3.3.3 T_g -moisture relation for common beans

The variation of T_g with increase in moisture content for the different materials is shown in Fig. 6. For each material, T_g analysis was conducted in duplicate on independently prepared samples hence the results are shown independently for each treatment. For all materials, the increase in moisture content was coupled to a decrease in T_g . This relation is attributed to the mobility-enhancing effect of water, commonly referred to as plasticizing effect, in hydrophilic food components. This property is facilitated by the low molecular weight of water which increases the free volume of the system thus enhancing its mobility (Al-Muhtaseb *et al.*, 2002).

Below 12% moisture, the T_g values of whole bean and cotyledon powders were similar and higher than those for seed coat powders. However, a tendency for similar T_g values for all bean materials was observed at moisture content greater than 12%. These results suggest that at low moisture content, dry matter composition differences may possibly significantly influence T_g rather than moisture content since the compositions of whole bean and cotyledon powders are comparable but very distinct from that of seed coat powders. This implies a possibility that beans of a specific moisture content stored at a particular temperature can have seed coats in the viscoelastic state (above T_g) whilst the cotyledons are in a glassy/elastic (below T_g) or rubbery/plastic (around T_g) state. Above 12% moisture, the T_g was highly influenced by moisture content and less material dependent. Since results for whole beans and cotyledons were similar at all moisture contents, we can conclude that the state of cotyledons (whether below or above T_g) has a critical contribution for storage stability of whole beans.

3.3.4 Is the T_g data obtained relevant?

In order to determine whether the T_g results obtained in this study (Section 3.3.3) are practically relevant or not, whole beans and cotyledons conditioned to different moisture contents through storage for 3 months were assessed for HTC development by performing cooking experiments (Section 2.4.). Using the moisture contents of these materials, their positions on the T_g -moisture graph were determined and are encircled in Fig. 6. It is clear that at the lowest moisture content (8.1%), the whole beans and cotyledons were in a glassy state. Cotyledons with 11.7% moisture were below T_g but whole beans with the same moisture content had seed coats around T_g but cotyledons below T_g . Materials with the highest moisture content (~15%) were clearly above the T_g of both cotyledons and seed coats and hence were in a viscoelastic state.

The cooking behavior (Supplementary Fig. 1) of these stored materials was assessed by comparison of their softening rate constants (Table 3) in order to investigate HTC development during storage. For both cotyledons and whole beans, the k values decreased with increase in moisture content indicating a decrease in cooking quality as moisture content increased during storage. Non-stored materials (~8% moisture) were regarded as control materials. Upon cooking, cotyledons and whole beans that contained 8.1% moisture had similar softening rate constants as their corresponding non-stored materials. Therefore, their cooking behavior was similar, signifying absence of HTC in these materials. Surprisingly, the k value for cotyledons that contained 11.7% moisture was significantly lower than that of the control cotyledons. Therefore, these cotyledons developed HTC because their cooking quality deteriorated. This was an unexpected result given the position of these cotyledons in Fig. 6 which indicates that they were in a glassy state during storage. Since T_g is a range rather than a point, it could be that these cotyledons were possibly in the vicinity of their T_g , in other words they could have been in a rubbery state.

Whole beans that contained the same moisture content showed different cooking behavior from that of the control whole beans but their k value was not significantly different from that of whole beans that were stored with 8.1% moisture. This result suggests that the whole beans with 11.7% moisture were possibly in a rubbery state during storage. Their position in Fig. 6 which shows that they were above the T_g of seed coats but below that of cotyledons could be the reason for the suggested rubbery state during storage. This is an interesting result given that our previous work (Chigwedere *et al.*, 2018) showed that cotyledons significantly contribute to HTC development unlike seed coats. Therefore, results from this study suggest that for storage stability of common beans in the context of their cooking quality, mobility should be infeasible in

both cotyledons and seed coats, that is, both macrostructural components have to be in a glassy state. However, there is need to explore the mechanism by which the seed coat in a viscoelastic state would facilitate for HTC development in the bean when the cotyledons are in a glassy state. It can be hypothesised that since cotyledons consist of multiple components, some of their T_g values might have possibly facilitated HTC development given the state of the seed coat.

Cotyledons and whole beans that had the highest moisture content during storage had the lowest k values which were however not significantly different from the corresponding materials containing 11.7% moisture. Their higher moisture content facilitated mobility in the matrix, leading to deteriorative reactions that could have led to the highest extent of HTC development and hence the lowest cooking quality. Biochemical reactions hypothesised as mechanistic explanations for occurrence of HTC were reviewed by El-Tabey Shehata (1992), Reyes-Moreno *et al.* (1993) and Liu and Bourne (1995). These results confirm the polymer science hypothesis of stability below and instability above the T_g . Therefore, the T_g data generated in this study are relevant and the established T_g -moisture relation can be regarded as a stability map for postharvest storage of common beans.

Conclusions

For the first time, the present study provides, based on a material science approach, the appropriate storage conditions, that is, moisture content and temperature, that ensure storage stability of common beans in the context of aging and cooking quality. Results indicate that the hypothesis of stability below T_g is supported as long as all the components of the beans are below their T_g values. If this condition is not met, beans will be rendered unstable and liable to lose cooking quality. In addition, the established T_g -moisture relation was proven to be of practical relevance, hence it can be used as a stability map for storage of common beans. After harvest, moisture content of the beans can be determined and the temperature below which they should be stored to ensure preservation of cooking quality can be read-off from the stability map (T_g -moisture relation). The lower the moisture content of the beans, the higher the T_g values hence postharvest drying becomes a prerequisite for enhanced stability of beans. This is an advantage for common bean farmers in temperate regions because they can store their beans at room temperatures but not so much for those in tropical regions who may require cool conditions for bean storage during the summer period. Among the commonly consumed legumes, the susceptibility of HTC development and hence loss of cooking quality follows the

order: common beans > Bambara groundnuts (*Vigna subterranea*) > cowpeas (*Vigna unguiculata*). Therefore, it is interesting to determine also the stability maps of Bambara groundnuts and cowpeas and other legumes in order to prevent quality deterioration during storage.

Acknowledgements

Chigwedere C. M. is a Ph.D. fellow funded by the Interfaculty Council for Development Cooperation (IRO, Grant number 000000003746). Panozzo A. is a postdoctoral researcher in the Laboratory of Food Technology who assisted with method implementation. This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Declarations of interest: The authors declare that they have no conflict of interest.

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Tables

Table 1: Selected salts to impart different water activities in the range 0.1 to 0.99 in enclosed environments in order to generate bean materials of different moisture contents

Salt	Target a_w at saturation at 4 °C
Potassium hydroxide (KOH)	0.14 ± 0.017
Sodium iodide (NaI)	0.42 ± 0.099
Sodium bromide (NaBr)	0.64 ± 0.072
Ammonium sulphate ((NH ₄) ₂ SO ₄)	0.82 ± 0.068
Potassium sulphate (K ₂ SO ₄)	0.98 ± 0.091

Source: Greenspan (1977)

Table 2. Parameters estimated by the Guggenheim, Anderson, and de Boer (GAB) model for the adsorption isotherms of whole bean, cotyledon and seed coat powders. The M_g is the GAB moisture content corresponding to the monomolecular layer of water molecules and, C and K are energy constants related to the net heat of sorption of the monolayer and multilayers, respectively. Analyses were conducted in duplicate and are presented as such.

Material	R^2_{adjusted}	M_g	C	K
Whole beans 1	0.99	7.3 ± 0.24	123 ± 100	0.8 ± 0.01
Whole beans 2	1.00	7.4 ± 0.20	74.3 ± 30.6	0.8 ± 0.01
Cotyledons 1	1.00	8.6 ± 0.44	33.6 ± 10.8	0.7 ± 0.03
Cotyledons 2	1.00	7.9 ± 0.19	66.2 ± 20.9	0.8 ± 0.01
Seed coats 1	1.00	8.2 ± 0.37	42.9 ± 14.9	0.7 ± 0.02
Seed coats 2	1.00	8.1 ± 0.41	42.8 ± 17.2	0.7 ± 0.03

507

508 **Table 3.** Parameter estimates ($\pm$ standard errors) for softening of whole beans and cotyledons
 509 with different moisture content stored at 35 °C for three months. The k is the estimated softening
 510 rate constant and H_0 is the normalized initial hardness.

Material	R^2_{Adjusted}	k^1 ($\times 10^2$) min^{-1}	H_0 (g)	RMSE
<i>%Moisture content of stored cotyledons</i>				
7.90 $\pm$ 0.0050 (control)	0.99	2.44 $\pm$ 0.19 ^a	1.01 $\pm$ 0.04	0.041
8.10 $\pm$ 0.0004	0.99	1.95 $\pm$ 0.07 ^a	1.00 $\pm$ 0.02	0.020
11.7 $\pm$ 0.0001	0.99	1.07 $\pm$ 0.12 ^b	1.05 $\pm$ 0.06	0.069
15.1 $\pm$ 0.0002	0.99	0.76 $\pm$ 0.03 ^b	1.00 $\pm$ 0.02	0.025
<i>%Moisture content of stored whole beans</i>				
8.10 $\pm$ 0.00700 (control)	0.99	1.48 $\pm$ 0.12 ^A	0.99 $\pm$ 0.04	0.045
8.10 $\pm$ 0.00006	0.99	1.11 $\pm$ 0.15 ^{AB}	1.06 $\pm$ 0.07	0.080
11.7 $\pm$ 0.00020	0.99	0.68 $\pm$ 0.12 ^{BC}	0.92 $\pm$ 0.07	0.078
14.5 $\pm$ 0.00000	0.99	0.37 $\pm$ 0.03 ^C	0.96 $\pm$ 0.04	0.044

¹ Different small and capital superscript letters show significant differences among k-values for cotyledons and whole beans, respectively

Figures

Fig. 1 Schematic illustration of the experimental approach of this study.

Fig. 2. Particle size distribution of powdered whole beans, cotyledons and seed coats after equilibration above phosphorus pentoxide in order to attain the minimum possible moisture content. Solid and broken lines of the same color represent duplicates of the same bean material.

Fig. 3. Sorption isotherms for whole bean, cotyledon and seed coat powders at 4 °C modeled with the GAB equation.

Fig. 4. Displacement of the probe during heating of skimmed milk powder at different heating rates under a compression force of 30 N (A) and corrected displacement for skimmed milk powder at the selected heating rate (1 °C/min) (B) using oven-dried microcrystalline cellulose.

Fig. 5. Compressibility curves of whole bean powders of different moisture content (A) and how T_g values were obtained from them as illustrated for whole bean powder with 11.1% moisture (B).

Fig. 6. Variation of glass transition temperature with moisture content (symbols not encircled) of whole bean, cotyledon and seed coat powders. The materials were analyzed in duplicate from independent treatments hence the results are presented in duplicate for each material. Encircled colored triangular symbols represent the conditions of whole beans and cotyledons stored at different moisture content (shown in the legend) at 35 °C for three months in order to determine relevance of T_g data.

Supplementary Figure

Supplementary Fig. 1. Cooking behavior of cotyledons (A) and whole beans (B) conditioned to different moisture contents through storage above different saturated salt solutions at 35 °C for 3 months. Data was modeled following first order kinetics. M.C. is moisture content.