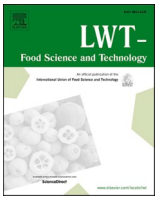
LWT - Food Science and Technology 190 (2023) 115571

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt



Modelling cowpea beans α-galactosidase inactivation dependence on temperature and moisture content

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ARTICLE INFO

Keywords:
Oligosaccharides
Enzyme kinetics
Hydrothermal process
Pulses

ABSTRACT

Hydrothermal treatment improves the digestibility of pulses through the coupled phenomena of enzymatic degradation and diffusion of galacto-oligosaccharides, despite the thermal inactivation of α-galactosidase at mild temperatures. To better understand the key process parameters that influence the balance between enzyme activity and its inactivation, the kinetics of α-galactosidase inactivation were investigated in a cowpea-water system at a broad range of temperatures (45 °C $\leq T \leq$ 60 °C), moisture contents (0.16 g/g dry basis, d.b. $\leq W \leq$ 0.85 g/g d.b.) and treatment times (5 min $\leq t \leq$ 7.5 h). For moisture contents ranging from 0.25 to 0.85 g/g d.b., the higher the temperature the faster the inactivation of α-galactosidase; while for 0.16 g/g d.b., the relative enzymatic inactivation was not temperature-dependent. A first-order model was successfully fitted to the experimental data, whereas the temperature dependence of inactivation rate followed the Arrhenius model. The influence of moisture content on the enzymatic inactivation rate was remarkable. The inactivation rate constant at excess water (0.85 g/g d.b.) was five times higher than the samples at limited water (0.35 g/g d.b.) condition. The results showed moisture-dependent α-galactosidase inactivation kinetics mainly at mild temperatures (55 and 60 °C), with implications for the representation of diffusion-reaction phenomena during hydrothermal processing of pulses.

1. Introduction

The question of how to achieve more sustainable food systems has emerged in the context of climate change and the necessity to develop renewable food sources (Augustin & Cole, 2022; Knorr, Augustin, & Tiwari, 2020). Dry seed legumes (i.e. pulses) are an example of a food source with potential for the development of sustainable diets thanks to its low environmental footprint, and its positive impact on human health due to the presence of essential amino acids, minerals, vitamins and phenolic compounds (Chigwedere, Njoroge, Van Loey, & Hendrickx, 2019; Röös et al., 2020).

On the other hand, pulses contain a subcategory of short-chain carbohydrates named α-galactosides (ajugose, verbascose, stachyose, manninotriose, raffinose and melibiose) that cause abdominal discomfort and bloating when ingested by humans due to the absence of an endogenous enzyme (i.e. α-galactosidase) (Henn, Goddyn, Olsen, & Bredie, 2022). Gibson and Shepherd (2005) hypothesized that excessive intake of these fermentable oligo-, di- and mono-saccharides triggers the development of Crohn's disease. Further, recent studies have associated α-galactosides with worsening symptoms of irritable bowel syndrome by increasing the volume of water in the small intestine and the production of colonic gas (Halmos, Power, Shepherd, Gibson, & Muir, 2014; van Lanen, de Bree, & Greyling, 2021).

Since α-galactosides are water-soluble compounds, hydrothermal processing of pulses (i.e. soaking, pre-cooking, cooking, germination or solid-state fermentation) is widely used to reduce their presence (Thirunathan & Manickavasagan, 2019). When subjected to hydrothermal treatment, pulses undergo a spatial-temporal evolution in temperature and water content, prompting physical-chemical modifications. These changes include the solubilization of α-galactosides due to structural changes in the pulses, coupled with hydrolysis of α-galactosides through the activity of α-galactosidase. Consequently, accurate prediction of the hydrolysis of α-galactosides during the hydrothermal processing of pulses relies on modelling α-galactosidase activity as a function of temperature, moisture content and time.

Although α-galactosidase activity is extensively documented in the literature in a variety of pulses under iso-thermal and iso-moisture conditions (Alani, Smith, & Markakis, 1989; Barham, Dey, Griffiths, &

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https://doi.org/10.1016/j.lwt.2023.115571
Received 22 September 2023; Received in revised form 14 November 2023; Accepted 18 November 2023
Available online 22 November 2023

Pridham, 1971; Coffigniez et al., 2018; Dey, Del Campillo, & Lezica, 1983; Dey & Pridham, 1969; Falkoski et al., 2006), experimental data that provide insights into enzyme activity dependence on moisture are lacking. The aim of this work was thus to model α-galactosidase inactivation kinetics caused by the combined effects of temperature and moisture content in cowpea beans, to enable better modelling of the degradation of α-galactosidase and the biosynthesis of the desirable compounds (i.e. monosaccharides) during processing (e.g. soaking, pre-cooking – at mild temperatures, either during germination or solid-state fermentation).

2. Materials and methods

2.1. Sample preparation

Cowpea beans (*Vigna unguiculata* (L.) Walp., var. *Wankoun*) harvested in the year 2022 were purchased at a local market in Abomey-Calavi (Benin) and stored in vacuum packs at – 20 °C until use. The frozen beans were disintegrated in a hammer mill (Perten 3100, PerkinElmer, Stockholm, Sweden) with a grid size of 0.5 mm, enabling rapid hydration of the cowpeas and reducing the thermal gradient (no heating lag period). The initial moisture content (W_0) and initial water activity at 20 °C ($a_{w,0}$) of cowpea bean powder were 0.13 ± 0.01 g/g d.b. and 0.59 ± 0.01, respectively.

Prior to thermal treatment, the cowpea-water system was prepared daily at different water activity levels by adding distilled water, as follows:

i) For excess water conditions, suspension samples were prepared by mixing 1 g cowpea powder with 5 mL distilled water. The sample was then placed in a plastic pouch (40 mm wide, 300 mm high and 0.09 mm thick) and heat sealed;
ii) For high water activity ($a_w = 0.9$ at 20 °C), cowpea-water dough was prepared by mixing 10 g cowpea powder and 2 mL distilled water in a porcelain mortar and pestle until complete homogenization. To reach internal moisture equilibrium, the dough was shaped into ball and stored in plastic pouch at room temperature for 1 h, after which the dough was divided into samples each weighting 1 g. Water activity values were then measured using a water activity meter (Aqualab 4 TE, Decagon Devices, Pullman, USA), then placed in plastic pouches and heat sealed;
iii) For intermediate water activity levels ($a_w = 0.8$ and $a_w = 0.7$ at 20 °C), 10 g of cowpea powder was homogenized with respectively, 1 mL or 0.1 mL distilled water. The mixing process was carried out using a porcelain mortar and pestle. The sample was stored in a plastic pouch for 1 h at room temperature to reach moisture equilibrium. Like the samples at $a_w = 0.9$, the sample was then divided into 1 g portions, measured a_w value at 20 °C, placed in plastic pouches and heat sealed.

2.2. Adsorption of moisture by the cowpea powder

The correlation between water activity ($0.6 \leq a_w \leq 0.9$) and moisture content (0.13 ± 0.01 g/g d.b. $\leq W \leq 0.31 \pm 0.02$ g/g d.b.) of the cowpea powder was experimentally determined using a dynamic hydration procedure. A thin layer of cowpea powder (5 g) was placed in a climate chamber (KBF 240, Binder GmbH, Tuttlingen, Germany) set at a relative humidity of 95 % and a temperature of 20 °C. After different holding times (5 min $\leq t \leq$ 20 h), the hydrated sample was weighed then stored in a heat-sealed plastic pouch for 2 h to stabilize. Subsequently, water activity was measured at 20 °C using the water activity meter (Aqualab 4 TE, Decagon Devices, Pullman, USA). The procedure was performed to obtain three replicates.

The adsorption behaviour of cowpea powder at 20 °C was characterized using the Oswin model (Eq. (1)). The model parameters (A and B) were optimized by minimizing the Sum of the Squared Errors (SSE) between experimental and predicted moisture contents ($SSE = 3.90 \bullet 10^{-3}$ and $R^2 = 0.950$) using the "fminsearch" optimization function in MATLAB (R2020b, MathWorks, Natick, USA).

$$W = A\left(\frac{a_w}{1 - a_w}\right)^B \tag{1}$$

where W is the moisture content (g/g d.b.), a_w is the water activity (−), A (0.105 ± 0.011 g/g d.b.) and B (0.546 ± 0.068) are the adjusted parameters. The confidence bounds for the adjusted parameters were calculated:

$$\text{parameter} \pm t_{Student}\sqrt{S} \tag{2}$$

where $t_{Student}$ is the inverse cumulative function of Student's t distribution on the confidence level (95 %, bilateral), S is the vector of the diagonal elements from the covariance matrix (σ). For a nonlinear regression, the covariance matrix of the adjusted parameter was calculated by the Jacobian matrix of the fitted parameters (J), its transpose (J^T) and the standard deviation of the residuals (sdr), as presented in Eq. (3).

$$\sigma = \left(J^T J\right)^{-1} sdr^2 \tag{3}$$

2.3. Iso-thermal treatments

The treatments were performed by immersing the heat-sealed plastic pouches containing the samples at different water activity values in a thermostatic water bath (Kiss E, Peter Huber Kältemaschinenbau SE, Offenburg, Germany). These treatments were carried out at different temperature (45 °C $\leq T \leq$ 60 °C) and time (5 min $\leq t \leq$ 7.5 h) combinations. Once the desired holding time at a given temperature was reached, the sample was immediately submerged in an iced-water bath and stored at 4 °C for less than 2 h until the enzyme assay.

2.4. Enzymatic activity assessment

α-Galactosidase activity was assayed by monitoring the initial rate of hydrolysis of *p*-nitrophenyl-*α*-galactopyranoside (PNP, Sigma-Aldrich, Buchs, Switzerland) as reported by Dey et al. (1983). The assay mixture consisted of 1 g of the sample at a selected water activity value, 20 mL of 0.1 mol/L sodium phosphate buffer (pH 6.5 at 25 °C) and 30 mL of $1.0 \bullet 10^{-3}$ mol/L PNP. This mixture was incubated at 30 °C for 10 min under magnetic stirring. To stop the reaction, 25 mL of $2.0 \bullet 10^{-1}$ mol/L sodium borate buffer (pH 9.8 at 25 °C) was added. The assay mixture was centrifuged at 2057 g for 5 min at 4 °C and then the supernatant was collected. The concentration of released *p*-nitrophenol was quantified at 405 nm using a spectrometer (UV-2450, Shimadzu, Kyoto, Japan). One katal of *α*-galactosidase activity (kat) is defined as the quantity of enzyme capable of hydrolysing 1 mol of PNP per second at the given conditions. The measurement of enzymatic activity was repeated twice for each assay mixture. The inactivation of α-galactosidase (Eq. (4)) is expressed as relative enzyme activity (A_R):

$$A_R = \frac{A_t}{A_0} \tag{4}$$

where A_t is the enzyme activity at time t (nkat/g d.b.) and A_0 is the initial activity at time zero (i.e. before the thermal treatment) ($\overline{A_0} = 48 \pm 8.3$ nkat/g d.b.).

2.5. Mathematical modelling of α-galactosidase inactivation

The simplest mechanism of enzymatic inactivation (see scheme (I)) was assumed, disregarding intermediates, thus implying an apparent first-order reaction with a rate constant (k) proportional to the relative

enzyme activity (A_R) (Van Boekel, 2008).

$$\text{Native enzyme} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{Partly denatured enzyme} \xrightarrow{k} \text{Irreversibly denatured enzyme (I)}$$

The first-order differential equation (Eq. (5a)) and its explicit form (Eq. (5b)) used to describe enzyme inactivation at a constant temperature and a given water activity are introduced, as follows:

$$\frac{dA_R}{dt} = -kA_R \tag{5a}$$

$$A_R = \exp(-kt) \tag{5b}$$

with the initial condition:

$$A_R = 1.0 \text{ at time } t = 0 \tag{5c}$$

The thermodynamics of α-galactosidase inactivation was described according to the Arrhenius model (Eq. (6a)). Additionally, the influence of moisture content on the inactivation rate was depicted using a symmetrical sigmoidal equation (Eq. (6b)) and its effect on the energy of activation was characterized by a linear equation (Eq. (6c)).

$$k = k_{Tref,W} \exp\left(-\frac{Ea_W}{R}\left(\frac{1}{T} - \frac{1}{Tref}\right)\right) \tag{6a}$$

$$k_{Tref,W} = k_{Tref,0} - \frac{k_{Tref,0} - k_{Tref,sat}}{1 + \left(\frac{W}{b}\right)^a} \tag{6b}$$

$$Ea_W = a'W + b' \tag{6c}$$

where $k_{Tref,W}$ is the inactivation rate constant (1/s) at a given temperature (T_{ref}) and moisture content (W), Ea_W is the energy of activation (J/mol), R is the universal gas constant (8.314 J/mol K), W is the moisture content (g/g d.b.), $k_{Tref,0}$ is the inactivation rate constant at initial moisture content of (0.13 ± 0.01) g/g d.b (1/s), $k_{Tref,sat}$ is the $k_{Tref,W}$ at water saturation (1/s), and a (−), b (g/g d.b), a' (J g d.b./mol g) and b' (J/mol) are the fitted empirical parameters.

The identification of the parameters was carried out in two steps. First, the kinetic parameters ($k_{Tref,W}$ and Ea_W) were estimated by performing nonlinear regression analysis on the relative enzyme activity obtained at different moisture contents. These parameters were identified by minimizing the objective function (Sum of the Squared Errors, *SSE*) using "fminsearch" from MATLAB software (R2020b, MathWorks, Natick, USA). The confidence bounds (Eqs. (2) and (3)) for the fitted parameters ($k_{Tref,W}$ and Ea_W) were calculated in MATLAB (R2020b, MathWorks, Natick, USA). Second, the parameters of the sigmoidal function (Eq. (6b)) and linear equation (Eq. (6c)) were then estimated based on the inactivation rate constants ($k_{Tref,W}$) and energy of activation values (Ea_W) that were previously fitted in the first step. The parameters from Eqs. (6b) and (6c) were identified using the curve fitting toolbox of MATLAB software (R2020b, MathWorks, Natick, USA).

3. Results and discussion

The relative activity of α-galactosidase for cowpea powder at different moisture contents (W = 0.16, 0.25, 0.35, 0.85 g/g d.b.) after treatment at various temperatures (T = 45, 50, 55 and 60 °C) is presented in Fig. 1, illustrating the influence of moisture content and temperature on enzymatic inactivation. The higher the temperature, the faster the inactivation of α-galactosidase at moisture contents ranging from 0.25 g/g d.b. to 0.85 g/g d.b. At a moisture content of 0.16 g/g d.b, the relative α-galactosidase activity was notably higher and the inactivation rate lower than at other moisture contents and was temperature-independent.

The α-galactosidase found in cowpea beans is characterized either in three isoforms (I, II[1] and II[2]) (Alani et al., 1989) or two isoforms (I and II) (Coffigniez et al., 2018) with different pH optima at 30 °C (pH 3–4 and 6–7). The enzymatic activity in this study was evaluated at neutral pH (pH 6.5), which corresponds to the standard practice of soaking dried beans in tap water. It was thus assumed that a first-order model adequately represented the inactivation kinetics of α-galactosidase (isoform II) ($0.83 \leq R^2 \leq 0.97$). The iso-thermal curves in Fig. 1 were

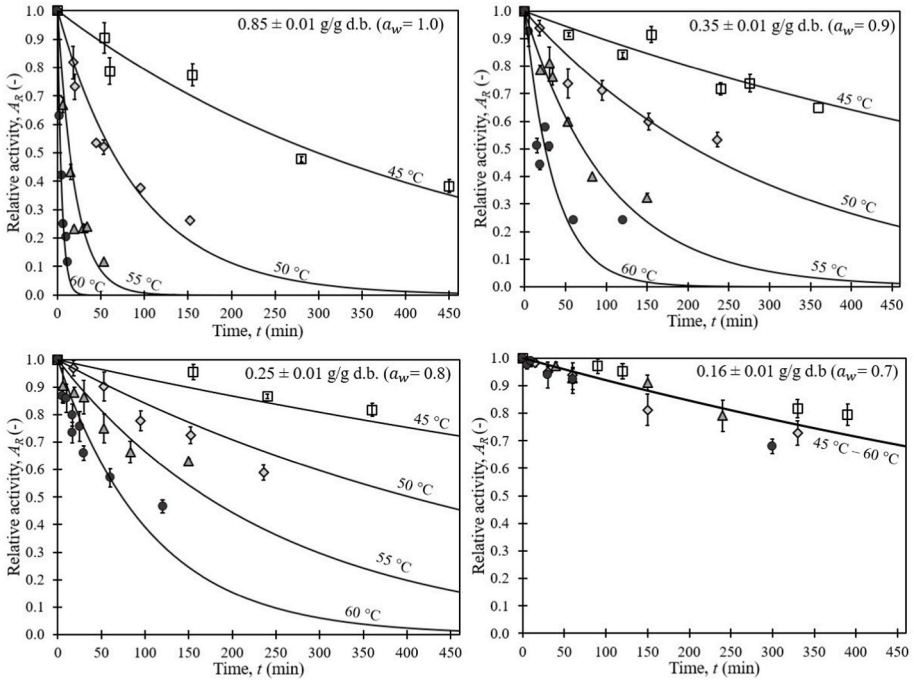

Fig. 1. Thermal inactivation of α-galactosidase of cowpea bean powder as a function of exposure time, temperature and moisture content. Data markers represent the observed relative activities of α-galactosidase (A_R) from two technical repetition of the same enzymatic assay mixture. Solid lines represent the iso-thermal inactivation curves (Eqs. (5b) and (6a)) with the adjusted parameters from Table 1.

calculated based on the kinetic parameters identified, which are summarized in Table 1. The temperature dependence of α-galactosidase inactivation rate ($k_{Tref,W}$) at a constant moisture content was described by the Arrhenius model (Eq. (6a)), resulting in activation energy values (Ea_W) between 152 kJ/mol and 264 kJ/mol. In their investigation of the thermal inactivation of α-galactosidase in heat-treated cowpea beans extract, Coffigniez et al. (2018) report an inactivation energy (103 kJ/mol) and an inactivation rate constant (0.09 min^{-1} at 56.6 °C) of the same magnitude as the values found in the present study.

The difference between the kinetic parameters as a function of moisture content shown in Table 1 is remarkable. For example, the inactivation rate constant ($k_{Tref,W}$) at 0.85 g/g d.b. increased 5.1-fold and the activation energy (Ea_W) increased 1.4-fold compared to the limited water condition (0.35 g/g d.b.), highlighting the dependence of the enzymatic inactivation rate on moisture content. Increased water content noticeably reduced the thermal stability of α-galactosidase, resembling the inactivation of lipase (Luyben, Liou, & Bruin, 1982) and β-galactosidase (Perdana, Fox, Schutyser, & Boom, 2012). The influence of moisture content (W) on the rate of enzymatic inactivation ($k_{Tref,W}$) at different temperatures is presented in Fig. 2, corresponding to a symmetrical sigmoidal function (Eq. (7)).

$$k_{Tref,W} = 1.40 \bullet 10^{-5} - \frac{1.40 \bullet 10^{-5} - k_{Tref,sat}}{1 + \left(\frac{W}{4.55 \bullet 10^{-1}}\right)^{5.79}} \quad (7)$$

where the $k_{Tref,0}$ was 1.40 • 10^{-5} s^{-1}, the steepness of the curve (a) was 5.79, the point of inflection (b) was 4.55 • 10^{-1} g/g d.b. and the $k_{Tref,sat}$ at 45 °C, 50 °C, 55 °C and 60 °C were 3.87 • 10^{-5} s^{-1}, 1.82 • 10^{-4} s^{-1}, 8.14 • 10^{-4} s^{-1} and 3.48 • 10^{-3} s^{-1}, respectively. The impact of moisture content (W, g/g d.b.) on energy of activation (Ea_W, J/mol) is given by Eq. (8).

$$Ea_W = 1.75 \bullet 10^5 W + 1.16 \bullet 10^5, R^2 = 0.971 \quad (8)$$

As discussed by Rahman and Labuza (2007), the rates of most chemical reactions, such as microbial activity, lipid oxidation, nonenzymatic browning and enzymatic activity, are affected by water activity of food. Enzyme activity generally increases as water activity rises. One hypothesis is that the enzyme activity depends on water content to dissolve the substrate and promote its diffusion into the enzyme's active site. The literature has reported the complexity of the role of water in enzyme-catalysed reactions, including changes in molecular mobility and solute-enzyme interactions (Bell, 2020; Rahman & Labuza, 2007).

In the same line of thought, it seems logical that cowpea α-galactosidase inactivation was intricately dependent on moisture dynamics. This influence can result in different local concentrations of enzyme activity in the pulse during hydrothermal processing. A better understanding of α-galactosidase activity, particularly concerning the spatial-temporal distribution of enzyme/substrate in the pulse, would facilitate efficient process design to achieve a final target concentration of α-galactosides. This, in turn, would maintain the prebiotic effect in the intestine and avoid intestinal discomforts (Martínez-Villaluenga, Frias, & Vidal-Valverde, 2008).

4. Conclusion

In this study, the inactivation kinetics of the cowpea α-galactosidase were well described by the first-order model at different temperature and moisture conditions. Predicted isothermal curves presented a sigmoidal dependence of inactivation rate on moisture content, while the dependency of reaction rate on temperature followed the Arrhenius equation. These findings showed that the water activity played a pivotal role in limiting the rate of α-galactosidase inactivation in cowpea powder, and hence, provided insight into the implications for the representation of the diffusion-reaction phenomena in pulses during hydrothermal processing, as well as underlining the importance of storing dried pulses in conditions that prevent α-galactosidase inactivation.

Table 1
Adjusted kinetic parameters ($k_{Tref,W}$ and Ea_W) for α-galactosidase inactivation in a cowpea-water system at different moisture contents (W) and water activity values (a_w) at a reference temperature (T_{ref}) of 55 °C.

W(g/g d.b)	a_W at 20 °C (−)	$k_{Tref,W}$• 10^5 (1/s)	Ea_W• 10^{-3} (J/mol)	SSE• 10^2 (−)	R^2 (−)
0.16	0.7	1.72 ± 0.23	N.A.	3.69	0.829
0.25	0.8	6.90 ± 0.81	149 ± 20.7	10.6	0.869
0.35	0.9	16.1 ± 1.35	188 ± 14.2	16.6	0.915
0.85	1.0	81.4 ± 6.39	264 ± 11.9	9.90	0.965

Parameters values are presented as mean ± standard error at 95 % confidence level (40 ≤ degrees of freedom ≤ 44). N.A.: not applicable. *SSE*: Sum of the Squared Errors. R^2: coefficient of determination.

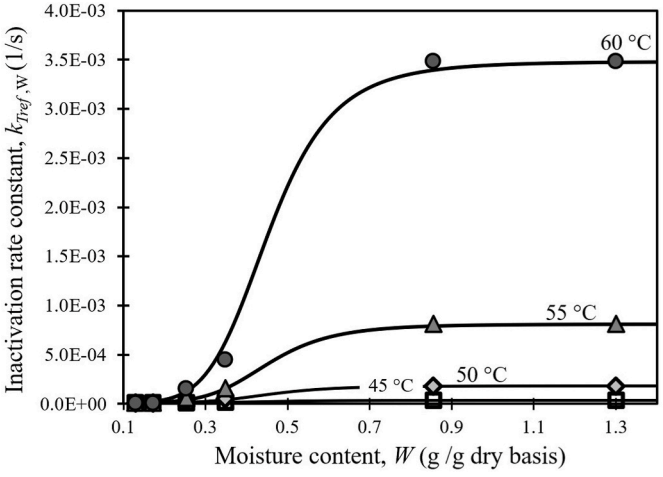

Fig. 2. Influence of moisture content (W) on the inactivation rate constant ($k_{Tref,W}$) at different temperatures. Data markers represent the identified inactivation rate constants at a given reference temperature and moisture content. Solid lines are sigmoidal curves fit from Eq. (7).

Funding sources

This study received the financial support from Agropolis Foundation (project #2102-001).

CRediT authorship contribution statement

Erica Siguemoto: Investigation, Methodology, Formal analysis, Writing – original draft. **Lamiae Atmani:** Investigation, Formal analysis. **Christian Mestres:** Supervision, Methodology, Formal analysis, Writing – review & editing. **Jean-Michel Meot:** Conceptualization, Supervision, Methodology, Formal analysis, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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