

## Degradation of $\alpha$ -Galactosides during the Germination of Grain Legume Seeds

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### Abstract

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Germination is one of the most effective ways of preparing grain legumes for consumption. Because it involves the total or partial elimination of some anti-nutritional compounds, it is also one of the simplest methods of enhancing the palatability of grain legumes, thereby increasing their consumption as a valuable source of nutrition. The main objective of this paper is to describe the changes that take place in  $\alpha$ -galactosides during germination. During germination, galactose molecules gradually become detached from  $\alpha$ -galactosides due to the effect of the enzyme  $\alpha$ -D-galactosidase activated during the process. To simulate the degradation of  $\alpha$ -galactosides during legume seed germination, we applied nine equations to the evaluation of the experimental data obtained with the germination of three types of grain legume seeds; mung bean, chickpea, and lentil.

**Keywords:**  $\alpha$ -galactosides; germination; grain legume seeds

The germination of legume seeds is a method that has been in existence for centuries, having been particularly developed using traditional procedures in the countries of the Far East and India (DESHPANDE & DESHPANDE 1991). During germination, the seeds (e.g. beans, peas, lentils) are transformed from the dormant state into a metabolically active state. This process involves intensive mobilisation of the stored reserves, which results in a rapid increase in respiration, the synthesis of proteins and nucleic acids, and the elongation and division of cells (GÓRECKI *et al.* 2000). The changes in the contents of the nutrient and non-nutrient substances are influenced by the germination conditions, including

the temperature, the presence or absence of light, the composition of the soaking and rinsing media, and the post-germination handling. Leguminous seeds must take up large quantities of water, typically 80–140% of their own weight, if swelling and germination are to occur. The germinated legume seeds and their sprouts contain significantly greater amounts of vitamins than do dry seeds, and are also an outstanding source of ascorbic acid (DESHPANDE & DESHPANDE 1991).

Apart from improving the sensory quality of leguminous seeds, the main advantage of germination lies in the full or partial removal of certain compounds, including phytic acid, tannins, and

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above all  $\alpha$ -galactosides (verbascose, stachyose, raffinose) which, when present, compromise the nutritional value of the seeds.

The energy sources required for germination are stored in the seeds themselves; one of these sources being  $\alpha$ -galactosides. The use of  $\alpha$ -galactosides as an energy source during germination means that the germination process is an efficient mechanism for  $\alpha$ -galactoside degradation. The degradation of  $\alpha$ -galactosides during the germination of leguminous seeds commences with inhibition, and continues intensively during the embryonic stage. In the case of soy, peas, and lupine seeds,  $\alpha$ -galactoside levels have been shown to decrease during the first two days of imbibition, with hydrolysis of the  $\alpha$ -galactosides in cells taking from 4–6 days, the enzyme  $\alpha$ -D-galactosidase catalysing the hydrolysis of the bond between  $\alpha$ -galactosides, the cell polysaccharide barrier, and the stored glycoprotein (KOSTER & LEOPOLD 1988; GÓRECKI *et al.* 1997a). Mature seeds usually contain isoforms of this enzyme, differing in activity and molecular weight (PRIDHAM & DEY 1974). In the developing seed,  $\alpha$ -D-galactosidase activity increases during intensive synthesis of  $\alpha$ -galactosides, reaching its maximum level at full seed maturity.

Because the enzymes of the human gastrointestinal tract are unable to break them down  $\alpha$ -galactosides, these are metabolised by the bifidobacteria and other bacteria of the colon, which results in the production of gases ( $\text{CO}_2$ ,  $\text{CH}_4$ ,  $\text{H}_2$ ) that are the primary cause of flatulence and other digestive disorders associated with the consumption of grain legumes seeds. Significantly, these problems do not occur after the consumption of germinated grain legume seeds with low  $\alpha$ -galactoside contents. However, a drawback of the germination process is that it results in an increase in the total number of microorganisms present in the germinated seeds (ROBERTSON *et al.* 2002, 2005; BREMER *et al.* 2003; THOMAS *et al.* 2003; WARRINER *et al.* 2003).

## MATERIAL AND METHODS

**Plant material.** Chickpea seeds (*Cicer arietinum* L.), year of harvest 2002, country of origin Turkey. Lentil seeds (*Lens esculenta* MOENCH), country of origin Canada. Mung bean (*Vigna radiata* L. WILCZEK), country of origin Burma.

**Germination.** The samples were germinated at the Czech University of Life Sciences Prague, in

the Department of Plant Production. They were germinated in 5 aerated bottles, connected in sequence. One such bottle contained 50 g of seeds and 100 ml of tap water. The water in the bottles was changed every 24 h, the time of germination was 1–5 days. For each seed type, one bottle was removed after each day of germination and 100 g of the germinated sample was removed and stored in a freezer. After five days, all germinated samples were taken together for simultaneous analyses. The germination experiments were repeated three times for each seed type.

**Extraction and determination of soluble carbohydrates.** Approximately 5 g of ground dry sample was homogenised in 20 ml of ethanol:water (80:20, v/v) and refluxed for 60 minutes. The extract was cooled and filtered through a membrane filter (pore size 0.45  $\mu\text{m}$ ), and the residue was rinsed out with distilled water. The filtrate was evaporated on a vacuum evaporator (temperature 60°C, pressure max. 0.9 kPa) and the residue was diluted with demineralised water (5 g to 1000 ml), repurified by filtration using a C18 microfilter (Maxi-Clean Cartridges), and analysed by HPLC method.

**HPLC determination.** Carbohydrates (fructose, glucose, galactose, sucrose, and  $\alpha$ -galactosides – raffinose, verbascose and stachyose) were separated by HPLC method using refractometric detection (Shodex RI-SE 61, Showa Denko, Japan) and a Separon SGX  $\text{NH}_2$  column [(4  $\times$  250 mm) (Tessek Ltd., Czech Republic)]. HPLC conditions: mobile phase – mixture of acetonitrile and demineralised water (65:35, v/v); flow of mobile phase 0.8 ml/min; working pressure 9.8 MPa; sample volume 100  $\mu\text{l}$ ; laboratory temperature. The external standard method was used to quantify the analyses.

**Statistical evaluation applied.** All germination experiments were repeated three times and the analyses of soluble carbohydrates in all samples obtained were carried out twice. The mean values and standard deviations were calculated.

The equations of approximation were used to evaluate  $\alpha$ -galactosides degradation during the germination of grain legume seeds. The coefficients of these equations and the correlation coefficients ( $R$ ) were calculated by means of Excel software.

## RESULTS AND DISCUSSION

Table 1 displays the detection and determination limits for carbohydrates identification. The lowest determination limits were those for fructose,

sucrose, and glucose (0.03–0.04 g/100 g d.m.), the highest determination limits were those for stachyose, galactose, and verbascose (0.08–0.10 g/100 g d.m.). The example of a chromatogram of the analysis of chickpea seeds carbohydrates is shown in Figure 1. This figure demonstrates a very good separation and determination of all  $\alpha$ -galactosides present as well as of sucrose.

Glucose, fructose, galactose, sucrose, and  $\alpha$ -galactosides (raffinose, verbascose, stachyose) were found in the samples of all three seed types. In addition, the  $\alpha$ -galactoside ciceritol was determined only in the lentil seed samples. The average results (mean values and standard deviations) of the analyses from all three germination experiments for each seed type are presented in Table 2. In all seed types, the total content of  $\alpha$ -galactosides (raffinose, stachyose, verbascose, ciceritol) consistently decreased until the fifth day

of germination. Overall,  $\alpha$ -galactosides contents in the chickpea and lentil seeds decreased to 32% and 25% of their original contents, respectively, while no  $\alpha$ -galactosides remained in the mung bean samples after four days of germination. The same results, the elimination of  $\alpha$ -galactosides after the germination of pea seeds, were found out by DESHPANDE and DESHPANDE (1991); VIDAL-VALVERDE and FRIAS (1992) and TRUGO *et al.* (2000). The content of sucrose increased during germination, with maximum levels observed on the fifth day of germination. Monosaccharide contents also increased. The enhancement of sucrose and monosaccharides levels is explained by the enzyme  $\alpha$ -D-galactosidase activated, during the germination process, causing a gradual detachment of galactose from  $\alpha$ -galactosides. As a result, raffinose, is converted into sucrose, stachyose into raffinose and verbascose into stachyose. However,

Table 1. Detection and determination limits for carbohydrates identification

Carbohydrates	Detection limit (g/100 g dry matter)	Determination limit (g/100 g dry matter)
Fructose	0.0123	0.0307
Glucose	0.0169	0.0422
Galactose	0.0382	0.0956
Sucrose (suc)	0.0137	0.0341
Raffinose (raf)	0.0245	0.0614
Stachyose (sta)	0.0325	0.0813
Verbascope (ver)	0.0396	0.1013

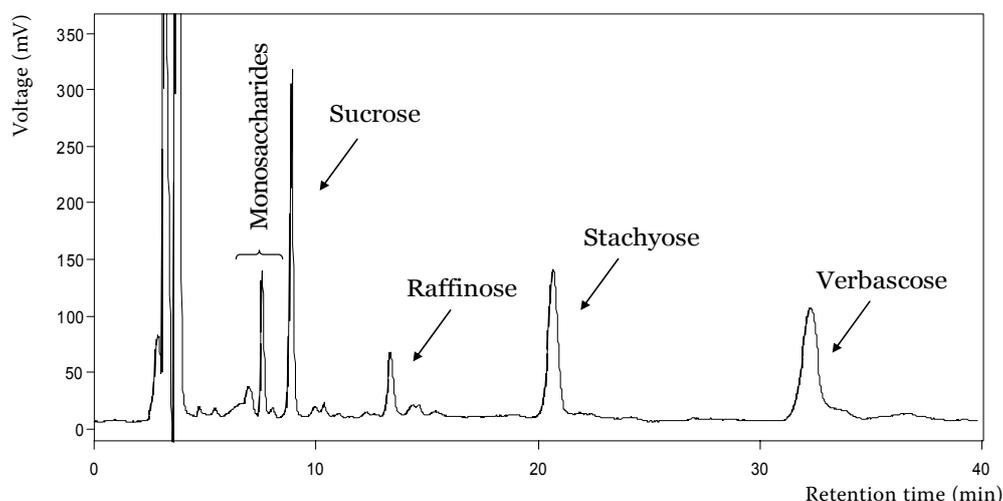


Figure 1. Example of chromatogram of carbohydrates analysis of chickpea seeds

Table 2. Changes in the contents of soluble carbohydrates (in g/100 g dry matter) during the germination of mung bean, lentil and chickpea seeds

Time of germination (days)	Fructose	Glucose	Galactose	Sucrose	Raffinose	Stachyose	Verbascose	Ciceritol
<b>Mung bean</b>								
0	0.00 ± 0.00	0.67 ± 0.01	0.05 ± 0.00	1.72 ± 0.01	0.48 ± 0.01	1.91 ± 0.02	2.47 ± 0.03	–
1	0.00 ± 0.00	0.45 ± 0.00	0.05 ± 0.01	1.51 ± 0.02	0.24 ± 0.01	1.52 ± 0.02	1.46 ± 0.01	–
2	0.10 ± 0.00	0.56 ± 0.02	0.10 ± 0.00	1.70 ± 0.03	0.20 ± 0.00	1.26 ± 0.04	1.20 ± 0.02	–
3	0.20 ± 0.01	0.81 ± 0.02	0.17 ± 0.00	3.10 ± 0.02	0.15 ± 0.00	0.64 ± 0.03	0.60 ± 0.01	–
4	0.47 ± 0.00	1.07 ± 0.01	0.29 ± 0.01	4.77 ± 0.01	0.10 ± 0.00	0.18 ± 0.01	0.16 ± 0.01	–
5	0.73 ± 0.02	1.27 ± 0.00	0.46 ± 0.00	5.80 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
<b>Lentil</b>								
0	0.23 ± 0.03	0.14 ± 0.01	0.26 ± 0.02	1.56 ± 0.02	0.36 ± 0.03	1.09 ± 0.03	2.32 ± 0.07	1.17 ± 0.01
1	0.23 ± 0.03	0.16 ± 0.02	0.24 ± 0.00	1.58 ± 0.05	0.31 ± 0.01	0.73 ± 0.02	1.54 ± 0.04	0.83 ± 0.03
2	0.39 ± 0.01	0.18 ± 0.02	0.20 ± 0.02	1.74 ± 0.01	0.37 ± 0.02	0.44 ± 0.01	0.87 ± 0.01	0.63 ± 0.01
3	0.36 ± 0.01	0.20 ± 0.02	0.21 ± 0.01	1.65 ± 0.03	0.39 ± 0.01	0.51 ± 0.01	0.84 ± 0.01	0.62 ± 0.02
4	0.70 ± 0.01	0.19 ± 0.01	0.28 ± 0.02	2.83 ± 0.05	0.41 ± 0.01	0.23 ± 0.02	0.48 ± 0.00	0.31 ± 0.01
5	0.65 ± 0.02	0.17 ± 0.01	0.32 ± 0.00	2.64 ± 0.06	0.41 ± 0.00	0.21 ± 0.00	0.37 ± 0.02	0.26 ± 0.02
<b>Chickpea</b>								
0	–	0.02 ± 0.00	0.00 ± 0.00	2.55 ± 0.14	0.67 ± 0.02	2.51 ± 0.08	0.08 ± 0.00	–
1	–	0.04 ± 0.00	0.08 ± 0.00	1.56 ± 0.05	0.68 ± 0.02	1.89 ± 0.03	0.07 ± 0.00	–
2	–	0.04 ± 0.00	0.04 ± 0.00	2.42 ± 0.07	0.60 ± 0.01	1.42 ± 0.02	0.06 ± 0.00	–
3	–	0.04 ± 0.00	0.02 ± 0.00	1.86 ± 0.05	0.45 ± 0.01	0.88 ± 0.01	0.05 ± 0.00	–
4	–	0.05 ± 0.00	0.03 ± 0.00	1.10 ± 0.03	0.30 ± 0.01	0.45 ± 0.01	0.03 ± 0.00	–
5	–	0.07 ± 0.00	0.05 ± 0.00	2.13 ± 0.06	0.44 ± 0.01	0.55 ± 0.01	0.05 ± 0.00	–

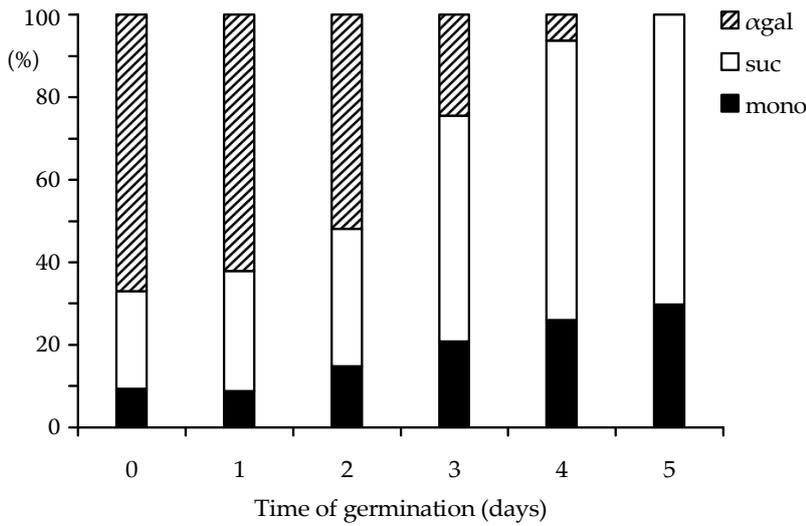


Figure 2. Relative content of each type of carbohydrates in germinated mung bean seeds ( $\alpha$ gal –  $\alpha$ -galactosides; suc – sucrose; mono – monosaccharides)

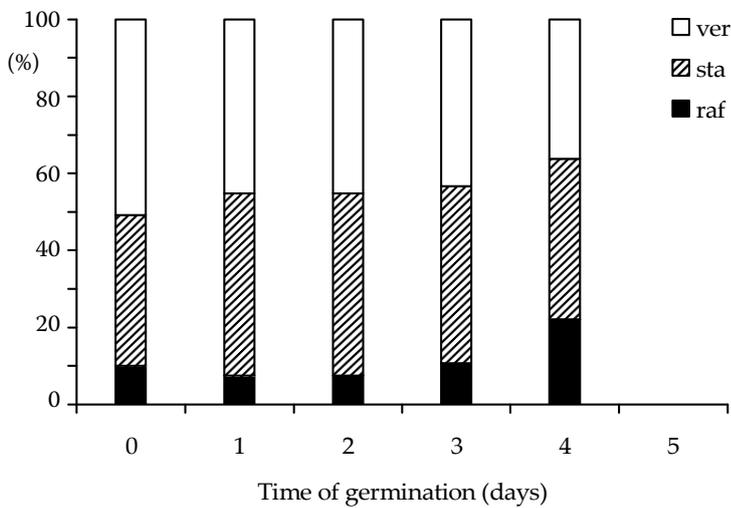


Figure 3. Relative contents of  $\alpha$ -galactosides in germinated seeds of mung bean (ver – verbascose; sta – stachyose; raf – raffinose)

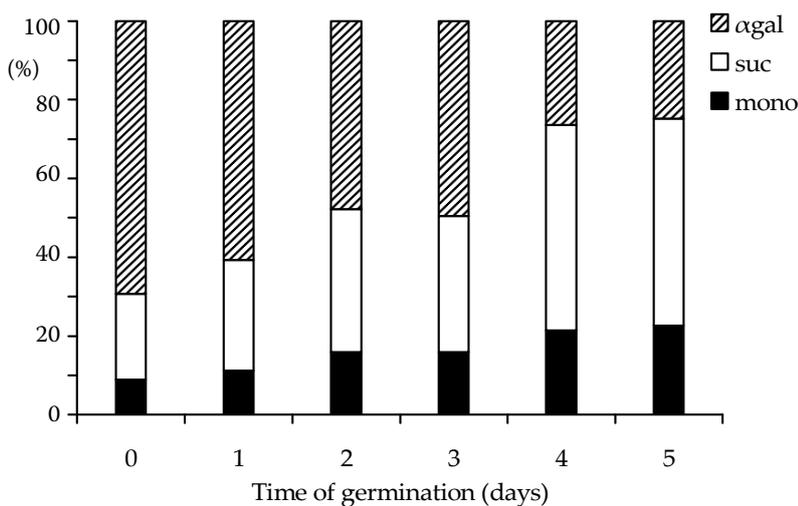


Figure 4. Relative content of each type of carbohydrates in germinated lentil seeds ( $\alpha$ gal –  $\alpha$ -galactosides; suc – sucrose; mono – monosaccharides)

as the monosaccharides are simultaneously metabolised, their contents do not increase in a simple relation to the corresponding reduction of  $\alpha$ -galactosides contents. These results are comparable

with the data from literature (VIDAL-VALVERDE & FRIAS 1992 – pea seeds, lentil; GÓRECKI *et al.* 1997b – lupine; TRUGO *et al.* 2000 – pea seeds). In general, the longer the germination period of

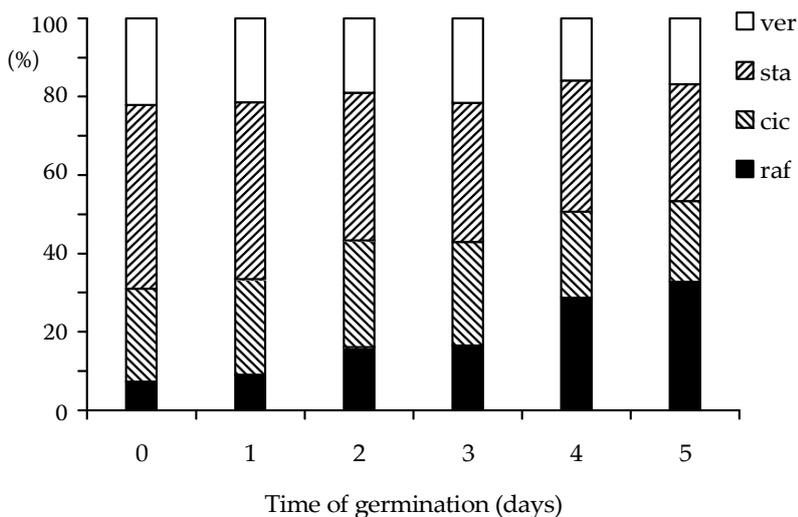


Figure 5. Relative contents of  $\alpha$ -galactosides in germinated seeds of lentil (ver – verbascose; sta – stachyose; cic – ciceritol; raf – raffinose)

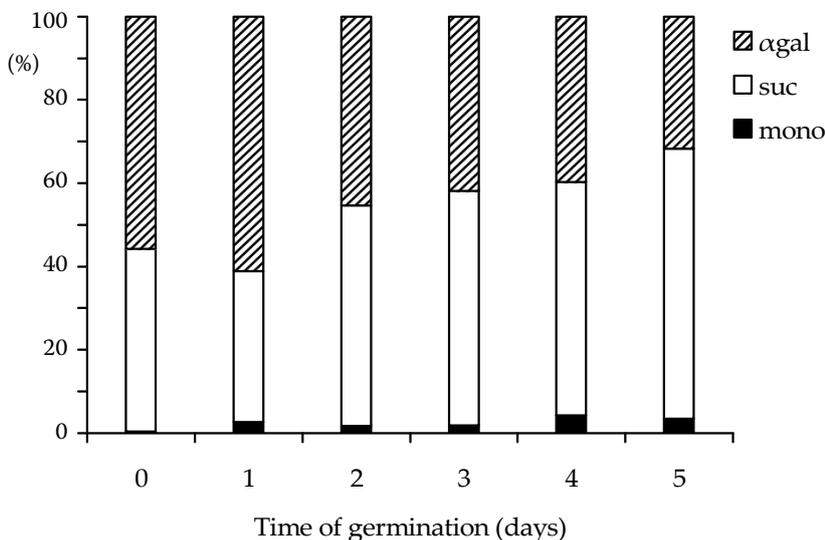


Figure 6. Relative content of each type of carbohydrates in germinated chickpea seeds ( $\alpha$ gal –  $\alpha$ -galactosides; suc – sucrose; mono – monosaccharides)

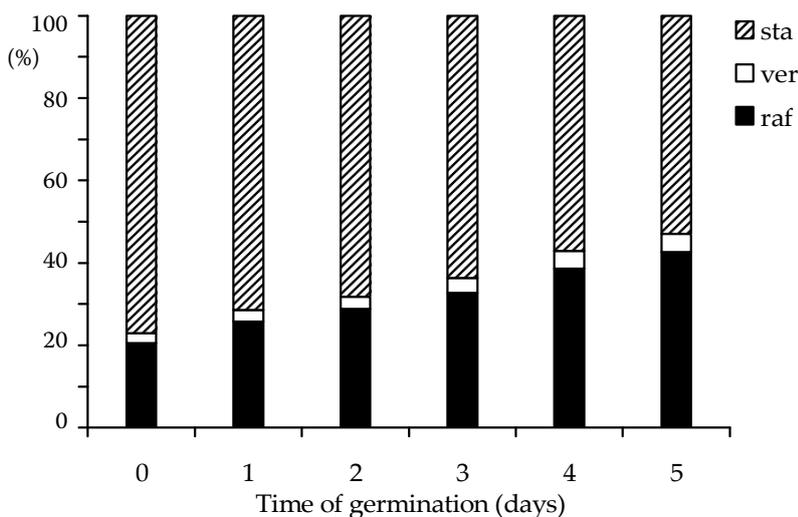


Figure 7. Relative contents of  $\alpha$ -galactosides in germinated seeds of chickpea (sta – stachyose; ver – verbascose; raf – raffinose)

leguminous seeds, the greater the reduction of  $\alpha$ -galactosides contents. The same conclusion, formulated by the sentence: “There is a positive

correlation between the decline in  $\alpha$ -galactosides content and the depression of seed longevity”, is shown in the book by GÓRECKI *et al.* (2000).

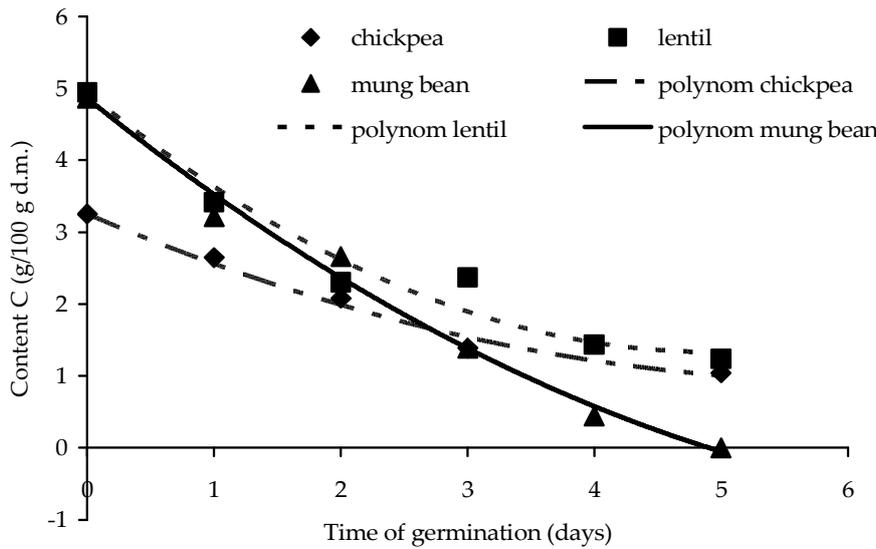


Figure 8. Plot of  $\alpha$ -galactosides content against germination time using Equation 2

Figure 2 shows, in percentages, the changes that took place in the relative content of each type of carbohydrates during the germination of mung bean seeds. It clearly shows that, over the course of five days of germination, the relative content of  $\alpha$ -galactosides in the mung bean samples decreased from 68% to zero. Conversely, the content of sucrose increased from 22% to 70%, while the monosaccharides contents also increased from 10% to 30%.

Figure 3 displays the changes in the relative contents of  $\alpha$ -galactosides (raffinose, stachyose, verbascose) during the first four days of mung bean germination. Overall, during this period, the content of raffinose enhanced from 10% to 22% while that of verbascose decreased from 52% to 37%, and the stachyose content varied across the range of 38–47%.

Figure 4 describes the changes that occurred in the relative content of each type of carbohydrates during the germination of lentil seeds. It shows that the proportion of  $\alpha$ -galactosides in the lentil samples decreased from 70% to 25% while, conversely, the sucrose content increased from 22% to 53%, and that of monosaccharides content from 9% to 22%.

The changes in the relative contents of  $\alpha$ -galactosides (raffinose, stachyose, verbascose, ciceritol) during lentil seed germination are shown in Figure 5. During five days of germination, the content of raffinose increased from 8% to 32%, the content of both ciceritol and verbascose varied across the range of 18–23%, and that of stachyose decreased from 47% to 30%.

Figure 6 displays the changes that took place in the relative content of each type of carbohydrates

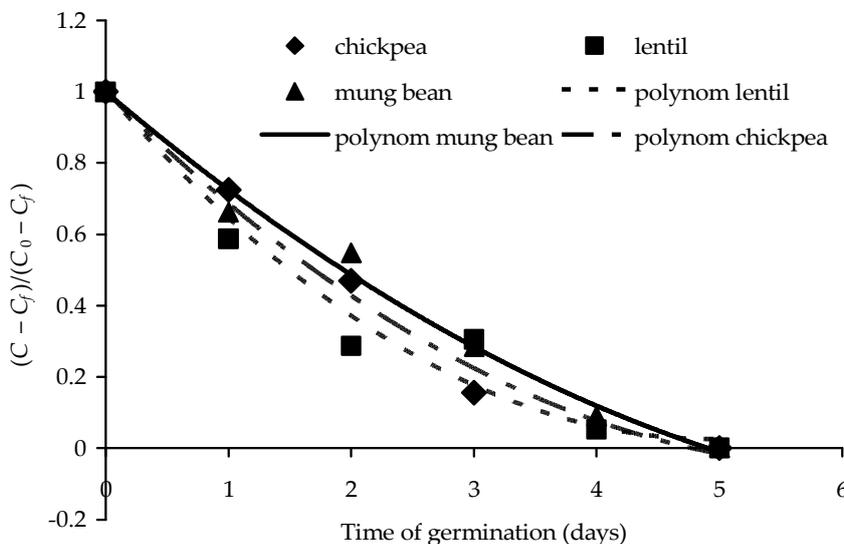


Figure 9. Plot of  $(C - C_p)/(C_0 - C_p)$  values against germination time using Equation 8

during the germination of chickpea seeds. Overall, it shows that the  $\alpha$ -galactosides content in the chickpea samples dropped from 56% to 32%, while, conversely, the sucrose content increased from 44% to 65%, and the monosaccharides content from zero to 3%.

The changes in the relative contents of  $\alpha$ -galactosides (raffinose, stachyose, verbascose) during chickpea germination are shown in Figure 7. During the five-day germination period, the content of raffinose rose from 20% to 42%, while that of verbascose, already minimal in chickpea, increased only by just 2%, to 5%. Meanwhile, the relative content of stachyose, the dominant  $\alpha$ -galactoside in chickpea, decreased from 77% to 53%. FRIAS *et al.* (1999) and VIDAL-VALVERDE *et al.* (1993) mentioned the content of raffinose in chickpea being in the range of 0.6–2.0 g/100 g d.m. and verbascose be in a present only in trace amount which completely corresponds with the results of these experiments.

### Modelling of degradation kinetics

To evaluate the degradation efficiency, it is crucial to know not only the extent of the changes in the  $\alpha$ -galactosides contents during germination, but also the rate at which they occur. These changes can be described by the application of the following zero order (Eq. I), first order (Eq. II) or second order (Eq. III) equations:

$$C = C_0 - k \tau \quad (\text{I})$$

$$C = C_0 \exp(-k \tau) \quad (\text{II})$$

$$1/C = 1/C_0 + k \tau \quad (\text{III})$$

where:

$C_0$  – initial content

$\tau$  – time (min)

$k$  – reaction rate constant (per min)

$C$  – calculated content

The fractional conversion model (Eq. IV) has also often been applied to describe the changes in the relative contents of compounds:

$$(C - C_f)/(C_0 - C_f) = \exp(-k \tau) \quad (\text{IV})$$

where:

$C_f$  – final equilibrium content

The same equations are frequently used to formulate the heat inactivation of microorganisms and enzymes, as well as the quality attributes of

Table 3. Correlation coefficients calculated for the equations applied to  $\alpha$ -galactosides degradation during legume seed germination

Equation	Left side	Mung bean	Chickpea	Lentil	$R^2$
1	C	$= -1.0626 \tau + 4.86$	$= -0.5018 \tau + 3.25$	$= -0.8554 \tau + 4.94$	0.9210
2	C	$= 0.0882 \tau^2 - 1.4235 \tau + 4.86$	$= 0.0609 \tau^2 - 0.7533 \tau + 3.25$	$= 0.1471 \tau^2 - 1.457 \tau + 4.94$	0.9876
3	C	$= 4.86 \exp(-1.0442 \tau)$	$= 3.25 \exp(-0.2394 \tau)$	$= 4.94 \exp(-0.2903 \tau)$	0.9729
4	1/C	$= 0.3405 \tau + 0.21$	$= 0.1253 \tau + 0.31$	$= 0.1137 \tau + 0.2$	0.9605
5	1/C	$= 0.2085 \tau^2 - 0.3545 \tau + 0.21$	$= 0.0086 \tau^2 + 0.09 \tau + 0.31$	$= 0.01 \tau^2 + 0.0728 \tau + 0.2$	0.9753
6	1/C	$= 0.21 \exp(-0.4928 \tau)$	$= 0.31 \exp(-0.2373 \tau)$	$= 0.2 \exp(-0.2936 \tau)$	0.9729
7	$(C - C_f)/(C_0 - C_f)$	$= -0.2186 \tau + 1$	$= -0.2273 \tau + 1$	$= -0.2311 \tau + 1$	0.9199
8	$(C - C_f)/(C_0 - C_f)$	$= 0.0182 \tau^2 - 0.2932 \tau + 1$	$= 0.0278 \tau^2 - 0.3419 \tau + 1$	$= 0.0399 \tau^2 - 0.3942 \tau + 1$	0.9876
9	$(C - C_f)/(C_0 - C_f)$	$= \exp(-0.9007 \tau)$		$= \exp(-0.962 \tau)$	0.6695

the materials subjected to heat (AVILA & SILVA 1999).

To simulate the  $\alpha$ -galactosides degradation during the germination of grain legume seeds, the following nine equations were used:

$$C = C_0 - k \tau \quad (1)$$

$$C = a \tau^2 + b \tau + C_0 \quad (2)$$

$$C = C_0 \exp(-k \tau) \quad (3)$$

$$1/C = 1/C_0 + k \tau \quad (4)$$

$$1/C = a \tau^2 + b \tau + 1/C_0 \quad (5)$$

$$1/C = 1/C_0 \exp(-k \tau) \quad (6)$$

$$(C - C_f)/(C_0 - C_f) = 1 - k \tau \quad (7)$$

$$(C - C_f)/(C_0 - C_f) = a \tau^2 + b \tau + 1 \quad (8)$$

$$(C - C_f)/(C_0 - C_f) = \exp(-k \tau) \quad (9)$$

where:

$C_0$  – initial content of  $\alpha$ -galactosides

$\tau$  – time of germination (days)

$k$  – reaction rate constant (per day)

$C_f$  – final, post-germination, content of  $\alpha$ -galactosides

$C$  – calculated content of  $\alpha$ -galactosides

For each seed type, Table 3 shows the correlation coefficients ( $R$ ) calculated using all nine equations. In the case of all three seed types, the best approximations (correlation coefficients ranging from 0.96–0.99) were achieved using Eq. 2 (Figure 8) and Eq. 8 (Figure 9). The use of exponential functions (Eqs 3, 6 and 9) resulted in the poorest approximations.

## CONCLUSIONS

The main objectives of this paper are to describe the changes that take place in  $\alpha$ -galactosides during germination, and to calculate  $\alpha$ -galactosides degradation by applying various models. To simulate the degradation of  $\alpha$ -galactosides during the legume seed germination, we applied nine equations to the evaluation of the experimental data obtained in the germination of three types of grain legume seeds; mung bean, chickpea, and lentil. With all three types of seeds, the best approximation was achieved using the following equations:

$$C = a \tau^2 + b \tau + C_0$$

$$C - C_f/(C_0 - C_f) = a \tau^2 + b \tau + 1$$

where:

$C_0$  – initial content of  $\alpha$ -galactosides

$\tau$  – germination time (days)

$C_f$  – final, post-germination, content of  $\alpha$ -galactosides

$C$  – calculated content of  $\alpha$ -galactosides

The correlation coefficients of these dependences ranged from 0.96–0.99.

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