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Microwear textures associated with experimental near-natural diets suggest that seeds and hard insect body parts cause high enamel surface complexity in small mammals

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In mammals, complex dental microwear textures (DMT) representing differently sized and shaped enamel lesions overlaying each other have traditionally been associated with the seeds and kernels in frugivorous diets, as well as with sclerotized insect cuticles. Recently, this notion has been challenged by field observations as well as *in vitro* experimental data. It remains unclear to what extent each food item contributes to the complexity level and is reflected by the surface texture of the respective tooth position along the molar tooth row. To clarify the potential of seeds and other abrasive dietary items to cause complex microwear textures, we conducted a controlled feeding experiment with rats. Six individual rats each received either a vegetable mix, a fruit mix, a seed mix, whole crickets, whole black soldier fly larvae, or whole day-old-chicks. These diets were subjected to material testing to obtain mechanical properties, such as Young's modulus, yield strength, and food hardness (as indicated by texture profile analysis [TPA] tests). Seeds and crickets caused the highest surface complexity. The fruit mix, seed mix, and crickets caused the deepest wear features. Moreover, several diets resulted in an increasing wear gradient from the first to the second molar, suggesting that increasing bite force along the tooth row affects dental wear in rats on these diets. Mechanical properties of the diets showed different

correlations with DMT obtained for the first and second molars. The first molar wear was mostly correlated with maximum TPA hardness, while the second molar wear was strongly correlated with maximum yield stress, mean TPA hardness, and maximum TPA hardness. This indicates a complex relationship between chewing mechanics, food mechanical properties, and observed DMT. Our results show that, in rats, seeds are the main cause of complex microwear textures but that hard insect body parts can also cause high complexity. However, the similarity in parameter values of surface textures resulting from seed and cricket consumption did not allow differentiation between these two diets in our experimental approach.

KEYWORDS

hard-object feeding, mechanical properties, dental wear, material properties, microwear

Introduction

The reconstruction of paleodiets is a central aspect of paleoecology. Diet provides insights not only to an animal's ecology but also, indirectly, to aspects of its habitat and predominant climatic conditions (e.g., closed vs. open, arid vs. humid) (e.g., Merceron et al., 2007a,b; Rivals et al., 2008) and on how these are linked to the evolution of different taxa (e.g., Teaford and Ungar, 2000). Thus, methods for paleodietary inference have always been of interest to the scientific community, but even more so when they allowed inferences about our ancestors and human evolution (e.g., Walker, 1981). However, when hominids are involved, not only is the interest but also the potential for debate high, as new results may challenge long-standing paradigms (Lee-Thorp, 2011). One important question is whether seeds played a prominent role as a key dietary component responsible for distinct morphological adaptations like thick enamel, enlarged, bunodont teeth, and complex microwear patterns. It applies to primates in general and hominids in particular.

Extant primates (Kay, 1981; Dumont, 1995; Shellis et al., 1998; Martin et al., 2003) and bats (Dumont, 1995) that incorporate a large proportion of so-called hard objects into their diet possess thick enamel. Hence, the presence of thick enamel has also been interpreted as an adaptation toward hard-object feeding in fossil species (e.g., Alba et al., 2010), including hominids (e.g., Martin et al., 2003). The relationship is, however, not straightforward, as thinner enamel may also be suitable for processing hard objects, as seen, for instance, in sea otters (Constantino et al., 2011). It seems that tooth crown morphology, enamel distribution, and enamel microstructure also need to be considered to predict the resistance of teeth during hard-object feeding (Constantino et al., 2011; Ungar, 2011; Schwartz et al., 2020).

Microwear and dental microwear texture (DMT) are often-applied “proxies” that are analyzed to infer the mechanical properties of the foods consumed (Calandra and Merceron, 2016). By directly observing the two-dimensional (pits and scratches) (Rivals et al., 2007; Semperebon and Rivals, 2007) or three-dimensional (roughness, complexity, anisotropy) patterns on enamel wear facets in extant species, inferences can be drawn about the dietary habits of extinct species (e.g., Scott et al., 2005; Ungar et al., 2008, 2010; DeSantis et al., 2012). One long-standing paradigm regarding specifically primate and hominid diets has been that hard object feeding (i.e., seeds and nuts) results in pitted enamel surfaces (Grine and Kay, 1988) with high complexity (Scott et al., 2005, 2012; Daegling et al., 2011).

However, there are cases when dental morphology and observed microwear features seem to be incongruent. The genus *Paranthropus* is an excellent example of how similar morphologies may have been used in different dietary specializations (Ungar et al., 2008; Sponheimer et al., 2022). There is an ongoing debate whether their enlarged molars with thick enamel caps were an adaptation for processing bulk low-quality forage (e.g., grasses). The similarity of the enlarged molars to extant hard object-feeders (e.g., otters, peccary) suggests that fracture resistance while processing nuts and seeds was the driving force behind the evolution of this particular tooth morphology [see Sponheimer et al. (2022) for a comprehensive review].

Both eastern (*Paranthropus boisei*) and southern (*Paranthropus robustus*) members of the genus *Paranthropus* possessed enlarged, bunodont molars with thick enamel caps, yet their reconstructed diet differs drastically. Dental microwear texture analysis (DMTA) (Ungar et al., 2008; Ungar, 2012) and isotope evidence (van der Merwe et al., 2008) suggest that *P. boisei* was primarily feeding on C₄/CAM plants (and/or meat of animals that consumed C₄ grasses), which is considered

tough but not hard food—an interpretation not easily reconciled with their thick enamel cusps (Sponheimer et al., 2022). By contrast, *P. robustus* is reconstructed as a hard-object feeder (Constantino et al., 2018), based on microwear and DMTA that reveal high complexity but low anisotropy values, both typically associated with hard-object feeding (Scott et al., 2005; Ungar et al., 2008; Ungar and Berger, 2018). Until recently, the diet of *P. robustus* was less subject to debate, as most studies (microwear, DMTA, tooth macrowear) suggested hard objects as an important dietary component. When talking about hard objects in an herbivorous diet, we usually imply seeds and nuts (Daegling et al., 2011; Ungar and Sponheimer, 2011).

Recently, this notion has been challenged by an experimental *in vitro* study by van Casteren et al. (2020). *In vitro*, they brought endocarps into contact with isolated human teeth and found that the endocarp *per se* was not able to cause deep lesions but only rubbing (plastic deformation) of the enamel. Thus, they concluded that seeds could not be the source of the high complexity in surface textures that were conventionally associated with hard-object feeding. Moreover, they suggested that this would bring seeds back on the table as a possible diet for *Paranthropus boisei*, as the lack of complexity in microwear textures would no longer be indicative of an absence of seeds in the diet. This is not the first debate that has arisen in the dental wear community. Similarly, the discussion of whether phytoliths (amorphous silica bodies inside the plant tissue) or external grit and mineral dust are more responsible for abrasive wear has been a long one as controlled feeding experiments provided abundant evidence for the importance of both as dietary abrasives (Merceron et al., 2016; Winkler et al., 2019a, 2020a; Schulz-Kornas et al., 2020a). Similarly, controlled feeding experiments demonstrated that hard-object (nut) feeding results in new dental microwear features after only one feeding bout in capuchin monkeys (Teaford et al., 2020), and the inclusion of seeds or nuts into otherwise identical diets results in distinct DMT in pigs (Louail et al., 2021), but without resolving whether high complexity is linked to seed consumption.

In the present study, we conducted a feeding experiment with rats to better determine which naturally ingested animal and plant diets can produce high complexity (in absence of external abrasives). We chose rats because they are omnivorous and can deal with various diets. Rat molar teeth have served as models for human molars in numerous studies, although evident differences in masticatory behavior and dental morphology are acknowledged (Ohba, 1974; Nishijima et al., 2007, 2009; Dammaschke, 2010). Rats cannot serve as a model organism for replication of the *Paranthropus* masticatory behavior. What rats can do, however, is to show what kind of diets do result in more complex microwear textures that are traditionally associated with herbivorous hard-object feeding.

Diets of omnivores are naturally diverse and complex in composition and are thus likely diverse in mechanical

properties, and we did not aim at mimicking the natural diet of either rats or primates. Rather, we aimed to design experimental diets that resemble a specialized feeding type. Thus, we used several herbivore and faunivore diets with different components that remain constant over the duration of the experiment: a vegetable mix, a fruit mix (whole fruits with seeds), a seed mix (legume, C₃ and C₄ grass, and sunflower seeds), whole house crickets (hereafter: crickets), whole black soldier fly larvae (hereafter: BSFL) and whole day-old chicks (hereafter: daychicks). We subjected these diets to tests of mechanical properties that can be obtained through compression tests to relate them to the observed DMT. The mechanical properties we focused on were as follows: Young's modulus (*E*), yield stress, and texture profile analysis (TPA) hardness, because they are well-represented in biomechanical anthropological studies (Young's modulus, yield stress) (e.g., Lucas et al., 2009; Thompson et al., 2014; Berthaume, 2016) or food texture studies (TPA hardness) (e.g., Nishinari et al., 2019). In addition, they can be obtained with a relatively simple analytical setup. We note that this approach is simplified and does not cover other commonly used measures of mechanical properties (e.g., obtained through wedge or scissor tests) or potential cuticle hardness in insects (Evans and Sanson, 2005) and have discussed the benefits and limitations.

In a previous study on guinea pigs, we found that, for hard and brittle, pelleted diets, a DMTA gradient indicating increasing wear from front to rear along the molar tooth row could be observed (Winkler et al., 2021), while natural plant diets displayed no such gradient or even showed an opposite trend of decreasing wear from the fourth premolar toward the third molar. As the rats in the present study were subjected to diets of very different mechanical properties, we were interested to see if they would also show tooth position-specific differences that reflected specific mastication behavior on different diets. We thus compared DMTA not only between diet groups but also between the first and second upper molars within each diet group.

Materials and methods

The feeding experiment was approved by the Swiss Cantonal Animal Care and Use Committee Zurich (animal experiment license No. ZH135/16) and was conducted during February/March 2018 at the Vetsuisse Faculty, University of Zurich. All animals in this experiment were female adult WISTAR (RjHan:WI) rats (*Rattus norvegicus* forma domestica; *n* = 36; initial body mass = 216.5 ± 16.5 g; initial age 12–14 weeks). Rats were kept in groups of 3 in indoor stables (0.58 m² each), each equipped with one or two large food dishes containing their assigned experimental diet and two nipple drinkers of tap water. Stables were enriched with dust-free softwood granulate, a shelter, a climbing frame, two tubes,

and a running plate (Frei et al., 2021). Two groups (i.e., 6 animals in total) received the same diet for 37 consecutive days. Initially, the breeder diet was provided in addition to the experimental diet and phased out until the 5th day of the experiment. Following the conclusion of the experiment, animals were euthanized with carbon dioxide and enzymatic maceration of the skulls was conducted at LIB Hamburg [former Center of Natural History (CeNak) of the University of Hamburg]. For the vegetable and fruit groups, one specimen each lost its label during maceration and could not be assigned without uncertainty to the correct diet group. Thus, the groups of vegetable and fruit eaters are only comprised of five individuals each.

Experimental diets

The six experimental diets consisted of a vegetable mix, a fruit mix, a seed mix, crickets, black soldier fly larvae, and day-old chicks. Weight percentages of each main dietary component are given in [Table 1](#). Diets were designed to be isocaloric and to contain all nutrients to sustain healthy growth in a 250 g rat. Rats received their designated diets one time per day in the morning. Each diet was supplemented by a specifically balanced supplement powder that was admixed to a quark (a type of dairy product, also known as curd or curd cheese) or a quark and sunflower oil base ([Table 1](#)), which were chosen as additional protein and fat supply that could not be chewed but had to be lapped up by the animals. The composition of each supplement powder is given in the [Supplementary Table 1](#).

Mechanical properties testing and limitations

Several diets included a mixture of food items, with likely different mechanical properties. Thus, rats could theoretically choose foods that they preferred over foods they disliked. Such behavior was observed in the group receiving a vegetable mix, where several rats would avoid eating root celery (pers. observation DEW). The groups receiving crickets and black soldier fly larvae had no such choice, except for avoiding eating body parts of the crickets (e.g., legs). Similarly, the daychick group could selectively feed on certain parts of the whole daychicks, but only left the beak and parts of the integument with feathers (pers. observation DEW). In the groups receiving fruit mixture and seed mixture, no preferences were observed, as food was consumed completely. However, we cannot rule out that individual rats had specific preferences and avoided specific items, while other rats consumed them. Therefore, we expect more variability of DMT parameters in groups that received a mixture of food items (vegetables, fruits, and seeds) as compared to more homogenous diets (crickets, BSFL, and daychicks).

Although the behavior and individual preferences of the rats could likely affect the items they consumed (and thus the tooth wear they developed), we conducted compression tests to derive quantitative mechanical properties of the items. Through the compression tests, we obtained stress-strain curves from which representative mechanical properties, i.e., Young's modulus, yield stress, and TPA hardness, were calculated. Due to limitations in the preparation of analyzed samples, these three mechanical properties could not be obtained simultaneously for all items but had to be derived from linear regressions with other highly correlated mechanical properties (see Statistics section).

For obtaining Young's modulus and yield stress, samples of fruit and vegetables were cut into rectangular, circular, or cubic pieces; seeds were tested as whole grains and insects as whole specimens. In crickets, we additionally tested the head capsule and the gizzard separately. For both, an approximately circular shape was assumed. The surface area of whole insects and grains was approximated from a rectangular shape. For daychicks, it was not possible to obtain circular or rectangular pieces, thus we only subjected them to TPA hardness tests (see below). The vegetable diet included two leafy greens (parsley, spinach) which are not suitable for compression tests. We did not assess the mechanical properties of these two items. Similarly, for the fruit mix, we restricted testing of figs to the isolated fig seeds, as the available figs were too soft to be cut into circular or rectangular pieces. Fig seeds are expected to be the hardest component of the fruit mix diet and, consequently, to contribute more to the observed wear differences than the soft parts of the figs.

Testing was conducted over several days of one week, in the same laboratory environment, to reduce variations in humidity and temperature. Each sample was placed on a circular platform of 60 mm diameter under a force gauge and compressed in a cutoff setup until breaking or until the force gauge registered an overload (due to contact with the platform). We used circular solid testers of different diameters (60 mm, 15 mm, 3 mm) but ensured that the tester was larger than the sample for obtaining Young's modulus and yield stress so that compression would be evenly exerted over the complete surface area of the sample. The movement speed of the circular solid tester was set to 600 mm/min and the test speed (from contact point on) to 120 mm/min. We used two different force gauges, HF-100 and HF-10 (JISC, Japan Instrumental Systems Inc., Nara, Japan), attached to an automated servo stand (JSV-H1000, JISC, Japan Instrumental Systems Inc., Nara, Japan). The HF-100 has a maximum load capacity of 1000 N and a resolution of 0.1 N, while the HF-10 has a maximum load capacity of 100 N and a resolution of 0.01 N. Thus, for soft, easily deformable samples, the force gauge HF-10 with a higher precision was employed. For fruit and vegetables that were fed including the skin, we conducted multiple compression tests both from the skin side or from the side without skin. A representative stress-strain diagram and the results of mechanical properties testing are shown in [Figure 1](#). Detailed results of mechanical properties

(Young's modulus, yield stress, false yield stress, TPA hardness) are illustrated in [Supplementary Tables 2–4](#). We note that values obtained for Young's modulus are negative, because we used a compression setup. However, the sign is usually omitted in the literature, thus when we are referring to larger Young's modulus, we would refer to a more negative value. To avoid confusion, we presented all values as positive numbers.

Additionally, the food texture tester TEX-100 (JISC, Japan Instrumental Systems Inc., Nara, Japan) was used to obtain the standardized parameter TPA hardness. In food science, TPA hardness is measured by compressing a sample two times for the same compression depth or ratio, with the peak force occurring during the first compression divided by the tester area being the hardness (Bourne, 2002; Nishinari et al., 2019). An example curve is represented in [Supplementary Figure 1](#). All raw measurement results are given in [Supplementary Table 4](#). We used a 3-mm circular solid tester (area = 7.069 mm²), at a test movement speed of 120 mm/min, and an indentation depth of 2 mm. We chose this small 3-mm tester following the advice from Nishinari et al. (2019) that the tester should be less than one-third of the diameter of the sample. The hold time between the first and second indentations was set to 3 s. This hardness measure is different from (micro) indentation hardness measures such as Vickers or Knoop hardness. As TPA hardness should only be measured on food items with a certain elasticity so that repeated compression would be possible, we did not test individual items from the seed mix, the cricket head capsules, and the cricket gizzards using this procedure, as they fractured during the first indentation.

Dental microwear texture measurements

Measurements were conducted at the LIB Hamburg [former Center of Natural History (CeNak) of the University of Hamburg], Germany, on a μ surf Custom (NanoFocus AG, Oberhausen, Germany) confocal disc-scanning microscope, equipped with a blue LED (470 nm) and high-speed progressive-scan digital camera (984 × 984 pixel), a 100x long working-distance objective (resolution in $x, y = 0.16 \mu\text{m}$, step size in $z = 0.06 \mu\text{m}$), following the published routine for DMT data (Schulz et al., 2010, 2013) with adjustment for rats (Winkler et al., 2016) and guinea pigs (Winkler et al., 2019a, 2020a, 2021). Measurements were performed on original tooth surfaces of the first and second upper molars on central anterior enamel bands. Depending on wear stage and preservation, up to four (minimum 2) non-overlapping scans were taken from each specimen. Forty-four DMT parameters were calculated after filtering surfaces [leveling, spatial filtering (denoising median 5 × 5 filter size and Gaussian 3 × 3 filter size

with default cut-offs), filling of non-measured points, noise-reduction by thresholding (upper and lower 0.5%), removal of outliers (maximum slope of 85%), 2nd order form removal] and cropping them to 60 × 60 μm in MountainsMap v.9.0.9878. From the two to four scans per specimen, median values were calculated for each parameter to adequately represent the surface texture of the enamel and avoid bias by picking particularly worn or unworn locations. Parameter results for each specimen are given in [Supplementary Table 5](#).

Statistics

Descriptive and test statistics were computed in JMP Pro v. 16.0. A non-parametric, heteroscedastic pairwise comparison test (Wilcoxon test) was performed for DMTA of all dietary pairs. The application of multiple comparison tests increases the probability of Type I error; however, conservative corrections for multiple testing would inflate the probability of Type II error in small samples such as ours. We hence applied the Benjamini–Hochberg procedure with an accepted false discovery rate (FDR) of 0.25. We therefore note that the results of diet group comparisons presented here were prone to include false positives but accepted this on the basis of it being an exploratory study and including both raw p -values and critical p -values using an FDR of 0.25 into the supplements ([Supplementary Table 10](#)).

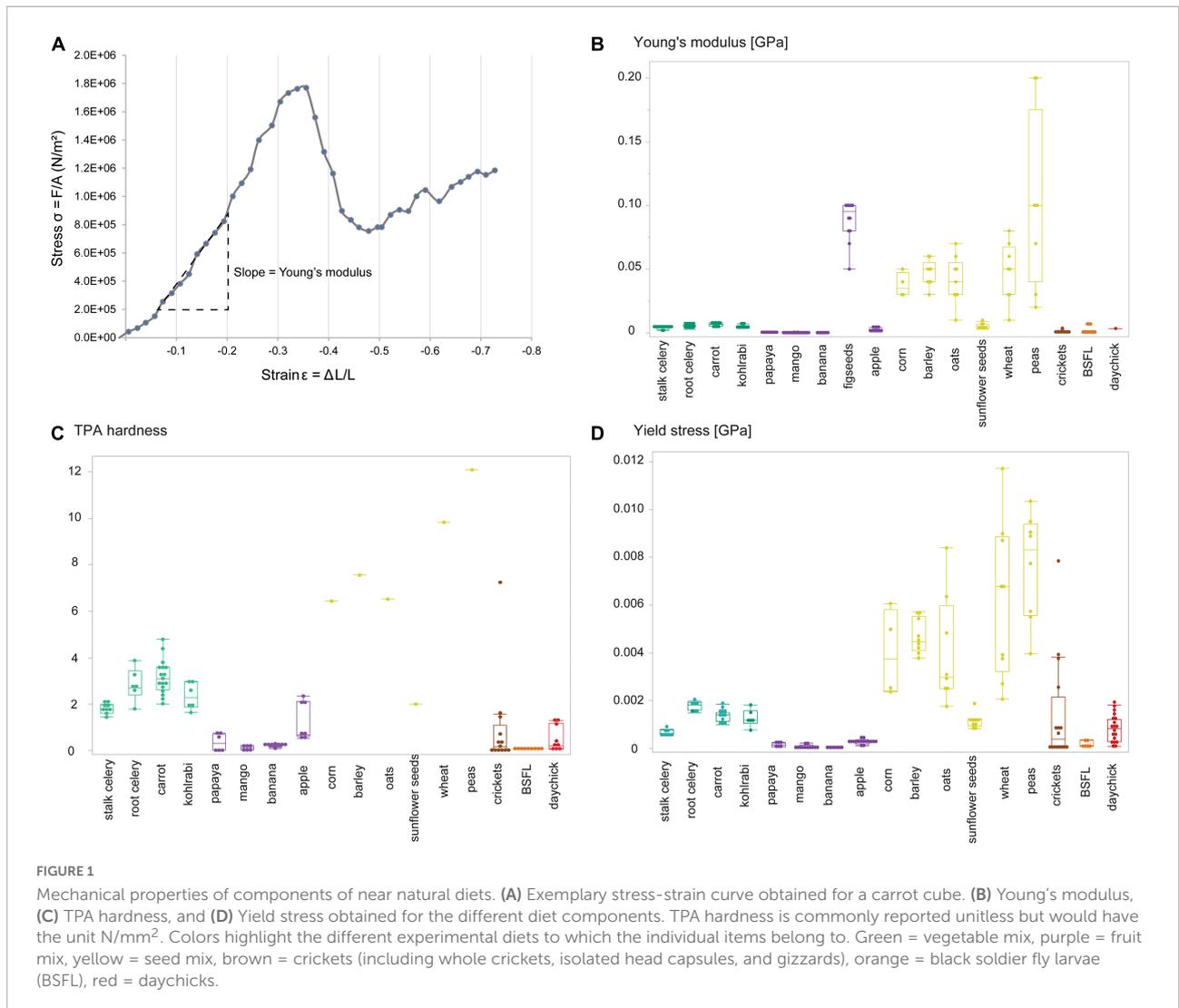
Mechanical properties parameters (Young's modulus, yield stress, TPA hardness) were repeatedly measured for multiple individual samples (4–20 repetitions) and then averaged for each dietary item. Individual measurements are given in [Supplementary Tables 2–4](#). Some diets were composed of several different items (vegetable mix, fruit mix, seed mix), while others were composed of one item only (BSFL, crickets, and daychicks). To generate representative mechanical properties from each diet, we calculated two values for each mechanical properties parameter: a weighted mean using relative proportions of diet items and a maximum using the largest value obtained for an individual diet component. For crickets, we tested whole specimens, head capsules, and gizzards and calculated a weighted mean based on weight percentages ([Supplementary Tables 6, 7](#)). For the diets BSFL and daychicks, the weighted mean and maximum value were identical.

As we could not obtain either Young's modulus and yield stress for daychicks or TPA hardness for the seed mix, cricket heads, and cricket gizzards, we used linear regressions to establish the nature of the relationships between measurable mechanical properties and used these regressions to infer the parameter values that we were unable to measure.

A fourth parameter, false yield strength, was employed to estimate yield stress for daychicks. We calculated false yield strength during compression of irregularly shaped objects as maximum force recorded divided by the area of the circular

TABLE 1 Composition of experimental diets (weight %) and supplementation in % fresh weight.

Diet name	Composition						Supplementation		
Vegetable mix	Celery root (32.2%)	Carrot (27.1%)	Stalk celery (10.9%)	Kohlrabi (10.7%)	Parsley (10.1%)	Spinach (9.00%)	Quark (7.3%)	Sunflower oil (1.2%)	Suppl. powder (0.8%)
Fruit mix		Figs (37.20%)	Banana (22.0%)	Papaya (17.0%)	Apple (12.7%)	Mango (11.2%)	Quark (20.1%)	Sunflower oil (1.1%)	Suppl. powder (1.1%)
Seed mix	Wheat (25%)	Barley (20%)	Dried peas (20%)	Oats (15%)	Corn (10%)	Sunflower kernels (10%)	Quark (22.2%)	Suppl. powder (7.7%)	
Crickets			Whole frozen crickets				Quark (9.8%)	Suppl. powder (3.4%)	
Black soldier fly larva			Whole frozen black soldier fly larvae				Quark (12.1%)	Suppl. powder (4.3%)	
Daychicks			Whole frozen day-old chicks				Quark (8.3%)	Suppl. powder (3.0%)	



solid tester (maxF/testerArea). The false yield was found to be significantly correlated with yield stress ($R^2 = 0.986$, $p < 0.0001$) for the dietary items in which we could obtain both. Yield stress for daychicks was inferred from the regression equation:

$$\text{Yield} = 0.000314 + 5.26 \times \text{falseYield} \quad (n = 18, r^2 = 0.683)$$

Young's modulus was also found to be significantly correlated with yield stress ($R^2 = 0.967$, $p < 0.0001$). Young's modulus for daychicks was obtained from the regression equation:

$$\text{Young's modulus} = 0.004499 - 10.22 \times \text{Yield} \quad (n = 18, r^2 = 0.774)$$

TPA hardness was significantly correlated with yield stress ($R^2 = 0.966$, $p < 0.0001$), and TPA hardness values for seeds, cricket heads, and cricket gizzards were obtained from the equation:

$$\text{TPA hardness} = 0.1966 + 1565.00 \times \text{Yield} \quad (n = 11, r^2 = 0.729)$$

It is important to note that, in the case of seeds and cricket gizzards, the extrapolated values were mostly outside of the range of the other measurements used to generate the regression equation and hence were particularly susceptible to error. Therefore, the following evaluation of these data was done using non-parametric tests only.

As mechanical properties parameters could not be normalized by common transformations, and DMTA parameters are also not normally distributed, we subsequently conducted Spearman correlations between all DMTA and mechanical properties parameters (Supplementary Tables 8, 9).

Results

Dietary differences

The vegetable mix, BSFL, and daychick diets caused visibly lower surface roughness and smaller wear features on the M1 (Figure 2). The groups receiving daychicks and BSFL were best distinguished from the other diet groups when the M1 is considered (Table 2 and Figure 3; Supplementary Figure 2). For the M2, the daychick diet was less distinct from the other diet groups. Seeds and crickets were not significantly different from each other for any parameter on both M1 and M2. Seeds and crickets showed the highest complexity values, but these were not significantly larger compared to the other diet groups due to the large variance observed. A detailed description of

DMTA parameter results for each diet group is given in the supplementary text in the **Supplementary material**. There was no trend of an increased variability in DMTA parameters for the more heterogeneous diets.

Tooth position-specific differences

For all diet groups, except fruit and BSFL, more than one DMTA parameter was significantly different between M1 and M2 within the same diet (Table 2). The differences were mostly due to height and volume parameters, with parameter values consistently increasing from M1 to M2.

Diet mechanical properties

Young's modulus was largest for dried peas and fig seeds (Figure 1B). Overall, the items from the seed mix had larger Young's modulus than other diets, resulting in the largest mean per diet. Insects (crickets and BSFL) and fruit mix showed the lowest mean Young's modulus, followed by vegetables and daychicks.

Using regression equations, it was inferred that TPA hardness was largest in all items from the seed mix except sunflower seeds and cricket gizzards (Figure 1C; Supplementary Table 4). The largest measured TPA hardness values were found for several vegetables (root celery, carrot, kohlrabi), followed by stalk celery and apple. Some individual samples of daychick body parts had higher values that were obtained from whole specimens, while on average, fruits, insects, and daychicks showed the lowest TPA hardness values.

The highest yield stress was found for items of the seed mix, with dried peas exceeding all other seeds, and cricket gizzards (Figure 1D; Supplementary Table 3). Sunflower seeds had much lower yield stress values, comparable to vegetables and daychicks. Fruit and whole insects (crickets and BSFL) had the lowest yield stress, while intermediate values were inferred for daychicks.

Correlations of mechanical properties and dental microwear textures analysis

For the M1, no significant correlation was found between any DMTA and mean mechanical properties parameter (Supplementary Table 8). When considering maximum values for each mechanical property, several significant correlations were found with DMTA parameters. Maximum Young's modulus was significantly correlated with the volume parameters V_v (void volume) and V_{vc} (void volume of the core) (Figure 4; Supplementary Table 8). Maximum TPA hardness was significantly correlated with 16 DMTA parameters

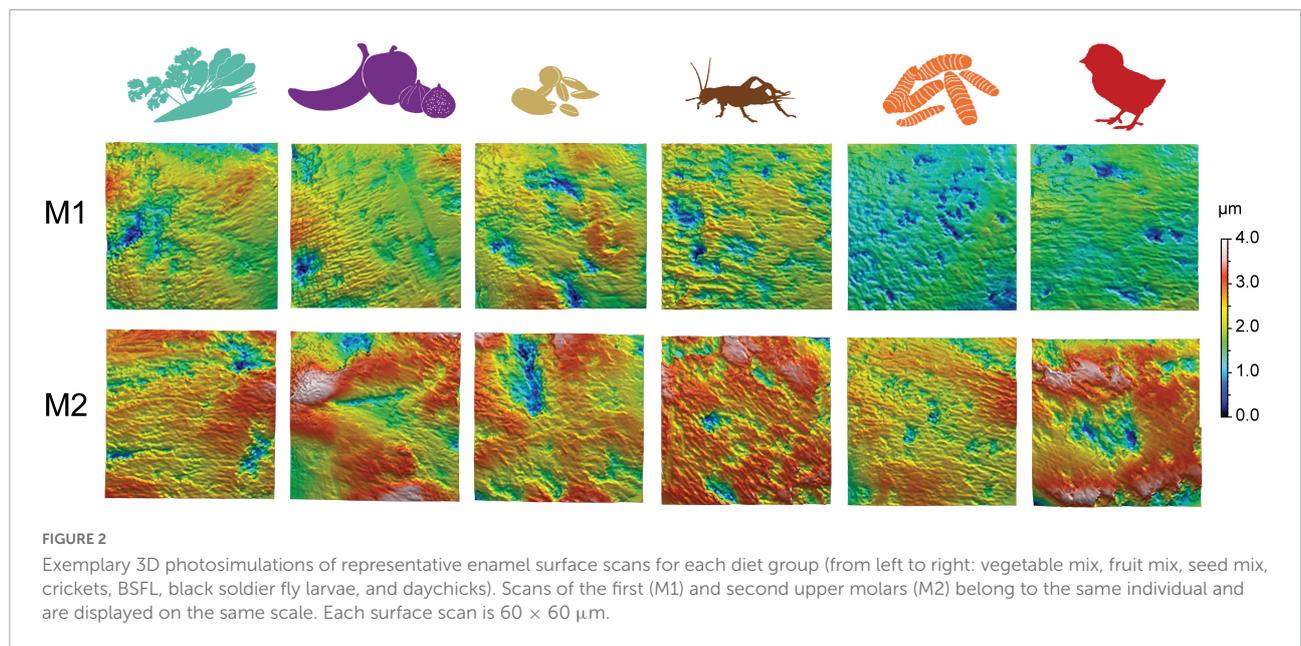
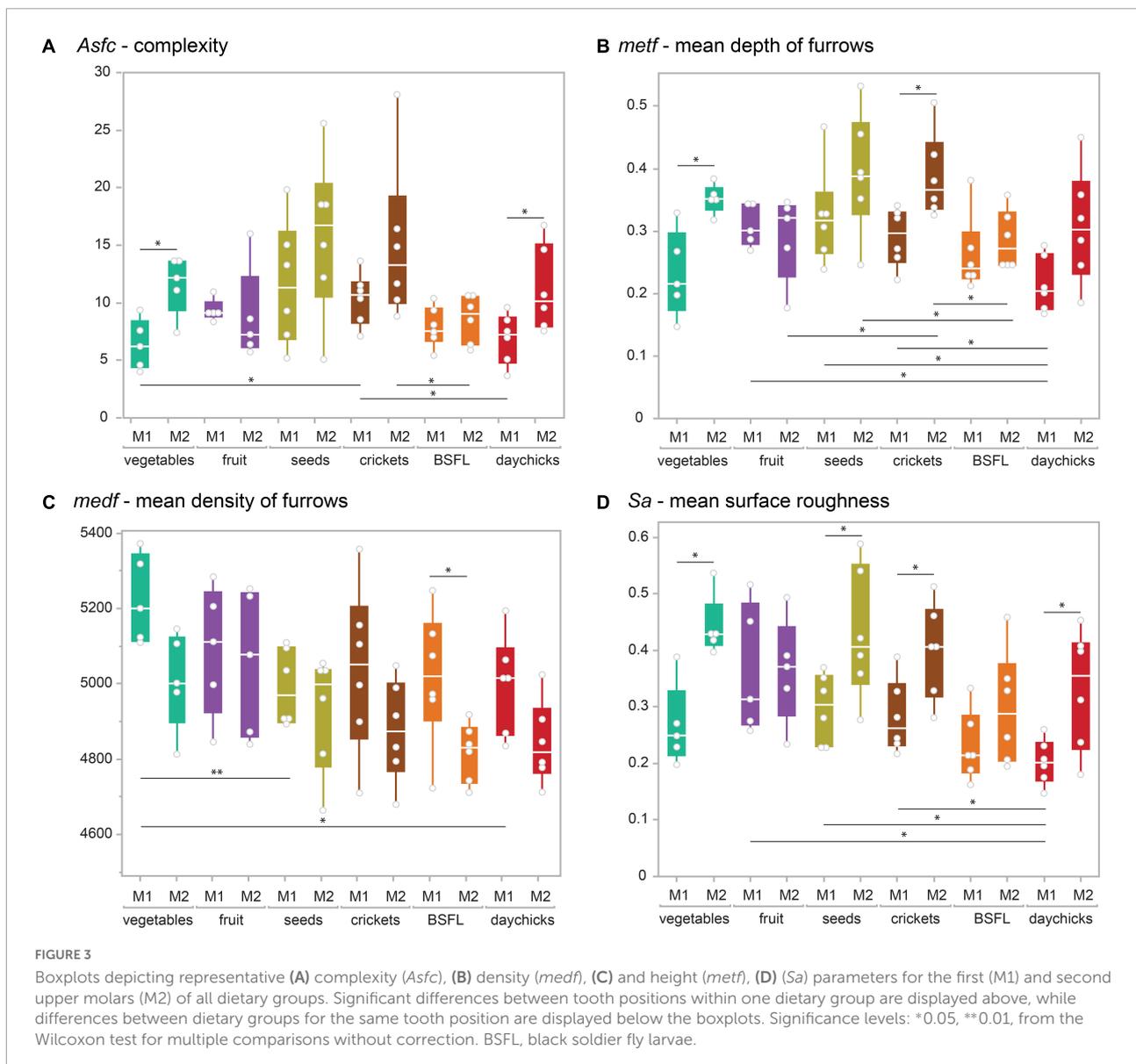


TABLE 2 DMTA parameters displaying significant differences after the application of the Benjamini–Hochberg procedure between diet groups for the same tooth position (white cells) and between tooth positions within the same diet group (gray shaded cells).

		M2					
		Vegetable mix	Fruit mix	Seed mix	Crickets	BSFL	Daychicks
M1	Vegetable mix	matf, metf, S5p, S5v, S10z, Sa, Sdq, Sdr, Sk, Smc, Sp, Spc, Sq, Sz, Vmc, Vvc	metf, Sa, Vmc	matf, Sku, Ssk	matf, metf, S5v, Sku, Smc	metf, S5p, S5v, S10z, Sa, Sdq, Sdr, Sk, Sku, Sp, Sq, Spc, Ssk, Vmc, Vvc	Sk, Sku, Smc, Vmc, Vvc
	Fruit mix	matf, metf, Sa, Sdq, Sdr		matf, metf, S5v, Sdq	IsT, matf, new_epLsar, Sdq, Sdr, Spc		IsT, S5v, Spc
	Seed mix	matf, S5v, Sdq, Sdr, Ssk	Ssk	matf, S5v, Sa, Sk, Smc, Spc, Sq, Vmc, Vvc		matf, metf, S5v, S10z, Sa, Sdq, Sdr, Sp, Spc, Sq, Vmc	matf, Sku
	Crickets	matf			matf, metf, new_epLsar, S5p, S5v, S10z, Sa, Sdc, Sdq, Sdr, Sk, Smc, Sp, Spc, Sq, Vmc, Vvc	metf, S5p, S5v, S10z, Sa, Sdc, Sdq, Sdr, Sp, Spc	matf
	BSFL	Sku, Ssk	matf, S5p, S5v, S10z, Sa, Sk, Smc, Sp, Sq, Ssk, Vmc, Vvc	matf, S5v, S10z, Sa, Sdc, Smc, Sq, Vmc	Vmc		matf, Spc
	Daychicks	Sku, Smc, Ssk, Vmc, Vvc	matf, metf, S5p, S10z, Sa, Sdc, Sdq, Sdr, Sk, Sku, Smc, Sq, Ssk, Vmc, Vvc	matf, metf, S5v, S10z, Sa, Sdc, Sk, Smc, Sq, Vmc, Vvc	matf, Sa, Sdc, Sdq, Sk, Sq, Vmc, Vvc		matf, metf, S5p, S5v, S10z, Sa, Sdc, Sdq, Sdr, Sk, Smc, Sp, Spc, Sq, Vmc, Vvc

Due to using an FDR of 0.25, several *p*-values > 0.05 were judged significant. Significance levels of raw *p*-values were as follows: > 0.05 = gray, 0.05 = regular, 0.01 = *italics*, 0.001 = **bold**.



representing height, complexity, volume, and area parameters (Figure 4; Supplementary Table 8). There were no significant correlations between any DMTA parameter for M1 and yield stress.

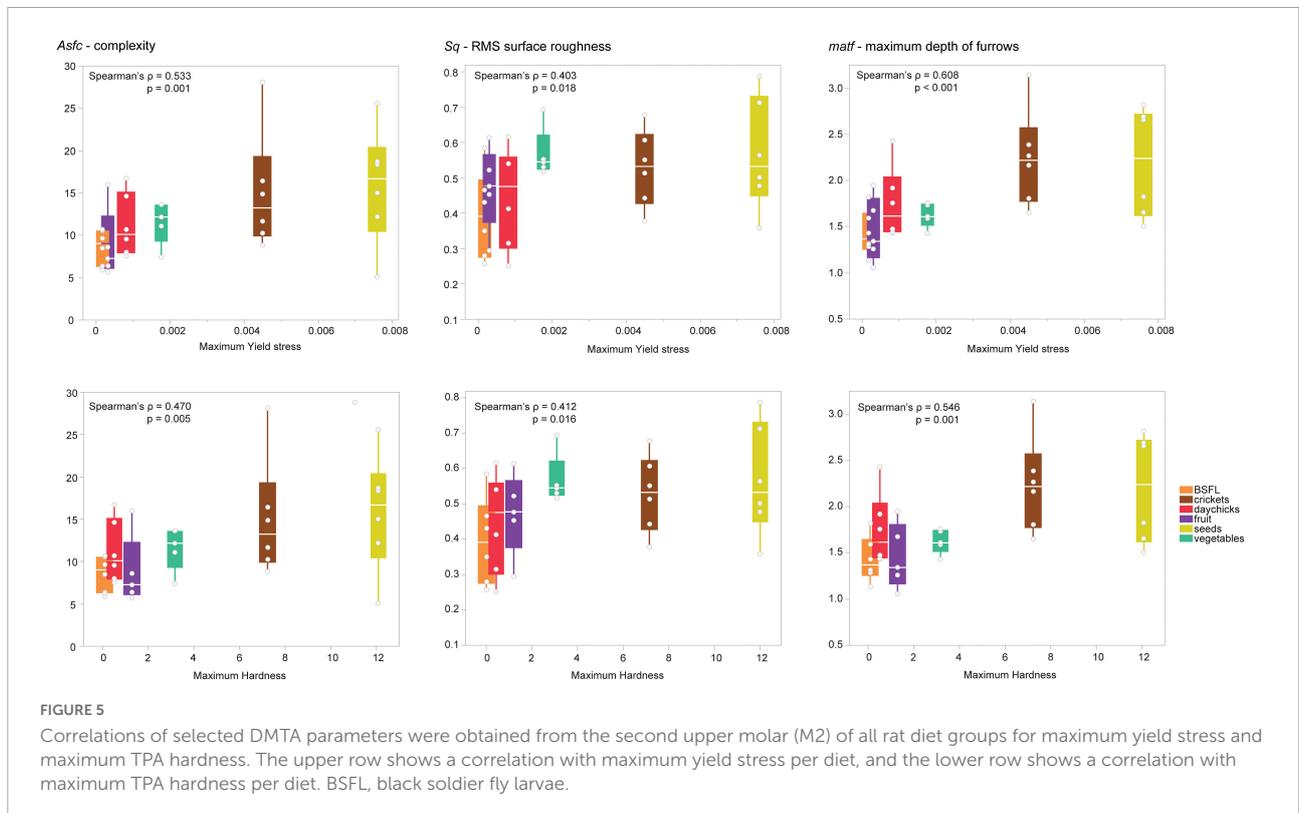
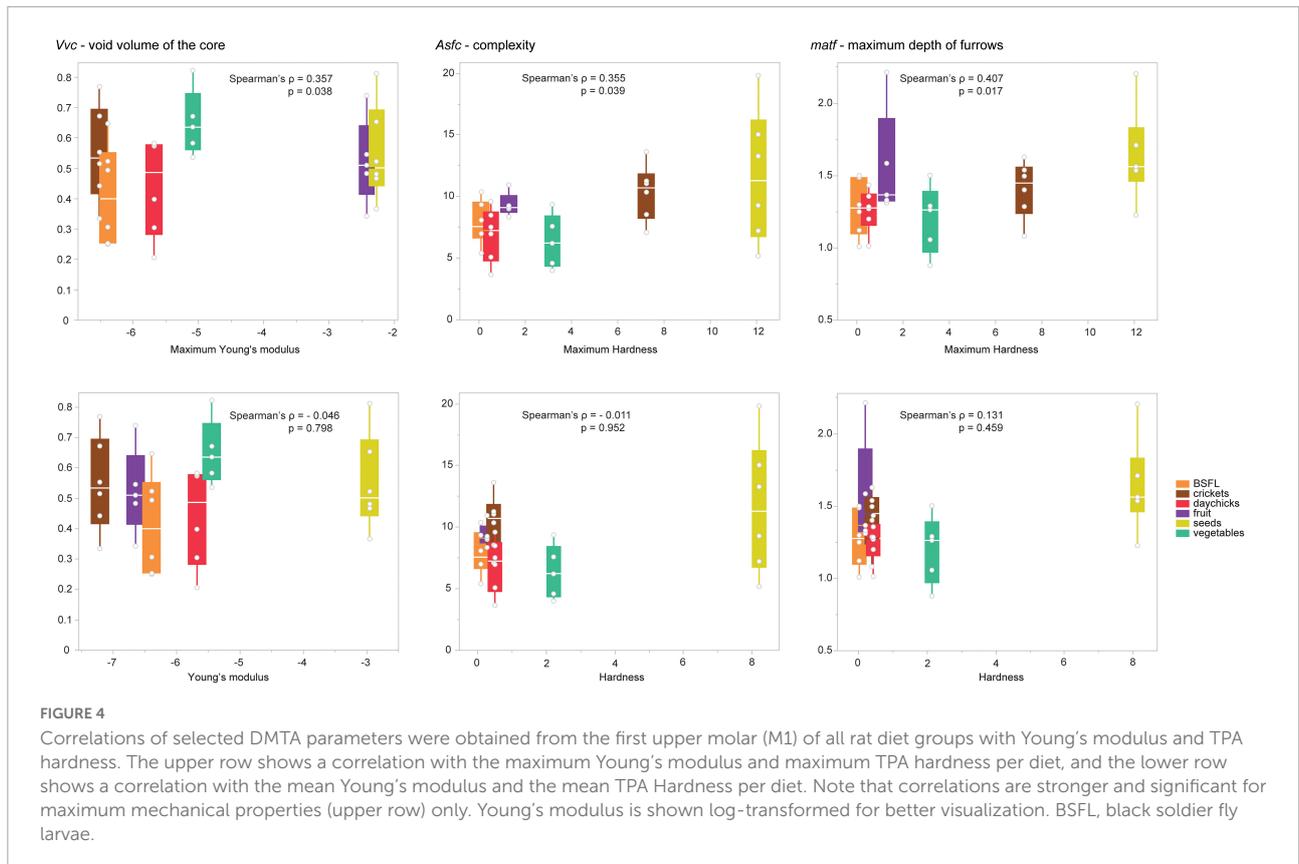
For the M2, mean TPA hardness and maximum TPA hardness showed multiple significant correlations (17 and 20, respectively) with several height, volume, slope, and complexity parameters (Figure 5; Supplementary Table 9). Mean yield stress showed no significant correlations, while for maximum yield stress, 21 DMTA parameters showed significant correlations (Figure 5; Supplementary Table 9). There were no significant correlations of DMTA parameters with mean Young's modulus, and only one parameter (*IsT* – texture isotropy) was significantly correlated with maximum Young's modulus (Supplementary Table 8).

Discussion

Limitations of the experimental setup

We have to note that several circumstances of the experimental setup likely introduced a source of variability and uncertainty. Therefore, all results obtained have to be interpreted with caution.

Firstly, rats received diets while housed in groups of 3 in bulk one time per day. Hence, for the mixed diets (vegetable mix, fruit mix, seed mix), individual rats were free to choose among the diet items offered. We could not control if each individual consumed equal amounts of each dietary component, as either dietary preferences or dominance of other individuals may have affected food choice. This is likely a source of the



observed variability in the DMTA data and may have influenced clearer separation between diet groups. We thus suggest that future feeding experiments should focus on one food item, or more homogenous food mixes, instead of trying to create a more natural diet mix.

Second, the components of the vegetable and fruit mix were purchased on a day-to-day basis, over the course of 37 days, to provide fresh food every day. The source of vegetables and fruits may have differed, as well as their degree of ripeness, water content, or other parameters, which might have added variability to the mechanical properties of these diets and could have influenced dental wear. The measurement of mechanical properties took place after the feeding experiment, hence—except for the seed mix—not on the same batches of food that the rats received. The seed mix was stored in a cool, dry place after the experiment and used for mechanical properties testing. Still, the storage time could potentially have altered the water content of the kernels to some degree (even though it was initially low). We purchased the same dietary items the rats received and treated them the same way (buying frozen crickets, black soldier fly larvae, and daychicks and defrosting them before testing). Still, the mechanical properties obtained may slightly differ from the mechanical properties of the original experimental diets but should nevertheless provide a good approximation of these food items.

Third, we excluded parsley and spinach from the mechanical properties testing, because their geometric shape does not allow for compression tests. Additionally, for daychicks and the seed mix, not all mechanical property parameters could be obtained but had to be inferred from linear regression equations.

Finally, choosing parameters for mechanical properties testing is similar to opening Pandora's box, as numerous methods for items with different structural properties (homogenous vs. inhomogeneous, brittle, tough, elastic, etc.) and an equally large number of opinions on which are the best practices exist [see [Berthaume \(2016\)](#) for a review]. The selection of testing methods and parameters presented in our study followed two principles: simplicity and feasibility. By only conducting compression tests, we limited our obtained parameters to Young's modulus and yield stress, hereby omitting other often measured parameters like energy release rate or toughness (e.g., [Strait and Vincent, 1998](#)). Our setup required only a force gauge, stiff testers, and measurement of the sample area and can hence be easily repeated. Regarding hardness testing, several methods are available but require sophisticated equipment such as diamond indenters and thin sections of the material (Vickers and Knoop hardness). Still, it has been questioned whether the observed hardness is an intrinsic mechanical property of the material or a product of the local testing environment, as argued by [Berthaume \(2016\)](#). We did not wish to engage in this discussion but did hope to simply find a hardness measure that was obtainable with our setup. Therefore, we chose TPA hardness. Parameters

from food texture studies (TPA) have been subjected to criticism because calculations of these parameters are often not consistently applied by researchers or details of the testing setup are insufficiently reported (e.g., size of the tester, test speed). However, TPA hardness has been evaluated as “probably the most reliable TPA parameter” by [Nishinari et al. \(2019\)](#). The general pitfall is that TPA intends to mimic human masticatory behavior using an instrument and compress (indenting) a food sample two times and that this measure only works for elastic foods. For hard and brittle items, such as the seeds in our experiment, TPA hardness thus had to be inferred, which introduced further uncertainty. This setup is an oversimplification of the actual masticatory process. In the case of our study, we did not intend to mimic masticatory behavior of the rats but to gain quantitative results related to the diet's intrinsic physical properties. The interaction between tooth morphology, bite force, mastication behavior (movement), lubrication of the food through saliva, and many more aspects cannot be accounted for in our approach but will all affect the observed tooth (micro)wear ([Schulz-Kornas et al., 2020b](#)). Therefore, our characterization of diet mechanical properties is not extensive but can still highlight several correlations between observed DMTs and diet mechanical properties.

Tooth position-specific dental microwear texture

The controlled rat feeding experiment of various near-natural diets revealed several systematic dental wear patterns. Comparable to guinea pigs feeding on pelleted diets ([Winkler et al., 2021](#)), overall surface roughness, including depth of wear features, increased from M1 to M2 in rats ([Figure 3](#); [Supplementary Figure 2](#)). The only exception is the fruit diet, which showed similar roughness values on M1 and M2. The most pronounced increase was seen for vegetables, seeds, crickets, and daychicks. Due to the variable magnitude of increase in surface roughness from M1 to M2, differences observed between the diet groups were more pronounced when only considering the M1 and weaker when considering only the M2. Overall, the number of significantly different DMTA parameters between tooth positions on the same diet is even larger than between diet groups for the same tooth position ([Table 2](#)). This finding highlights that DMTA is likely tooth-position-specific—an observation made before for ungulates ([Schulz et al., 2010](#)) and macropods ([Arman et al., 2019](#))—and cautions against lumping tooth positions together to increase sample sizes (for example in fossil samples). It might be that, in small mammals, DMTA conducted on anterior cheek tooth positions better reflects dietary differences than when using posterior teeth. This observation is in agreement with [Winkler et al. \(2021\)](#), who found that the fourth premolar showed the best dietary discrimination

in guinea pigs receiving natural plant diets of different abrasiveness.

When focusing on the M1, the six experimental diets can be roughly separated into three groups according to their resulting DMT patterns. However, differentiation between diets within these groups is difficult. Group one includes fruit mix, seed mix, and crickets, which are showing the highest surface complexity (*Sdr*, *Asfc*) and largest height differences (e.g., depth of furrows, peak height, mean surface roughness). These are the groups containing diet components that are often considered hard objects. Group two is composed of BSFL and daychicks, which had the lowest complexity, height, and volume parameter values. Black soldier fly larvae can be considered, in contrast to crickets, as soft-bodied insects, as they are not covered by a hard, sclerotized cuticle (Chapman, 2013a). Strait and Vincent (1998) tested the toughness of insects through scissor tests. They found beetles to be tough and brittle, while caterpillars were soft and compliant. We would suggest that crickets might be more comparable to beetles, while BSFL bear more resemblance to caterpillars (Evans and Sanson, 2005).

Finally, the vegetable mix diet would fall into group three, which is characterized by intermediate height and volume parameter values, but has distinctly higher anisotropy (*new epLsar*, *Sfrax epLsar*) and mean density of furrows. In herbivorous ungulates, high anisotropy values are characteristic of grazing species (Ungar et al., 2007), while in guinea pigs, the differences between browse and grass diets were less pronounced (Winkler et al., 2021). For rats, it seems that mastication of the vegetables required a different mastication mode as compared to the other diets. We believe that the more fibrous vegetables may require more frequent shearing motions, while diets such as fruit, seeds, and insects may require crushing movements. The stronger alignment (anisotropy) and higher density of wear features would hence result from such a repeated shearing motion.

What causes high complexity?

Patterns of complex surfaces have been associated with hard-object feeding not only in primates (Scott et al., 2005, 2012; Ungar et al., 2008, 2010; Ungar and Berger, 2018) and carnivorous mammals (DeSantis et al., 2012), but also in lepidosaurs (Winkler et al., 2019b), archosaurs (Bestwick et al., 2019), and fish (Purnell and Darras, 2015). Rats are no exception, as they also display the highest surface complexity (both by *Asfc* and *Sdr*) on the hardest diet (seeds) after feeding on their designated diet for 37 consecutive days. Yet, how complexity develops over longer feeding periods cannot be predicted. van Casteren et al. (2020) argued that “occasional contacts between seeds and irregular spicules of enamel could result in the latter being fractured, but over time this process should result in a decrease, rather than increase, in texture

complexity.” We cannot rule out that our experimental animals would have displayed a change in complexity values over time. However, from the current viewpoint of DMTA, it is likely that texture turnover in (small) mammals is fast (Winkler et al., 2020b) and continuously feeding on the same diet will produce similar DMTA over and over again, thus resulting in a short-term dietary proxy that reflects the last few weeks of an individual’s diet. We therefore conclude that the connection between high surface complexity and hard-object feeding is independent of body size, chewing mode, and taxonomic affinity [a similar conclusion was reached by Purnell and Darras (2015) for fishes], and thus a universal wear pattern following the oral processing of stress-limited foodstuffs.

However, we note that our hardness measure yielded low values for crickets, which resulted in the second highest surface complexity observed in this experiment. Overall, the mechanical properties of the two insect diets (crickets, BSFL) were found to be similar when testing whole insects, while the DMT pattern diverged completely. Cricket feeding resulted in high complexity, surface roughness, and deep and voluminous wear features, while BSFL feeding resulted in the lowest complexity, surface roughness, and lowest depth of wear features observed among all diet groups. For insectivorous bats, Purnell et al. (2013) found that species consuming “harder” prey (e.g., coleoptera) showed larger volume parameter values than species consuming “softer” prey, which is consistent with the disparity in our results observed between supposedly harder crickets and softer BSFL. It is therefore evident that our approach toward measuring mechanical properties of foodstuffs is not sufficient to describe the essential differences between these two insect diets. When testing individual cricket body parts, the head capsule displayed much higher TPA hardness than the abdomen, and a part of the digestive system (the gizzard) showed mechanical properties comparable to seeds (see raw data, Supplementary Tables 2–4). The gizzard is lined with strong cuticular plates or “teeth” in house crickets and other Orthoptera (Kirby et al., 1982 and references within, Elzinga, 1996; Chapman, 2013b). When considering maximum yield stress and maximum TPA hardness values for crickets (that were derived from regression equations for the gizzards), we found strong correlations with a variety of DMTA parameters. Although the hardness data were inferred from the regression equation, and the extrapolated values were mostly outside of the range of the other measurements (see Statistics section), these results suggest that crickets are much more heterogeneous as compared to BSFL, and likely, their sclerotized body parts greatly exceed less sclerotized body parts (like the abdomen) in hardness. Also, Young’s modulus of individual body parts has been found to differ greatly in locusts (over seven orders of magnitude, Li et al., 2020). Such subtle differences were not well-captured by our experimental approach but are likely responsible for the pronounced differences in DMT observed between crickets and BSFL.

Dental microwear texture and mechanical properties

Maximum values of mechanical properties have a stronger impact on the DMTA of M1. We found maximum Young's modulus to be significantly correlated with 2 DMTA parameters, while mean Young's modulus was not significantly correlated with any DMTA parameter (**Supplementary Table 8**). For maximum TPA hardness, M1 showed 16 significant correlations, while mean TPA hardness was not significantly correlated with DMTA parameters at all (**Supplementary Table 8**). M2 showed more significant correlations between material properties parameters and DMTA than M1. Here, maximum yield stress and maximum TPA hardness showed abundant strong correlations with DMTA parameters, and mean TPA hardness was significantly correlated with 17 DMTA parameters (**Supplementary Table 9**). In conclusion, differences in (maximum and mean) Young's modulus between diet items did not explain differences observed in DMTA, while maximum TPA hardness was strongly correlated with DMTA on both M1 and M2. For the M2, also maximum yield stress and mean TPA hardness were equally well-correlated with DMTA parameters, which may result from different chewing behavior when processing harder items. If harder items would be primarily processed using the M2, the observed wear might be more correlated with yield stress, which determines the fracture point of the diet item and overall hardness. Although our methodology has several limitations, this general trend highlights that mechanical properties and tooth position-specific wear are interrelated.

Parameters associated with increased abrasion such as height, volume, and complexity parameters increased from M1 to M2 on most diets. The stronger correlation of maximum mechanical properties indicates that M1 dental microwear may be more sensitive toward detecting inhomogeneous diets, more singular feeding events, and thus potentially fallback feeding events (Lambert et al., 2004). The M2, on the other hand, is strongly affected by overall TPA hardness and maximum TPA hardness of the diet. This may indicate that stiff, resistant food items (commonly termed hard objects) are more frequently and thoroughly processed using the M2 (and hence in the rear of the dentition). This is in accordance with observations from guinea pigs (Winkler et al., 2021), for which it was suggested that increased bite force toward the rear of the dentition results in increased abrasive wear when feeding on hard pellets. Under the previously discussed limitations, our results here need to be treated with caution, but in the context of the increased wear on posterior teeth in guinea pigs, it is plausible that rats also exert stronger bite forces toward the rear of the dentition and utilize the posterior teeth to break harder foodstuff.

Outlook and conclusion

Different foodstuffs can likely cause high complexity of DMT; however, in our controlled feeding experiment seeds, fruit (with seeds) and harder insect body parts resulted in the highest recorded complexity. The findings of this study emphasize that DMT formation in small mammals (i.e., rodents) seems to be strongly connected to mastication behavior and mechanics (likely bite force) and is hence significantly different for different tooth positions. These results call for a more thorough investigation of DMT gradients along the tooth row in other taxa, including large mammals.

We attempted to associate quantitative food mechanical properties with quantitative DMT. We considered this approach relevant to better understand the interrelation between ingesta and observed dental (micro)wear, beyond the detection of differences between diet categories. As noted, the masticatory system is highly complex, and accounting for all possible factors that affect DMT formation is impossible in an experimental setup. However, we can approach this complex system by controlling isolated factors and quantifying them. Future research may further elucidate how food mechanical properties affect dental (micro)wear, but also manage to take into account different occlusal morphologies and chewing mechanics, including movement, speed, bite force, fluid dynamics on the occlusal surface, and many more. At the moment, we are still only scratching the surface of understanding dental wear.

Data availability statement

The dataset presented in this study is permanently stored in the following online repository: ZFDM online repository of the University of Hamburg, doi.org/10.25592/uhhfdm.10235.

Ethics statement

The animal study was reviewed and approved by Swiss Cantonal Animal Care and Use Committee Zurich animal experiment license No. ZH135/16.

Author contributions

DEW, MC, MOK, ES-K, and TT: conceptualization. DEW, MC, MOK, and ADC: methodology. DEW: formal analysis and visualization. MOK, TMK, and AT: resources. DEW, MC, and MOK: writing—original draft. TT, DEW, and TK: funding acquisition. All authors: writing—review and editing and approve the submitted version.

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Conflict of interest

Author AT was employed by Granovit Zoofeed, Granovit AG.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2022.957427/full#supplementary-material>

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