INTRODUCTION

During the past years, many food products containing sprouted grains appeared on the market. Consequently, the use of sprouted grain to produce bread, pasta, breakfast cereals, biscuits, and porridge was studied and discussed (Richter, Christiansen, & Guo, 2014; Singkhornart, Gu, & Ryu, 2013). In order to achieve this desired incorporation in a controlled manner, it is important to understand how the raw material changes during the sprouting process. This is necessary to understand the possible consequences of the incorporation of sprouted grains for final product properties.

The term sprouted grains is used to refer to germinated grains with radicles and coleoptile with a length greater than that of the most important reference product, the green malt in malting for brewing purpose. The coleoptile of green malt is only allowed to grow to a maximum of two-thirds of the grain length under controlled conditions. In contrast, during sprouting of grains further growth of the seedling is tolerated up to the onset of the photosynthetic metabolic activity.
Sprouting, hence further progressed germination, gives rise to significant metabolic changes. This offers the opportunity to produce sprouted grains according to desired objectives, such as improved nutritional profiles.

Basically, during sprouting the embryo generates a new plant by metabolizing carbohydrates, allowing the growth of the radicles and coleoptile (Kunze, 2011). Therefore, hydrodrolases, for example, amylases and proteinases, are secreted from the aleurone layer into the endosperm of the grain. As a consequence, starch and proteins are degraded in the endosperm into transportable sugars, for example, glucose, peptides, and amino acids. In the embryo, these substances are transported into the growing regions for the synthesis of a first leaf and radicles (Bewley, 2001). Sprouting only takes place at a sufficiently high moisture content (>30%), at beneficial temperatures, and under aerobic conditions. Controlling these conditions, the biological processes in the grain can be directly affected (Narziss & Back, 2012).

It is well established that the sprouting process positively affects the nutritional value of the grains. This fact is used in different technical applications, for example, the hydrothermal activation of grains for the bread production (DE3038463A1, 1980). The activation of the grain results in an enhancement of the vitamin and mineral content in the bread. Moreover, the taste of the bread is improved.

A significant increase in the vitamin content in grains due to sprouting was also reported in many other studies (Harmuth-Hoene, Bognar, Kornemann, & Diehl, 1987; Yang, Basu, & Ooraikul, 2001; Žilić et al., 2014). Tian et al. (2010) found that, next to vitamins, also the total polyphenol content in oat increased by 100% after 3 days of spraying at 16°C. Xu et al. (2009) also studied the changes in the phenolic acids after different steeping and spraying times of huskless oat. They found a 60% increase in the total phenol content after 2 days of spraying at 16°C. Due to physiological changes during spraying, stress is exerted on the grain and the redox equilibrium is disturbed. This way, the formation of secondary metabolites like antioxidants such as phenolics and vitamins is stimulated to protect the seedling (Świeca & Dziki, 2015). It was stated that sprayed grains with increased levels of antioxidants can be applied in products to suppress rancidity and color changes (Xu et al., 2009).

An additional benefit of the sprouting process is the reduction of the phytic acid content (Tian et al., 2010). Since phytic acid hampers the bioavailability of minerals, its content in bread is typically reduced during the sourdough leavening process (Schuchmann & Schuchmann, 2012). In product concepts not suited for sourdough processing, the usage of flour from sprayed grains could be a means to reduce the level of phytic acid.

The sprouting process has, however, not only positive effects. During the spraying process, cell wall material, especially β-glucan, a soluble fiber with health benefits, is degraded as well. β-glucan increases the viscosity in the intestine and causes a retarded absorption of glucose and hence reduced surges of the blood sugar level (Anttila, Sontag-Strohm, & Salovaara, 2004).

Wood et al. (1994) studied the acid hydrolysis of oat gum drinks for 15 and 60 min. The acid-hydrolyzed drinks and the reference had the same β-glucan concentration but differed in viscosities and showed different glucose responses. The strong correlation between glucose response and reduced viscosities found indicates that not only the total β-glucan concentration but also the molecular weight distribution of β-glucan is important.

Wilhelmson et al. (2001) studied degradation of β-glucan during spraying of oat grains as a function of temperature and time depending. The β-glucan content was most reduced, by 75%, after 3 days of spraying at 25°C. Under the same conditions, the average molecular weight reduced by 38% compared to the initial value.

Another problem arising during spraying is microbiological activity. The usual spraying conditions, such as long steeping stages, high moisture contents, and possible temperatures of 25–30°C, benefit the growth of microorganisms and can result in unwanted fermentation processes. Consequently, bacteria, mold, and yeast growths were observed during spraying and dormant spores might be activated as well (Helland, Wicklund, & Narvhus, 2002; Wilhelmson et al., 2001).

Tian et al. (2010) studied the length of the coleoptile and radicles of 30 sprayed oat kernels by ruler and did not find any correlation with other grain characteristics except for color changes. The work presented here documents an attempt to systematically relate the changes in the composition of sprayed grains to the progression of the spraying process. Grains were analyzed after spraying for defined time-temperature combinations. In order to quantify the degree of spraying, a new method based on the length of the coleoptile was developed and employed in an effort to correlate progress of the spraying process and composition. The targeted simple approach to categorize the sprayed material would have the advantage to define and standardize specifications for sprayed material, which are directly verifiable and yet ensure a successful product application.

2 MATERIALS AND METHODS

The huskless oat “Gehl,” cultivated in 2016 in Canada, was used throughout the study. The grains were stored in sealable containers at 10°C.

During preliminary studies, different methods to evaluate the spraying progress were tested. Finally, the grains were sprayed at different temperatures (10, 14, 20, 25, and 30°C) and for different times (1, 2, and 3 days). A temperature of
14°C is applied because this setting is usually used in the malting step for brewery purposes (Jacob, 2016). During the sprouting process, the changes in the content of respectively vitamin C, β‐glucan, and reducing sugar were monitored. Additionally, the α‐amylase activity was studied as a marker for the total enzyme activity.

2.1 Standard sprouting process at laboratory scale

For the steeping and sprouting process, 500 g of oat grains was washed for 30 min under running tap water in order to clean the grain surface from microorganism to minimize microbiological growth. Afterward, the grains were steeped in closed containers filled with water (so that all grains were covered with water): 4.5‐hr wet steeping, 19‐hr air rest, and 4‐hr steeping, all at 20°C (Jacob, 2016). After the steeping step, the grains were drained and put on a metal sheet. The steeped grains were covered with cling film and put in a climate cabinet (Lovibond 220P‐02) in the dark. During the sprouting step, the grains were washed once a day using a sieve. Due to the washing process, the water content was kept constant and checked by using the moisture analyzer MA35 (Sartorius).

At the end of the different sprouting periods, the grains were deep‐frozen (Siemens Comfort Plus, −20°C) and subsequently freeze‐dried (Beta 1‐16—Christ) for approximately 60 hr until a final moisture content between 4% and 8% was reached.

Prior to analysis, the oat grains were ground in a speed rotor mill (Pulverisette 14—Fritsch) with a sieve ring of 0.5 mm at 3220 g.

2.2 Study of different methods to characterize grain growth progress

By use of visual and gravimetric measurements using different sprouted oat material, an attempt was made to find an easy systematic characterization method for the quantification of the progression of the sprouting process.

For the different samples, the 1,000 kernel weight was determined gravimetrically by counting 100 kernels and multiplying the result by 10. The dry matter was analyzed using the moisture analyzer MA35 (Sartorius). Determinations were done in duplicate.

The mass percentage of the radicles and coleoptile of the total grain was determined by cutting off radicles and coleoptile with a dissecting needle and weighing the undried grain with and without the radicles and coleoptile. The determination was performed in duplicate.

The degree of sprouting was determined by visually classifying the length of the coleoptile and radicles of 100 kernels by dividing them into the six categories shown in Figure 1. The determination was done in triplicate. On each day of sprouting, an average degree of sprouting was calculated as the sum of relative occurrence of the different classes (DoS) multiplied by its respective degree of sprouting (i):

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\text{Average DoS} = \sum_{i=0}^{5} i \cdot \text{relative occurrence (DoS)}
\]  

2.3 Determination of the α- and β-amylase activity

The α- and β-amylase activities were determined by using the Megazyme (2012) Malt‐Amylase assay procedure K-MALTA 05/15. Determinations were done in duplicate.

2.4 Determination of the ascorbic acid content

The vitamin C content was determined according to the Indophenol Method (Nielsen, 2003). For the determination of the vitamin C content in flour, a modified dilution factor of eight had to be applied. The sprouted flour sample was dispersed in a mixture of 30 g/L metaphosphoric acid and 8% (v/v) acetic acid and centrifuged at 3,000 g. A volumetric sample of the resulting supernatant was diluted and used for titration according to the method. The procedure was executed in duplicate per specimen.

2.5 Determination of the reducing sugar content

The reducing sugar content was determined using DNS (3,5 dinitrosalicylic acid) in combination with a colorimetric
analysis. Determinations were done in duplicate. In detail, the sprouted flour samples (1 g) were mixed with 5 ml of a mixture of 40% (v/v) ethanol and 60% (v/v) of a 10 mM copper chloride solution to extract the reducing sugars. After shaking the mixture for 10 min, the suspension was centrifuged for 15 min at 3,000 g (Heraeus, Labofuge 200). This procedure was repeated twice. The collective supernatant, three subsequent extractions of the same material, were diluted and filled to 25 ml with the above-mentioned ethanol/copper chloride solution mixture. By addition of 0.625 g polyvinylpolypyrrolidone to the solution, all phenols present were precipitated. The precipitate was filtered off (Tian et al., 2010), and 1 ml of the filtrate was mixed with 1 ml of an aqueous solution containing 10 g/L DNS, 16 g/L sodium bicarbonate, and 300 g/L potassium sodium tartrate. After shaking this mixture for 10 min at 100°C, the solution was diluted with 5 ml distilled water. The reducing sugar content was determined spectrophotometrically (Spekol 1300—Analytik jena) at 545 nm. For the calibration, different concentrations of maltose monohydrate were used. The reducing sugar content determination is thus related to the reducing potential of maltose. However, glucose exhibits a reducing potential, which is identical to maltose per molecule.

### 2.6 | Determination of β-glucan content

The β-glucan contents of the differently sprouted oat samples were determined by using the Megazyme (2017) Mixed-Linkage Beta-Glucan assay procedure (McCleary Method—K-BGLU 02/17; AACC Method 32-23.01). Determinations were done in duplicate.

### 2.7 | Statistical evaluation (ANOVA)

The statistical evaluation of the methods tested to evaluate the sprouting process, the temperature and time effect on selected grain properties, and the dependence of selected grain properties and the degree of sprouting was performed using Microsoft Excel 2016. The p-value (probability of error) was calculated by analyzing the experimental data by means of ANOVA (analysis of variance) using a single-factor variance analysis. A level of significance (α) of .05 was chosen.

The significance of the temperature effect was analyzed by using the data of the 3-day sprouted samples, and the significance of the temperature effect was analyzed by comparing the data of the samples sprouted at 20°C.

### 3 | RESULTS AND DISCUSSION

#### 3.1 | Statistical evaluation of experimental data

Table 1 presents the p-values of all properties considered. These were calculated as part of the ANOVA. Since all p-values are lower than the level of significance, the found difference can be considered significant.

#### 3.2 | Evaluation of different methods to characterize the sprouting progress

Different properties of the grains were evaluated for their usability to characterize the progress of the sprouting process. This evaluation of the different approaches was done based on the β-glucan content as an output property. This marker was chosen because of its importance due to health-promoting and functional property effects (Choi et al., 2012). A first approach to characterize the progress of the sprouting process is based on the visual inspection of the lengths of the coleoptile and radicles.

In Figure 1, the definition of the degree of sprouting as used in this study to identify the sprouting progress is shown and the different development stages of the oat grains during the sprouting process can be seen. The length of the coleoptile was selected as a criterion of the categorization of the degree of sprouting.
Grains of degree 0 do not show any radicle or coleoptile growth. Degree 1 characterizes grains with visible embryos (small white point), while the radicles and coleoptile are not visible. Degree 2 describes grains already showing a developed embryo emerging from the seed coat. The grains of degree 3 reveal coleoptile lengths of at least half the oat grain length. Degree 4 represents coleoptile lengths between half and a full grain length. Under degree 5, grains with a coleoptile longer than a full grain length are summed up.

Alternatively, to the degree of sprouting it is conceivable to determine the 1,000 kernel weight (dry matter based) to characterize differently sprouted grains. Another alternative to describe the progress of the sprouting process is the determination of the weight fraction of both the radicle and coleoptile of the full grain.

In Figure 2, the β-glucan content is shown as a function of three possible properties to characterize the progression of the sprouting process, respectively.

From the properties evaluated, the 1,000 kernel weight corresponds to the least with the β-glucan content ($R^2 = .63$). Both other methods show a high degree of correlation: degree of sprouting ($R^2 = .98$) and weight fraction of coleoptile and radicles ($R^2 = .90$). Even though both correlations with the β-glucan content were found to be very good, only the degree of sprouting was considered for further consideration. Reason to do so is the simplicity of the procedure, which renders it less prone to errors. An obvious downside is the integer nature of this parameter. However, the “degree of sprouting” method was used as lead parameter to correlate with changes in grain properties due to sprouting.

### Influence of sprouting temperature and time

The degree of sprouting as defined above for subsets of oats in a single sprouting process was used to quantify the oat sprouting process at different temperatures and for different periods of time. For each time–temperature combination for the sprouting process the grain population exhibited a specific distribution of the degree of sprouting.

The number fractions of the grains with different degrees of sprouting are shown in Figure 3. Here, the results obtained for the five different sprouting temperatures after 3 days of sprouting are depicted. From the distribution of degrees of sprouting within a sample, the average degree of sprouting can be derived according to Equation 1. These values are represented in Figure 3 by diamonds. The error bars are based on counting three independent samples from one sprouting experiment. For the 3-day sprouting period, the longest coleoptile was observed for sprouting at 25°C. Sprouting at a temperature of 20°C resulted in less vigorous sprouting. At 30°C, the oat grains did practically not show any radicle growth.

The data gathered also allowed to determine the germinability. The germinability is defined as the percentage of grains reaching a degree of sprouting above 0. For all temperatures investigated, the germinability after 3 days was about 99%.

Figure 3 also reveals that around 20% of the grains which were sprouted at 20 and 25°C have a coleoptile longer than a full grain length (degree of sprouting 5). Less long coleoptiles developed at the other sprouting temperatures studied.

No standard deviation of the average degree of sprouting is given in Figure 3, because the chart already illustrates the contribution of different degree of sprouting within the sample to the average degree of sprouting. This illustrates how homogeneously a sample had been sprouted. The consideration of the homogeneity is an important point with respect to a large-scale sprouting operation. A narrow distribution of the degree of sprouting within a production run would allow for better control of product properties.

The data gathered reveal that a high average degree of sprouting (e.g., 20 & 25°C) corresponds to a high standard deviation and thus a low homogeneity. Vice versa, processes with a low average degree of sprouting (e.g., sprouting at 30°C—average degree of sprouting 1.4) do not show a high variation and are rather homogeneous. This was also found for the standard malting temperature of 14°C. The choice for this temperature is probably motivated by low risk for microbiological spoilage, homogeneous radicle growth and limited losses due to radicle and coleoptile growth.

In Figure 4, the effect of the sprouting time and temperature on the average degree of sprouting is shown. One can see that the sprouting started fastest for the oat which sprouted at 20°C. The data indicate linear increase in the degree of
sprouting with time for the samples sprouted at 10, 20 and 25°C.

3.3.1 | Effect of sprouting time and temperature on oat properties

In Figure 5a–d, the effect of the sprouting temperature and time on the different quality parameters of the sprouted grains is illustrated.

In Figure 5a, the results of the α-amylase analysis are shown. The data reveal that after 1 day, the α-amylase activities between the different temperatures did not differ too much. After 3 days however, the α-amylase activities at 20 and 25°C increased significantly to values one order of magnitude larger than those for the other temperatures (10, 14, and 30°C).

In contrast to the increase in α-amylase activity during the sprouting process, the analysis of β-amylase activity revealed no changes (data not shown) and the enzyme was apparently not synthesized de novo.

In line with the data for the α-amylase activities, the content of reducing sugars in oat increased most at sprouting temperatures of 20 and 25°C (Figure 5b). Expectedly, the limited α-amylase activity at 10, 14, and 30°C corresponded to only subtle increases in the reducing sugar contents. The limited data available suggest a linear relation between the levels of reducing sugars and the duration of sprouting.

As can be seen in Figure 5c, the β-glucan content is also a function of the temperature and duration of the sprouting process. At all sprouting temperatures studied, the β-glucan content was decreased after 3 days of sprouting. For a sprouting temperature of 20°C, the degradation is most pronounced, almost halving the initial β-glucan content to 3.9%. At sprouting temperatures of 10 and 14°C, the β-glucan content only slightly decreased, confirming the findings by Wilhelmson et al. (2001) that the β-glucan content decreases less at low sprouting temperatures.

In line with Lintschinger et al. (1997), vitamin C was chosen as a marker for the general vitamin content because of its high reactivity. No ascorbic acid was present in the native grain. Upon sprouting, a significant increase in the ascorbic acid content was found (Figure 5d). The highest levels were found when sprouting at 20°C. Except for the sprouting temperature of 30°C, all other sprouting temperatures also resulted in increased levels of ascorbic acid.

3.3.2 | Discussion of the effect of sprouting time and temperature on property changes in oat grains

The results gathered reveal a rather consistent picture, revealing relations between the different grain properties. It was shown that the different properties changed systematically with temperature and duration of the sprouting process.

During the sprouting process, a de novo synthesis of α-amylase in the oat grain was observed. This is most
pronounced at a sprouting temperature of 20°C. Since the starch degradation relates to the α-amylase activity, the increase in the amount of reducing sugars followed a corresponding pattern. The relationship of certain properties appears more complicated considering that sugars are transported into the growth regions of the grain for further development of the coleoptile and radicles. This suggests that the coleoptile and radicle growth (input parameters for the degree of sprouting) and the reducing sugars and α-amylase activity are interdependent.

The acceleration of the sprouting process on increasing sprouting temperatures was also found for barley by Müller (2015). Varying the sprouting temperature from 16 to 24°C resulted in a reduction of the sprouting time of about 24 hr per 4°C. It was further found that sprouting at 20°C yielded best results, which is in line with the results presented here.

Progression of the sprouting process also resulted in changes of the ascorbic acid levels. Ascorbic acid is needed as protective antioxidant in the growing grain. The increase in the vitamin C contents of oat during sprouting (see Figure 5) is a function of several processes. In order to terminate the dormancy and start the sprouting process, reactive oxygen species have to be released. However, the presence of these species results in an oxidative stress in the cells. Moreover, the exposure to light also causes stress in the grain inducing the synthesis of antioxidants, for example, ascorbic acid (Pitzschke, Fraundorfer, Guggemos, & Fuchs, 2015).

With respect to improve the nutritional value of sprouted oats, the increase in vitamins is desired while the degradation of beta-glucan is considered a downside (El Khoury, Cuda, Luhovyy, & Anderson, 2012). Consequently, optimizing the sprouting process for best nutritional values would involve balancing the levels of these two nutrients.

### 3.4 Study of oat having different degrees of sprouting

Oat samples of varying degrees of sprouting were studied. These grains were sprouted at 20°C for 3 days and sorted according to their degree of sprouting. In Figure 6, the data on reducing sugar content, ascorbic acid content, β-glucan content, and α-amylase activity in oat samples with each a homogeneous different degree of sprouting (1–5) are shown.

In this context, a homogeneous sample means that it compromises only grains with the respective degree of sprouting. The data reveal a systematic evolution of the α-amylase activity, ascorbic acid, β-glucan, and reducing sugar contents with increasing degree of sprouting. One could argue about the correct choice of the mathematical function to be fitted to the experimental data. It appears to be beyond the scope of this work to formulate a model to describe the changes. The choice of the function to be fitted to the data remains thus
arbitrary. Even though linear regressions would also allow to describe the data quite well, it was chosen to use simple exponential functions. This choice was motivated by the nature of the properties of the described reaction products. The exponential fits show a quite strong correlation between the response parameters as a function of the degree of sprouting. It has to be noted though that the degree of sprouting is not a transformed reaction time.

Increased amounts of reducing sugars and ascorbic acid were found particularly in the radicles and coleoptile (data not shown). The ascorbic acid content in the radicles and coleoptile was four times higher than that in the grain without the radicles and coleoptile. Hence, for the production of oat flour having a high nutritional value it is of special interest to leave radicles and coleoptile at the grains. The oat grains which were sprouted for 3 days at 20°C had an average degree of sprouting of 3 (Figure 3); hence, the radicles and coleoptile contribute about 8% of mass.

These findings indicate that a fast visual determination of the degree of sprouting allows to estimate, for example, the ascorbic acid content without doing expensive experiments. Moreover, the sweetness of the product can be estimated based on the correlation between the degree of sprouting and the reducing sugar content.
3.5 Evaluation of the suitability of the degree of sprouting

In order to evaluate whether or not the degree of sprouting could be of any practical value in estimating characteristic data of sprouted material, calculated data were compared to experimental data (see Figure 7).

A set of 15 samples (five different sprouting temperatures, three different sprouting durations) was used for the evaluation. It has to be pointed out that the correlations between properties of samples and degree of sprouting, as displayed in Figure 6, were based on homogeneous subsamples of the spraying process at 20°C. The 15 samples differing due to variation of the process conditions are each inhomogeneous (see Figures 3 and 4). Consequently, the training set for the correlations and the evaluation set are not only independent from one another, but also differ with respect to homogeneity. The content of ascorbic acid, β-glucan, and reducing sugars and α-amylase activity were calculated as function of the respective degree of sprouting based on the functions displayed in Figure 6. It goes without saying that for an exponential function averaging the argument of the function yields a different result than averaging the values. Hence, good predictions are only possible by averaging the properties over the different homogeneous fractions constituting an inhomogeneous sample. The use of the average degree of sprouting would systematically yield overpredictions as a function of the homogeneity of the samples.

The parity plots depicted in Figure 7 show that the functions derived from the data shown in Figure 6 yield reasonably good predictions for the different properties of the inhomogeneous samples. Each data point represents the grain population generated by a specific process setting. For each sample, the distribution of sprouted grains over the different degrees of sprouting was determined (Figure 3). Per degree of sprouting, the respective property was computed. The property per sample was subsequently derived by proportional contribution from the different degrees of sprouting within a sample.

In detail, the α-amylase activities and contents of reducing sugars were predicted quite well by the approach outlined. The level of ascorbic acid appears to be underpredicted systematically. This is also true for the prediction of the β-glucan levels. In this case, it has to be noted that the experimental values did only vary in a limited range between the different samples. However, it appears fair to summarize that Figure 7 documents that the concept of the degree of sprouting can be used to predict the properties of a sprouted sample, taken that averaging is done in an adequate way.

4 CONCLUSIONS

The effect of temperature and duration of the spraying process was systematically studied for oats. Process temperatures between 10 and 30°C were studied for a duration of up to 3 days. The resulting samples of sprouted oats were studied for their concentrations of β-glucan, ascorbic acid, and reducing sugars. Additionally, the α-amylase activity was determined. It was found that the composition of oat changed in a rather systematic pattern. The obvious interdependency of reducing sugar content and α-amylase activity was verified. The degradation process of β-glucan seemed to correlate with the degree of sprouting as well. This is only true for a lesser extent for the presence of ascorbic acid. This might be due to complex processes in the growth process during which ascorbic acid is formed and later consumed. Overall, it is found that for a process duration of 3 days, temperatures between 20 and 25°C yield the most significant changes in the properties of sprouted grains.

In order to simplify the categorization of samples of sprouted materials, a correlation of the compositional properties mentioned above to an easy applicable descriptor of a sprouted grain sample was sought. Initial assessment revealed that the 1,000 grain weight is insufficiently linked to quality parameters. The mass fraction of radicle and coleoptile in the grain correlated very well with the β-glucan level. A similarly good correlation was found for the much easier applicable degree of sprouting. This DoS is derived based on the visual assessment of coleoptile length set into relation to the grain size.

The degree of sprouting was assessed for various samples, and it was found that for different process settings, a typical distribution of the degree of sprouting within a sample existed. Correlations between the measured compositional properties and the degree of sprouting were derived from subsets of grains for a single process condition (20°C, 3 days). The sample of grains was subdivided into homogeneous subsamples with identical degree of sprouting. Based on these homogeneous samples, functions to calculate the grain properties as a function of the DoS were derived. These functions were used to predict the properties of inhomogeneous samples originating from different spraying process settings. The surprisingly good predictive power found indicates that the application of the concept of degree of sprouting could develop into a reliable characterization method for sprouted grains usable for predicting compositional and nutritional changes of oats during sprouting and ultimately leveraging this information for product development and specification.

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