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# **Determining chewing efficiency using a solid test food and considering all phases of mastication**

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## Highlights

- Chewing ability is related to oral functioning and food preference.
- Using solid food, the median particle size,  $X_{50}$ , is measured with number of chews.
- Reduction in  $X_{50}$  is traditionally measured after the initial chewing phase.
- Initial particles of appropriate size, shape and amount enable to measure all phases.
- Testing of chewing ability needs less chews and bite force than traditionally used.

## Abstract

*Objectives:* Following chewing a solid food, the median particle size,  $X_{50}$ , is determined after  $N$  chewing cycles, by curve-fitting of the particle size distribution. Reduction of  $X_{50}$  with  $N$  is traditionally followed from  $N \geq 15-20$  cycles when using the artificial test food Optosil<sup>®</sup>, because of initially unreliable values of  $X_{50}$ . The aims of the study were (i) to enable testing at small  $N$ -values by using initial particles of appropriate size, shape and amount, and (ii) to compare measures of chewing ability, i.e. chewing efficiency ( $N$  needed to halve the initial particle size,  $N(1/2-X_0)$ ) and chewing performance ( $X_{50}$  at a particular  $N$ -value,  $X_{50,N}$ ).

*Design:* 8 subjects with a natural dentition chewed 4 types of samples of Optosil particles: (1) 8 cubes of 8 mm, border size relative to bin size (traditional test), (2) 9 half-cubes of 9.6 mm, mid-size; similar sample volume, (3) 4 half-cubes of 9.6 mm, and 2 half-cubes of 9.6 mm; reduced particle number and sample volume. All samples were tested with 4  $N$ -values. Curve-fitting with a 2<sup>nd</sup> order polynomial function yielded  $\log(X_{50})-\log(N)$  relationships, after which  $N(1/2-X_0)$  and  $X_{50,N}$  were obtained.

*Conclusions:* Reliable  $X_{50}$ -values are obtained for all  $N$ -values when using half-cubes with a mid-size relative to bin sizes. By using 2 or 4 half-cubes, determination of  $N(1/2-X_0)$  or  $X_{50,N}$  needs less chewing cycles than traditionally. Chewing efficiency is preferable over chewing performance because of a comparison of inter-subject chewing ability at the same stage of

food comminution and constant intra-subject and inter-subject ratios between and within samples respectively.

*Keywords:* chewing efficiency, chewing performance, food comminution, mastication,

*Running title:* Determination of chewing efficiency

## **1. Introduction**

A major function of mastication is to prepare food for swallowing (van der Bilt et al., 2006). Furthermore, mastication influences the release of flavour. Before a coherent bolus is formed which keeps food particles together, comminution of a solid food during a chewing cycle includes collecting and transport of particles by the tongue from the oral cavity to the occlusal area of the posterior teeth and subsequently breakage of part of these particles between antagonistic teeth while closing the jaw (Hiemae & Palmer, 2003). Every chewing cycle begins thus with selection, in which food particles have a chance to be placed between the teeth in such a way that they are at least damaged, if not broken by the subsequent breakage process (Lucas, 2004). For any particle size, the selection chance can be defined as the weight of fragments with respect to the total weight of damaged and non-damaged particles. Because of the essential initial role of the tongue, the break-down of solid foods is most challenging, in particular for subjects whose chewing ability is impaired. For example, in wearers of full dentures without fixation by implants or adhesives, the tongue assists in stabilizing the denture, which disturbs its role in collecting and transporting particles. For serving feasibility in subjects in which apart from tongue function, the delivery of force by jaw muscles may be impaired, chewing tests have been developed using a soft bolus made of a colour-changeable or two-coloured chewing gum (Komagamine et al., 2011; Schimmel, Christou, Herrmann & Müller, 2007), or wax (Sato et al., 2003; Speksnijder et al., 2009). However, such tests measure a subject's ability of mixing a semi-solid artificial test food between the teeth, or between tongue and palate, rather than an integrated functioning of all

oral structures which are involved in the breakdown of solid foods. A test using a solid food remains relevant, the more as an impairment of chewing such foods will inevitably cause diet restrictions.

When chewing starts on a sample of single-sized particles, the comminution process is reflected in the reduction of the median particle size ( $X_{50}$ ) with the number of chews ( $N$ ). Furthermore, food comminution changes the variation in particle size which is reflected in the broadness ( $b$ ) of the size distribution. For a particular  $N$ -value,  $X_{50}$  and  $b$  can be determined by curve-fitting of the particle size distribution by cumulative weight, hence volume, using the Rosin-Rammler equation (Olthoff, van der Bilt, Bosman & Kleizen, 1984). Chewing performance is quantified by  $X_{50}$  at a particular  $N$ -value,  $X_{50,N}$ , and chewing efficiency by the number of chews needed to achieve an  $X_{50}$  value that equals half of the initial particle size,  $N(1/2-X_0)$  (van der Bilt et al., 1987). A larger chewing performance corresponds with a smaller value of  $X_{50,N}$ , and a larger chewing efficiency with a smaller value of  $N(1/2-X_0)$ .

Regardless of the measure of chewing ability, a test on food comminution which includes solely short chewing sequences will be most feasible because it requires the least endurance of a subject. Furthermore, the initial phase of chewing is important to be considered rather than being ignored (see below), because (1) most reduction in particle size occurs here, and (2) this phase includes a transition from a slower rate of reduction to a faster rate which reflects the influence of selection in particular (cf. Discussion). To date chewing sequences in tests have been longer than necessary for some reasons.

First, the present study will show that it is important to choose an initial size of the single-sized particles on which chewing is started which is a mid-size of the upper class of sizes. However, in several studies using sieving to separate size classes of an artificial test food (Optosil®), chewing has been started on cubes, which had an edge size of either 8.0 mm (Olthoff et al., 1984; Slagter, Olthoff, Steen & Bosman, 1992a; van der Bilt et al., 1987) or 5.6 mm (Barbosa et al., 2013; Caputo et al., 2012; Eberhard et al., 2012, 2015; Fontijn-Tekamp et al., 2000; Gomes et al., 2010; Gonçalves, Viu, Gonçalves & Garcia, 2014; Marquezin et al., 2013; Mendonça et al., 2009; Pereira et al., 2012; Slagter, Bosman & van der Bilt, 1993; Soares et al., 2017). These particle sizes corresponded with the aperture of a wire sieve (included in a stack of sieves) that retains the original particle size. However, cubes of 8.0 or 5.6 mm have a border size rather than a mid-size with respect to the limits of the size classes 8.0-11.3 mm and 5.6-8.0 mm respectively, which are determined by successive sieve apertures. In order to avoid bias in the estimation of  $X_{50}$  and  $b$  while initial particles are still

present, a determination of chewing performance has been carried out when the initial phase of chewing had passed in all subjects, *i.e.* at least after 15-20 chewing cycles.

Second, the first larger sieve aperture through which the total weight of all particles and fragments will pass, should be included as a data point with the value 1.0 in the cumulative underweight distribution. Hence, the relevance will be shown of considering the first empty sieve as the top sieve of the series used, rather than the sieve below (the sub-top sieve) on which most if not all initial particles are retained in an initial phase of chewing.

Third, a fairly large amount of initial particles has been used in the abovementioned studies using Optosil, *i.e.* 8 cubes of 8 mm or 17 cubes of 5.6 mm. Because of a limited number of posterior teeth and predominantly one-sided chewing, such numbers of large particles (which are easily transported by the tongue) will initially saturate the breakage sites on the teeth (van der Glas, van der Bilt & Bosman, 1992; van der Glas, Kim, Mustapa & Elmanaseer, 2018). The number of selected cubes per chew is then limited to a maximum. This maximum, the number of breakage sites on the teeth, is about 5 particles for a size of 8.0 mm and 8 particles for a size of 5.6 mm, which is smaller than the number of offered particles. The selection chance of the initial particles will therefore be reduced, yielding initially a lower rate of reduction in  $X_{50}$ . Another reason for a lower rate of size reduction is that initially the selection of small fragments will be hampered by the presence of large cubes (van der Glas et al., 2018; cf. Discussion). A particular stage of particle size reduction can be attained after less chews by reducing the number of particles on which chewing is started (Baragar, van der Bilt & van der Glas, 1996, theoretical study; van der Bilt, van der Glas & Bosman, 1992; Voon et al., 1986, simulation studies).

Another advantage for limiting the amount of initial particles is reducing the bite force which is required to fracture particles. This bite force is approximately proportional to the number of selected particles, which decreases with smaller numbers of offered particles (van der Glas et al., 1992; van der Glas et al., 2018). Thus in order to enhance feasibility of a chewing test by shortening chewing sequences, it will be advantageous to limit the number of offered particles for avoiding an initial saturation of the breakage sites, a delayed selection of the fragments, and for reducing the amount of force needed for fracturing.

Limiting the required bite force can further be attained by using half-cubes rather than cubes as initial particles. With the same percentage of deformation needed to initiate fracture, the work (force x displacement) needed to initiate fracture will be half for half-cubes than for cubes of the same size. Another advantage of using half-cubes is that the volume of a half-cube corresponds more than that of a cube with the mean volume of irregularly shaped flakes,

which are formed during chewing on Optosil (van der Bilt, van der Glas, Mowlana & Heath, 1993; Eberhard et al. 2012).

The first aim of the present study is to enhance feasibility of carrying out a test on chewing ability using a solid test food without ignoring the initial phase of chewing. To that end, the effect of using initial particles of appropriate shape, size and amount will be examined on the quality and validity of curve-fitting with the Rosin-Rammler equation and on relationships between  $X_{50}$  and  $N$ . Apart from chewing ability, summarizing the size distributions during all phases of chewing using the Rosin-Rammler equation with reliable values of  $X_{50}$  and  $b$ , is relevant for computer simulation studies. Simulation studies may give insight, for example, into flavour release during chewing. In order to decide whether chewing efficiency or chewing performance may be preferred as measure of chewing ability, the second aim is to examine whether intra-subject and inter-subject ratios in chewing ability are constant between and within types of particle samples respectively.

## 2. Materials and Methods

### 2.1. Subjects

The study was carried out in compliance with the Helsinki Declaration, and approved by the University Ethics Committee (Ref no. 2017060201). Eight students from the School of Food Science and Biotechnology, Zhejiang Gongshang University (4 males and 4 females), gave informed consent, and participated in the chewing experiments. The mean age was 23.6 years (SD 1.3, Table 2). The subjects had a good general health (no medication), and a sufficiently complete natural dentition (allowing missing third molars) with normal occlusal relationships. Jaw muscle pain and/or pain in the temporomandibular joint, or disturbances of intra-oral or peri-oral sensory function were absent.

### 2.2. Test food

Using brass moulds, cubes with an edge size of 8.0 mm and half-cubes with a larger edge size of 9.6 mm were made of Optosil® (Bayer, Germany; version 1980), a silicone dental impression material which has a constant consistency, and is not affected by saliva. Versions of Optosil with similar or reduced strength have been used as an artificial test food in many previous studies (cf. Introduction). The procedure of preparing Optosil particles has been described in detail previously (van der Glas, Al-Ibrahim & Lyons, 2012). The ratio between Optosil base and catalyst ((Heraeus Kulzer GmbH, Hanau, Germany) was 0.02477 in the present study (24.77 mg catalyst to 1 gram of base).



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Table 1 about here  
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### 2.3. Chewing experiments

Table 1 shows the test conditions for the various particle samples. Chewing on samples of 8 cubes with an edge size of 8 mm (sample volume: 4.1 cm<sup>3</sup>), a traditional type of test (Olthoff et al. 1984), was compared with chewing on samples of half-cubes with a larger edge size of 9.6 mm. In contrast to cubes of 8 mm, chewing was started with mid-sized particles between two successive sieve apertures, using half-cubes of 9.6 mm. By using mid-sized half cubes, curve-fitting of the particle size distribution with the Rosin-Rammler equation will yield unbiased estimates of  $X_{50}$  and  $b$ , even for small  $N$ -values (cf. Results). The numbers of half-cubes tested were 9, 4 and 2 with sample volumes of 4.0, 1.8 and 0.9 cm<sup>3</sup>. While the sample volume of 9 half-cubes (4.0 cm<sup>3</sup>) was similar to the sample volume of cubes (4.1 cm<sup>3</sup>), the sample volume was smaller for the other numbers of half-cubes to determine the extent to which the rate of food breakdown is increased by decreasing the initial number of particles. Shorter chewing sequences would then be sufficient for determining chewing ability. For each type of particle sample, four numbers of chewing cycles,  $N$ , were applied with a range in which the value of chewing efficiency,  $N(1/2-X_0)$ , could be expected for the various subjects. These  $N$ -values were performed before the food was expectorated by the subject for subsequent data analysis. More trials were applied for smaller numbers of  $N$  to ensure that fluctuations in the way that a limited number of initial particles are handled by the tongue and the teeth, were averaged out sufficiently. Particle samples,  $N$ -values and repetitions (trials) were applied randomized, in a session with a duration of 1½ hours.

Each particle sample was weighted with an accuracy of 1 mg, and placed in a separate labelled cup. Furthermore a series of labelled containers was prepared for collecting the outcome particles after chewing. These containers were each provided with a household sieve by which a labelled coffee-filter (with a round bottom) was supported. The subjects were blinded for the labelling on the cups, containers and coffee-filters.

The observer was seated at a distance of 2 meters from the subject, for observing the movement of a marker on the subject's chin during chewing. The subject was sitting upright in a comfortable chair. An assistant who was seated alongside the subject handled the cups with the particle samples, while the observer provided the subject with the right combination of container, household sieve and coffee-filter. The chewing outcome belonging to a particular condition of particle type and number was pooled across the various trials in the

same coffee-filter. When the same container was needed by chance, the observer carried out a fake change of containers to keep the subject blinded for the conditions of the next chewing trial. Observer and assistant had an identical list to communicate about the progress of a session by subsequently mentioning a stage number.

The assistant transferred the samples one-by-one to a soup spoon, sprayed some water on the particles, and handed the filled spoon to the subject. The subject was instructed by the observer to transfer the particles from the spoon to the tongue, and was then instructed to start chewing. Before the chewing experiments were started, subjects were instructed to chew in a habitual way and not to swallow during chewing sequences.

The observer followed the cycle numbers using a custom-made spreadsheet in Excel<sup>®</sup> as a cycle counter (cf. Appendix, section A.1. “A counter of chewing cycles”). After the final cycle number, the observer immediately instructed the subject to halt chewing. Then, the subject spat out the chewing outcome in the appropriate coffee-filter, rinsed the mouth with water for clearing the mouth from all particles and fragments, and spat this rinse water also out in the coffee-filter. A few chewing trials were exercised at the start of a session before the actual experiments were carried out.

#### *2.4. Data processing for determining chewing performance and chewing efficiency*

The particles in the coffee-filters were cleaned using a diluted solution of a dish-washing detergent in hot water (80°C) and by rinsing with hot clean water. The coffee-filters were then folded as bags with the particles inside, which were dried overnight in an oven at 60°C. Each dried bag was weighted and emptied in a stack of 10 wire sieves (diameter 100 mm), with sieve apertures of 8.00, 5.60, 4.00, 2.80, 2.00, 1.40, 1.00, 0.71, 0.50, and 0.25 mm (in general, a factor  $\sqrt{2}$  between successive apertures). While the stack was placed on a sheet of smooth baking paper to recover fragments which might fall outside the stack, a bag was emptied in the stack by tapping the bottom while carefully opening the bag hold up-side-down. Furthermore, following initial emptying, the interior of the bag was gently brushed to release all small fragments. The emptied coffee-filter was weighted and the weight of the chewing outcome equalled the difference between the weight of the bag including content and the weight of the emptied coffee-filter.

Following the procedure of Olthoff et al. (1984, meeting international standards), mechanical stack sieving was continued by hand-sieving for each sieve from top to bottom of the stack. Hand-sieving was carried out above a sheet of smooth baking paper to recover the passing fragments for the remaining sieve stack. Furthermore, each sieve was emptied on such

a sheet, for collecting its content for weighing. The weights of the Optosil particles and fragments on the various sieves were converted to underweight fractions as a function of size  $X$  (sieve aperture) to which curve-fitting was applied using the Rosin-Rammler equation (Olthoff et al., 1984; van der Bilt et al., 1987):

$$Q_W^-(X) = 1 - 2^{-(X/X_{50})^b} \quad \text{equation (1)}$$

where  $Q_W^-(X)$  is the weight fraction of particles with a size smaller than  $X$  (underweight),  $X_{50}$  the median particle size by weight (volume), and  $b$ , the broadness, is a measure of the extent to which the particles are equally sized. The value of  $b$ , which is related to the gradient of the  $Q_W^-(X) - X$  relationship, is inversely related to the extent of variation in  $X$ . The data processing including curve-fitting was programmed in a custom-made spreadsheet of Excel®, using the Solver to determine the least residual sum of squares. The quality of curve-fitting was assessed by the value of  $R^2$ , and by judging whether the outcome of  $X_{50}$  was realistic. Subsequent data processing was also programmed in custom-made spreadsheets of Excel.

For each subject ( $n=8$ ), 16 relationships between underweight and sieve aperture were obtained corresponding with 4 numbers of chews from each of 4 types of particle samples. Curve-fitting using the Rosin-Rammler equation yielded estimates of  $X_{50}$  and  $b$  for all 16 relationships. For each subject, relationships between  $\log(X_{50})$  and  $\log(N)$  and between  $b$  and  $\log(N)$  were then determined for each of 4 types of particle sample. Relationships between  $\log(X_{50})$  and  $\log(N)$  will be further emphasized because determining of chewing ability is a main issue of the present study.

Following some chews,  $X_{50}$  declines with  $N$ , according to a power function (Olthoff et al., 1984; van der Bilt et al., 1987):

$$X_{50} = c \cdot N^{-d} \quad \text{equation (2)}$$

The range of non-initial  $N$ -values for which equation (2) is valid, is revealed in  $\log(X_{50})$ - $\log(N)$  relationships by a linear part with  $-d$  as gradient.

Chewing performance ( $X_{50}$  at a particular  $N$ -value,  $X_{50,N}$ ) could be compared between all types of particle samples for  $N$ -values of 3 and 7 cycles. In order to determine  $N(1/2-X_0)$  of chewing efficiency by which initial cubes of 8.0 mm will be reduced to an  $X_{50}$  of 4 mm, or half-cubes of 9.6 mm to an  $X_{50}$  of 4.8 mm, curve-fitting with a 2<sup>nd</sup> order polynomial function was applied to each set of four  $[\log(X_{50}), \log(N)]$  data points. Such a curve-fitting accounted for the convex shape of  $\log(X_{50})$ - $\log(N)$  relationships for small values of  $N$  (cf. Results, Figs 6 and 8).  $N(1/2-X_0)$  was then determined by solving the 2<sup>nd</sup> order polynomial function for the intersection of the function with the  $\log(4.0 \text{ mm})$  or  $\log(4.8 \text{ mm})$  level respectively.

### 2.5. Determining selection of particles in the initial phase of chewing

Undamaged hence non-selected particles were still present in the chewing outcome of the smallest number of chews,  $N=1, 2$  or  $3$ , for the various types of particle samples (Table 1). Undamaged particles could afterwards be distinguished from damaged or broken particles by visual inspection, because all original particles had a regular shape (cube or half-cube). The number of selected particles equalled the difference between the number of offered particles and the number of non-selected particles. All numbers were converted to mean numbers per trial.

The selection chance across  $N$  chewing cycles,  $S_{(N)}$  was given by:

$$S_{(N)} = n_{s(N)}/n \quad (0 \leq S_{(N)} \leq 1) \quad \text{equation (3)},$$

in which  $n_{s(N)}$  is the number of selected particles per trial, across  $N$  cycles ( $N= 1, 2$  or  $3$ ), and  $n$  is the number of particles which was offered in the sample. Values of  $S_{(N)}$  could be mathematically converted into the average selection chance per chew,  $S_{(1)}$  (van der Glas, van der Bilt, Olthoff & Bosman, 1987):

$$S_{(1)} = 1 - (1 - S_{(N)})^{1/N} \quad \text{equation (4)}$$

$S_{(1)}$  approaches the selection chance in the first chewing cycle of a sequence. A decrease in  $S_{(1)}$  with an increase in  $n$ , reflects the effect of saturation of the breakage sites on the teeth.

The mean number of selected particles in a single chew,  $n_{s(1)}$ , is given by:

$$n_{s(1)} = S_{(1)}.n \quad \text{equation (5)},$$

The value of  $n_{s(1)}$  reveals the extent of reduction of bite force needed for fragmentation in the first cycle, which will be attained by a decrease in  $n$  (cf. Introduction).

### 2.6. Statistical analysis

Using Graphpad software (Graphpad Prism 7.03; Graphpad Software Inc., San Diego, CA), one-way ANOVAs for paired observations were applied for statistical testing of differences between three or four groups of data in which one factor was involved. Bonferroni's multiple comparison tests were subsequently used to determine significance of differences between pairs of data. A two-way ANOVA was applied when two factors were involved, with paired observations for both factors. The level of significance was 5%.

In order to enable comparisons of ratio values between chewing efficiency and chewing performance, all values of measures of chewing ability were logarithmically transformed (cf. section 3.5, Results). Regarding ratios in intra-subject values of chewing ability between types of particle samples, a linear regression analysis was carried out on log-

values of chewing ability from pairs of types of particle samples, considering gradients of the regression functions (Edwards, 1976). Furthermore, Pearson's correlation coefficients were determined between types of particle samples, for three measures of chewing ability, i.e.  $N(1/2-X_0)$  (chewing efficiency) and  $X_{50}$  at  $N = 3$  and  $N = 7$  respectively (chewing performance at two  $N$ -values).

### 3. Results

#### 3.1. weight loss during the procedure

Overall the weight loss (percentage of the baseline weight), was 0.66% before sieving (SD 0.63,  $n=128$ ) and 0.80% after sieving (SD 0.80). Hence, on average 82% ( $0.66/0.80 \times 100\%$ ) of the weight loss occurred before sieving. The maximal value of weight loss from all observations was 3.92%, which occurred for particle samples of 2 half-cubes in the latest chewing phase ( $N=7$  chews for this sample type). Like for the mean loss (82% before sieving), the maximal loss of 3.92% was mainly due to a loss before sieving; it corresponded to a maximal loss of 3.10% (79%) before sieving.

#### 3.2. Quality and validity of assessments of median particle size and size variation

Fig. 1A shows, as an example, the result of curve-fitting of an underweight distribution which was obtained following 3 chews by subject S06 on samples of 9 half-cubes of 9.6 mm. The sieve with aperture 11.3 mm was included as an empty top sieve from the series of sieves used, yielding a cumulative underweight fraction of 1.0 for this aperture. The weight on the sub-top sieve with aperture 8.0 mm included 18 out of 36 (50%) initially offered half-cubes (4 trials with 9 half-cubes) which were undamaged, hence non-selected for breakage. The value of  $R^2$  (0.9968) reflects a quality of curve-fitting which is overall fairly good. Most important is that the curve-fitting was good for the interval of sieve apertures between 8.0 and 11.3 mm, which was decisive for estimating  $X_{50}$  and  $b$ .  $X_{50}$  was assessed as being 9.1 mm by an adequate interpolation between the top sieve of 11.3 mm and the sub-top sieve with aperture 8.0 mm. The value of  $b$ , 8.6, reflects the steep gradient of the interval of sieve apertures used to estimate the value of  $X_{50}$ , and indicates correctly a small variation of particle sizes following a small number of chews ( $N = 3$ ).

Fig. 1B shows the result of curve-fitting in the example of Fig. 1A, without including an empty top sieve of 11.3 mm. Because the sieve with aperture 8.0 mm retained a large fraction of 0.801 (80.1%) of the entire weight of particles and fragments, its underweight fraction was only 0.199. Hence, the level of  $X_{50}$  at an underweight fraction of 0.500 was

located above the range of underweight levels of the sieves with apertures up to 8.0 mm. Curve-fitting of data points up to 8.0 mm therefore yielded an estimation of  $X_{50}$  by extrapolation. The large value of  $R^2$  (0.9993) indicates a very good overall quality of curve-fitting. However, the outcome of  $X_{50}$  (10.9 mm) was unrealistic large for particles with an initial size of 9.6 mm, and following some comminution by 3 chews. Furthermore, the value of  $b$  (3.6) was unrealistic small as it reflects the gradient of the curve, which approached by extrapolation an underweight of 1.0 at an sieve aperture which is larger than the empty sieve of 11.3 mm. The gradient was therefore less steep in Fig. 1B than in Fig. 1A. Hence, it is relevant to consider the first empty sieve with an underweight of 1.0 as the top sieve of the series used for avoiding bias in the values  $X_{50}$  and  $b$ . The first empty sieve was therefore always included in the data processing of the present study.

Fig. 2A shows, as another example, a relationship between underweight and sieve aperture (up to 11.3 mm), and the corresponding weights on the sieves, from subject S06 who had repeatedly (4 trials) chewed on a sample of 8 cubes of 8.0 mm for 3 cycles. In contrast to the half-cubes of 9.6 mm in the previous example (Fig. 1A) that had a mid-size with respect to sieve apertures of 8.0 and 11.3 mm, the cubes had a border size, corresponding with sieve aperture 8.0 mm. Although the weight on the sieve of 8.0 mm included 6 out of 32 (19%) initially offered cubes (4 trials with 8 cubes) which were undamaged, the quality of curve-fitting using the Rosin-Rammler equation (equation (1)) was excellent ( $R^2 = 0.9999$ ). However, the estimated value of  $X_{50}$  was 8.4 mm, hence unrealistic large for an initial cube size of 8.0 mm, and following comminution by 3 chewing cycles.

Fig. 2B shows the underweight-size relationship which simulates closely the relationship for subject S06 just before chewing was started under the same particle conditions. To that end, the entire weight across the various sieves in Fig. 2A (29.144 g) was placed on the sieve with aperture 8.0 mm, corresponding to placing 32 cubes of 8 mm (the total of 4 pooled trials of 8 cubes) on that sieve. Furthermore, a tiny negligible weight (1 per mil of the weight) was placed at the bottom of the sieve stack to construct a step-function between underweight and sieve aperture. Curve-fitting of this step-function using the Rosin-Rammler equation yielded an estimation of 9.6 mm of  $X_{50}$  which is the mid-size between the sieve apertures 8.0 and 11.3 mm, and not the edge size of 8.0 mm of the cubes. Hence, Figs 2A and 2B show that as long as initial cubes of border size 8.0 mm, are present on the sieve with the same aperture size, the value of  $X_{50}$  will be overestimated between 8.0 and 9.6 mm by curve-fitting using the Rosin-Rammler equation.

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Figs 1A, 1B and Figs 2A, 2B about here

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The examples in Figs 1 and 2 show that, apart from including the first empty top sieve in data processing, it is also important to choose an initial size of the single-sized particles which corresponds with the mid-size of the initial upper size class with particles. A mixture of irregularly shaped grains will occur in the middle of the chewing process, in which the particles of each size class will on average have a size which corresponds to the mid-size between two subsequent borders of size classes. Curve-fitting of the particle size distribution, using the Rosin-Rammler equation then yields unbiased values of  $X_{50}$  and  $b$ .

In order to examine whether the quality of curve-fitting is influenced by a pronounced presence of initial particles, values of  $R^2$  were compared between an initial stage of chewing and a non-initial stage. The number of chews from different particle samples were grouped according to similarly reduced values of  $X_{50}$  (cf. Fig. 6). Hence, the smallest number of chews for each type of particle sample ( $N = 1, 2$  or  $3$ ) was considered as initial chewing stage ('chewing stage 1'), and the 3<sup>rd</sup> number of chews in the series of the various sample types ( $N = 3, 7$  or  $14$ ) as a non-initial stage ('chewing stage 3'). Undamaged initial particles were present in stage 1 with a mean overall incidence of 24.9% in the pooled chewing results. In contrast, undamaged initial particles were nearly absent in stage 3 with a mean overall incidence of 0.5%. A two-way ANOVA for paired observations ( $n = 8$  subjects) with two factors, 'chewing stage' (2 levels) and 'particle sample' (4 levels) showed that effects on  $R^2$  of either chewing stage or particle sample were non-significant, indicating that there was no common direction of change in  $R^2$  between chewing stage 1 and stage 3 for the various types of particle samples (Fig. 3). Only the interaction between both factors was significant ( $p < 0.05$ ) which indicates that sample-specific changes in  $R^2$  occurred. The inter-stage increase in  $R^2$ , which occurred for samples of 9 half-cubes of 9.6 mm (Fig.3) was significant ( $p < 0.05$ ) in Bonferroni's multiple comparison tests. The differences in  $R^2$  values were notably small between subjects and conditions. Single  $R^2$  values varied within a range from 0.9794 to 0.9999. The minimal  $R^2$  value of all conditions (0.9794) occurred in subject S07 for chewing stage 1 with 9 half-cubes of 9.6 mm ( $N = 3$ ). Like for subject S06 in Fig. 1A, the overall quality of curve-fitting was fairly good, and the curve fitting was good for the interval of sieve apertures which was decisive for estimating realistic values of  $X_{50}$  and  $b$ .

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Fig. 3 about here

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### 3.3. Initial selection chance and number of selected particles

Non-selected particles from chewing stage 1 ( $N = 1, 2$  or  $3$ ) were detected by visual inspection. Fig. 4 shows the selection chance per single chew,  $S_{(1)}$  (cf. section 2.5.) for the various types of particle samples. A one-way ANOVA for paired observations ( $n = 8$  subjects) showed that  $S_{(1)}$  differed significantly ( $p < 0.0001$ ) between types of particle samples. In Bonferroni's multiple comparison tests, the difference in  $S_{(1)}$  was non-significant between 8 cubes of 8mm and 9 half-cubes of 9.6 mm, but  $S_{(1)}$  differed significantly ( $p < 0.0001-0.05$ ) between all other pairs of particle samples. The mean value of  $S_{(1)}$  was similarly small for 8 cubes and 9 half-cubes, and  $S_{(1)}$  increased towards a large mean value of 0.90 (close to the maximal possible value of 1.00) with a decrease in particle number of the half-cubes per trial, from 9 to 2 (Fig. 4).

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 Fig. 4 and Fig. 5 about here  
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Fig. 5 shows the number of selected particles during a single chew,  $n_{s(1)}$ , from chewing stage 1, for the 4 types of particle samples. A one-way ANOVA for paired observations showed that  $n_{s(1)}$  differed significantly ( $p < 0.05$ ) between types of particle samples. Significance between pairs of particle samples did not occur in Bonferroni's multiple comparison tests. However, the overall significance of inter-sample differences reflects that whereas  $n_{s(1)}$  was similar for 8 cubes of 8 mm and 9 half-cubes of 9.6 at a mean level of 2.9-3.0 particles,  $n_{s(1)}$  decreased gradually to a mean value of 1.8 particles when the number of half-cubes was decreased from 9 to 2.

### 3.4. Relationships between median particle size, broadness and number of chews

Fig 6 shows group results to summarize the  $\log(X_{50})-\log(N)$  relationships for the four types of particle samples. The first part of a mean  $\log(X_{50})-\log(N)$  relationship had a convex shape; the relationship approached thereafter a linear one for larger values of  $\log(N)$  when  $X_{50}$  decreased with  $N$  according to a power function (equation (2), cf. section 2.4.). The  $\log(X_{50})-\log(N)$  relationships were similar between samples of 8 cubes of 8.0 mm and those of 9 cubes of 9.6 mm, of which apart from particle number, the total particle volume was also similar (4.1 vs. 4.0  $\text{cm}^3$ ). The more the particle number was reduced in samples of half-cubes, the more the  $\log(X_{50})-\log(N)$  relationship shifted towards a range of smaller  $\log(N)$  values (a shift



to the left in Fig. 6). Hence, the rate of particle size reduction increased when the number of offered particles was reduced. The levels of the horizontal lines at the bottom of the arrows in Fig. 6 indicate the levels of  $\log(X_{50})$  at which the initial particle size was halved for half-cubes or cubes (at  $\log(4.8 \text{ mm})$  and  $\log(4.0 \text{ mm})$  respectively). In Fig. 6, the log-number of chews needed to halve the initial particle size,  $\log(N(1/2-X_0))$  (the log-value of chewing efficiency), corresponds for each type of particle sample with the intersection of its group function with the log-level of half its initial particle size. The shift of the group function of  $\log(X_{50})-\log(N)$  with the decrease in numbers of half-cubes in the samples was reflected in smaller  $\log(N(1/2-X_0))$  values and smaller values of  $N(1/2-X_0)$  according to these functions (Fig. 6, legend).

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 Fig. 6 and Fig. 7 about here  
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Fig. 7 shows group results of  $b-\log(N)$  relationships. Regardless of the type of particle sample, the value of  $b$  was initially large and decreased towards a level of about 2.2. An initially large value of  $b$  is self-evident because of a start of chewing on initially single-sized particles with hardly any variation in size.

Relationships between  $\log(X_{50})$  and  $\log(N)$  from individuals are of interest to determine chewing efficiency for each subject. Fig. 8 shows, as example, the  $\log(X_{50})-\log(N)$  relationships and functions from two subjects who differed most in chewing efficiency for samples of 4 half-cubes of 9.6 mm. Because of smaller values of  $\log(X_{50})$ , the intersection between the function and the level of half the initial size of the half-cubes ( $\log(4.8 \text{ mm})$ ) occurred at a small  $\log(N)$  value for subject S01 (Fig. 8A, dots), which corresponded with a small value of 2.7 cycles for  $N(1/2-X_0)$ . In contrast, the values of  $\log(X_{50})$  were larger for subject S06 (Fig. 8A, squares) so that the intersection of the  $\log(X_{50})-\log(N)$  function with the level of  $\log(4.8 \text{ mm})$  occurred at a larger value of  $\log(N)$ , corresponding with a larger value of 10.1 cycles for  $N(1/2-X_0)$ . It is notable that the  $\log(X_{50})-\log(N)$  relationship was approximately linear for the subject with a large chewing efficiency ( $N(1/2-X_0)=2.7$  cycles) and had a pronounced convex shape for the subject with a small chewing efficiency ( $N(1/2-X_0)=10.1$  cycles). Because of the incidence of linear  $\log(X_{50})-\log(N)$  relationships for subjects with a large chewing efficiency, the group function for samples of 4 half-cubes was less curved than those of the other types of particle samples (Fig. 6). Fig. 8B shows the log-values of  $X_{50}$  related to chewing performance of the two subjects at  $N=3$  and  $N=7$  respectively. These log-values were smaller for subject S01 than for subject S06, indicating a larger chewing performance of subject S01 for both numbers of chews. However, because of a

different shape of the two  $\log(X_{50})-\log(N)$  relationships, the distance between the log-values of  $X_{50}$  was larger at  $N=7$  than at  $N=3$ , reflecting a larger inter-subject ratio between the  $X_{50}$ -values of chewing performance at  $N=7$ .

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 Fig. 8, Table 2 and Table 3 about here  
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### 3.5. Comparisons between chewing efficiency and chewing performance

Table 2 shows the chewing efficiency,  $N(1/2-X_o)$ , for each subject, and the four types of particle samples. A one-way ANOVA for paired observations ( $n = 8$  subjects) showed that  $N(1/2-X_o)$  differed significantly ( $p < 0.0001$ ) between types of particle samples. In Bonferroni's multiple comparison tests,  $N(1/2-X_o)$  differed significantly ( $p < 0.0001-0.05$ ) between all pairs of particle samples.  $N(1/2-X_o)$  was always the largest for samples of 8 cubes of 8 mm, and the smallest for samples of 2 half-cubes of 9.6 mm. Furthermore,  $N(1/2-X_o)$  decreased continuously when the number of half-cubes was decreased from 9 to 2.

Table 3 shows values of chewing performance, *i.e.* values of  $X_{50}$  for two numbers of chewing cycles,  $N=3$  (Table 3, left) and  $N=7$  (Table 3, right), which were tested with all types of particle samples. A one-way ANOVA for paired observations ( $n = 8$  subjects) showed that  $X_{50}$  differed significantly ( $p < 0.0001$ ) between types of particle samples for  $N=3$  as well as for  $N=7$ . In Bonferroni's multiple comparison tests,  $X_{50}$  differed significantly ( $p < 0.0001-0.05$ ) between many pairs of particle samples (Table 3). At both  $N$ -values,  $X_{50}$  of chewing performance was the largest in 7 out of 8 subjects for samples of 9 half-cubes of 9.6 mm, and the smallest for samples of 2 half-cubes of 9.6 mm. Furthermore,  $X_{50}$  decreased continuously when the number of half-cubes was decreased.

In order to enable comparisons between chewing efficiency and chewing performance, all values of measures of chewing ability (cf. Tables 2 and 3) were logarithmically transformed. Differences in log-values correspond with log-values of ratios between non-transformed values. Log-values of ratios are linear and have a zero point which corresponds to a ratio value of 1. It was examined whether constant ratios were involved in intra-subject values of chewing ability (either from chewing efficiency or chewing performance) between the various types of particle samples. Furthermore, it was examined whether constant ratios were involved between inter-subject values of chewing ability within particle samples.

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 Fig. 9 A,B about here  
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Regarding ratios in intra-subject values of chewing ability between particle samples, Fig. 9A shows examples of relationships between log-values of chewing efficiency from the 8 subjects, for two pairs of particle samples. Fig. 9B shows these relationships for log-values of chewing performance at  $N=7$ . The scatter around the regression lines (the standard error of estimate,  $S_{Y.X}$ ) of the various inter-sample log-log relationships (in total 6 regressions with 4 types of particle samples) was always similar, regardless of whether chewing efficiency or chewing performance was involved.  $S_{Y.X}$  was on average 0.0701 log-units (SD 0.0121,  $n=6$ ) for chewing efficiency, 0.0620 log-units (SD 0.0225) for chewing performance at  $N=3$ , and 0.0646 log-units (SD 0.0204) for chewing performance at  $N=7$ . Similar gradients occurred in the group of regression lines for chewing efficiency (mean 0.976, SD 0.109,  $n=6$  regression lines), and also in the group for chewing performance following 3 cycles (mean 1.417, SD 0.253) or 7 cycles respectively (mean 1.257, SD 0.216). Similar gradients, hence approximately parallel regression lines reflects ratios between values of chewing efficiency or performance from different pairs of particle samples, which are specific for these pairs and nearly constant for the various subjects. For example, the X-distance between the two nearly parallel regression lines in Fig. 9A was 0.325 log-units at the mean Y-level of the data points from samples of 2 half-cubes. Samples of 2 half-cubes are common in the example of two regression functions in Fig. 9A for pairs of samples in which also 4 or 9 half-cubes are involved respectively. Hence, the X-distance of 0.325 log-units corresponds with an approximately constant ratio of 2.11 ( $=10^{0.325}$ ) between non-transformed values of chewing efficiency from the various subjects, between particle samples of 9 and 4 half-cubes. The coefficient of variation (SD/mean) was 0.112 (11.2% of the mean) for the gradients of the regression lines related to chewing efficiency and was 0.178 and 0.172 for the gradients related to chewing performance at  $N=3$  and  $N=7$  respectively. Hence, the regression lines were most parallel (least variation in gradient) for chewing efficiency and the inter-sample ratios of measures of chewing ability were therefore more constant for chewing efficiency than for chewing performance.

An SD-value related to the log-values of chewing ability from the various subjects within a type of particle sample, is a measure of the variation in inter-log values (hence inter-subject ratios) within that sample. These SD-values (Tables 2 and 3, bottom, bold) have been grouped in Table 4 according to type of particle sample and measure of chewing ability. A one-way ANOVA for paired observations ( $n = 4$  particle samples) showed that the intra-sample variation in log-values differed between measures of chewing ability ( $p < 0.01$ ). In Bonferroni's multiple comparison tests, the variation was larger ( $p < 0.05$ ) for chewing efficiency than for chewing performance, either at  $N=3$  or  $N=7$ . The variation differed also

( $p < 0.05$ ) between both measures of chewing performance. The larger variation for chewing efficiency (mean: 0.206 vs. 0.082-0.115 log-units for chewing performance) reflects a range of inter-subject log-values of chewing ability which was wider for chewing efficiency than for chewing performance (see also Figs 9A and 9B).

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 Table 4 and Table 5 about here  
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Apart from comparing the mean values of the measure of variation between log-measures of chewing ability, it is also of interest to consider the coefficient of variation ( $CV = SD/mean$ ) of the measure of variation. This because a small CV for a particular log-measure of chewing ability indicates more similar inter-subject log-values hence more similar ratios in the values of chewing ability for the various types of particle samples than a large CV. Table 4 shows that the inter-subject variation between different particle samples was more similar for chewing efficiency ( $CV = 0.068$ ; 6.8% of the mean) than for chewing performance at  $N = 3$  ( $CV = 0.393$ ; 39.3%) or  $N = 7$  ( $CV = 0.274$ ; 27.4%).

Less than 4 types of particle samples were tested at  $N = 14$  (3 types: CU-P8, HC-P9 and HC-P4; Fig. 6) and  $N = 28$  (2 types: CU-P8 and HC-P9), excluding an extensive statistical testing. However, the mean inter-subject variation of log-values was still smaller for chewing performance at larger number of chewing cycles of  $N = 14$  and  $N = 28$  (0.130-0.133 log-units) than that for chewing efficiency (0.206 log-units). However, CV became similarly small (0.067-0.050) for chewing performance as the CV for chewing efficiency (0.068).

A similar degree of scatter around the regression functions of relationships between log-values of chewing ability from pairs of particle samples, but a larger inter-subject range of these values yielded a degree of correlation of intra-subject log-values between particle samples which was larger for chewing efficiency than for chewing performance. Pearson's correlation coefficients ( $r$ ) have been grouped in Table 5 according to pairs of particle samples and measure of chewing ability. A one-way ANOVA for paired observations ( $n = 6$  pairs of particle samples) showed that the inter-sample correlation differed between measures of chewing ability ( $p < 0.05$ ). In Bonferroni's multiple comparison tests, the degree of correlation between pairs of particle samples was larger ( $p < 0.05$ ) for chewing efficiency than for chewing performance, either following 3 chewing cycles or 7 chewing cycles.

## Discussion

### 4.1 Validity of testing and data processing

The present study shows that the initial particle size should be a mid-size with respect to borders of the size-classes which are chosen. Furthermore, it is essential to include a lower border of an empty top class, yielding a value of 1.0 in the cumulative size distribution as a logic extension of a series of cumulative values including an inevitable end-value of 1.0. When both conditions are met, unbiased values  $X_{50}$  and  $b$  are consistently obtained for all chewing phases, following curve-fitting of a cumulative size distribution by weight (volume) using the Rosin-Rammler equation. The values of  $R^2$  are large and vary within a narrow range, indicating a curve-fitting which varies from fairly good (good for the size class which is decisive for assessing  $X_{50}$  and  $b$ ) to excellent, also for the initial chewing stage in which undamaged initial particles are present. Except for samples of 9 half-cubes, for which the quality of curve-fitting is slightly less optimal with a pronounced presence of initial particles, such an effect is either absent or non-significant otherwise.

In accordance with theoretical and simulation studies of chewing (Baragar et al., 1996; van der Bilt et al., 1992; Voon et al., 1986), reducing the number of particles in the initial samples is effective for increasing the rate of food comminution, which is reflected in pronounced shifts of  $\log(X_{50})-\log(N)$  relationships towards ranges of smaller  $N$ -values. The selection chance during a single initial chewing cycle increases on average from 0.33 to 0.90 when the number of half-cubes of 9.6 mm per sample is reduced from 9 to 2. The much smaller selection chance for 9 half-cubes of 9.6 mm which is similarly small (0.33) as the one (0.36) for 8 cubes of 8 mm, indicates an initial saturation of the breakage sites for such numbers of large particles.

In samples with only two half-cubes of 9.6 mm, the sample volume ( $0.88 \text{ cm}^3$ ) was much reduced, with respect to the sample volume of a traditional test using 8 cubes of 8 mm ( $4.10 \text{ cm}^3$ ) or 17 cubes of 5.6 mm ( $3.00 \text{ cm}^3$ ). Using a small sample volume has the risk of losing relatively more small fragments of Optosil during chewing and/or after spitting out the test food. The loss of fragments was, however, always small and negligible in the present study. For samples of 2 half-cubes, the total loss following the entire procedure including sieving, was on average only 1.4% for the latest stage of chewing ( $N=7$  for this sample type), with a maximum of 3.9%. Limiting the loss to a small amount is mainly due to the use of coffee-filters (with a round bottom) for collecting the chewing outcome, and to using sheets of smooth paper for collecting particles and fragments during the sieving procedure.

While the selection chance is increased from 0.33 to 0.90 by reducing the number of half-cubes from 9 to 2, the number of initially selected particles decreases from on average 3.0 to 1.8. This reduction in selected numbers by 40% yields a reduction of about 40% in bite

force (1.8/3.0\*100%) needed to fracture 1.8 selected particles. Maximal bite force is considerably reduced in various categories of subjects whose chewing ability is greatly impaired (35-63%; for an overview cf. van der Glas et al., 2012). A decrease of 40% in required bite force by offering only two half-cubes, will make a chewing test using Optosil suitable for subjects with an impaired chewing ability, the more since previous studies used cubes with an edge size of 8.0 or 5.6 mm rather than weaker half-cubes with a height of 4.8 mm. Further reduction of the required bite force can be attained by weakening Optosil by mixing the base with a non-prescribed catalyst (Optoweak; van der Glas et al., 2012; also a means of making rheological characteristics of current Optosil Comfort® similar to those of Optosil, version 1980), heating the base (Optosoft; van den Braber, van der Glas, van der Bilt & Bosman, 2001), or adding substances to the base (Optocal; Slagter, van der Glas, Bosman & Olthoff, 1992b; Pocztaruk et al., 2008).

The initial chewing phase is important to consider because much food comminution occurs initially with respect to an  $X_{50}$  of about 2 mm at swallowing. Like in previous studies (Olthoff et al., 1984; van der Bilt et al., 1987),  $X_{50}$  decreased in the present study from 8.0 mm (samples of 8 cubes) to on average 4.5 mm following 14 chewing cycles and to 2.8 mm following 28 cycles. Halving of the initial particle size occurs also in an initial phase of chewing, *i.e.* within a range of 7-26 cycles for samples of 8 cubes of 8.0 mm, and within a range of 2-7 cycles for samples of 2 half-cubes of 9.6 mm. Determining  $N(1/2-X_0)$  is of special importance because reaching the stage of halving an initially large particle size concurs with a phase transition from a lower to a higher rate of food comminution (see below).

Cumulative particle size distributions by weight, hence volume, are directly obtained using sieving. Such a distribution is of interest in sight of digestion and flavour release as the reversed value of  $X_{50}$  is related to the rate of total surface area produced during chewing (Lucas & Luke, 1984). As an alternative for sieving, optical scanning has been used as a less time consuming technique. Optical scanning yields particle size distributions by projected area to which curve-fitting has been applied using the Rosin-Rammler equation for assessing  $X_{50}$  and  $b$  by projected area (Hutchings et al., 2012; Yven et al., 2010). Although values of  $X_{50}$  by volume differs from those by projected area, both techniques have been used for measures of chewing ability. While sieving always yields a cumulative size distribution with discrete values, the cumulative distribution from optical scanning can be continuous following ranking of the size values of the individual particles. The recommendations from the present study regarding initial particle shape and amount remain then relevant for attaining a test on

chewing ability with short chewing sequences and with a reduced bite force needed. Following calibration, projected size and area data from optical scanning can be mathematically converted to a discrete cumulative size distribution by volume (Eberhard et al., 2012, 2015). All recommendations from the present study are then relevant, including those on matching a mid-size of the initial particles with respect to the border sizes of the upper class, and on including the value 1.0 of the upper border size of the upper class, in the cumulative size distribution by volume.

#### 4.2. background of $\log(X_{50})$ - $\log(N)$ relationships

The  $\log(X_{50})$ - $\log(N)$  relationships have initially a convex shape for large numbers (8-9) of cubes or half-cubes of which the size (8.0-9.6 mm) is relatively large with respect to the bucco-lingual dimensions of the posterior teeth (about 4-5 mm). This shape is in part due to an initial saturation of the breakage sites on the teeth. Saturation causes a reduced selection chance of the particles for subsequent breakage and therefore initially a reduced rate of food comminution. Furthermore, the rate of food comminution will initially also be reduced due to a one-way interaction between large particles and smaller fragments in their competition for occupying breakage sites (van der Glas et al., 2018). Large particles have a height advantage with respect to small ones to become engaged between antagonistic teeth. When small fragments are present only in small numbers in the initial phase of chewing, they cannot pile to compensate their height disadvantage so that large particles are engaged first during jaw closing and less breakage sites are left for the smaller particles. Hence the selection of small particles is then hampered by the presence of large particles and reversely not, yielding a one-way interaction in selection between particles of different size. When smaller particles become abundant in a later phase of chewing, they can compensate their height disadvantage by particle piling, and a mutual competition occurs then between larger and smaller particles for the breakage sites. A one-way interaction in selection as well as a two-way interaction have been theoretically modelled (van der Glas et al., 1992) and confirmed in one-chew experiments using simple mixtures with two particle sizes (van der Glas et al., 2018).

Apart from one-chew experiments, further evidence that the later phase of natural chewing is related to a two-way interaction in selection is given by the following findings.  $X_{50}$  decreases with  $N$  in a later phase according to a power function with  $-d$  as exponent (equation 2);  $\log(X_{50})$  decreases then linearly with  $\log(N)$  with  $-d$  as gradient (Olthoff et al., 1984; Figs 6 and 8). Simulation and theoretical studies (van der Bilt et al., 1992; Baragar et al., 1996) have shown that the value of  $d$  equals (largely because of some assumptions for

simplification) the inversed value of another exponent  $w$  (hence  $d \approx 1/w$ ), from the power function which describes the relationship between selection chance and particle size  $X$  during chewing, *i.e.*  $S(X) = v.X^w$  ( $0 \leq S(X) \leq 1$ ) (Lucas & Luke, 1983; van der Glas et al., 1987). Experiments with colour-and-form labelled particles show that the exponent  $w$  has a subject-specific value which remains constant between non-initial phases of chewing (van der Glas et al., 1987). A constant value of  $w$  indicates that the ratios in selection chance between two arbitrary sizes are then constant, a finding which is in agreement with the prediction of the theoretical two-way interaction model for selection but not with that of the one-way model (van der Glas et al., 1992). In sight of the close relationship between  $d$  and  $w$  and the constancy of  $w$  with  $N$ , the later phase of chewing (the linear part of a  $\log(X_{50})-\log(N)$  relationship), is related to a two-way interaction between larger and smaller particles in selection. The initial phase with a lower rate of reduction in  $X_{50}$  (the convex-shaped part of a  $\log(X_{50})-\log(N)$  relationship) is related to a one-way interaction in selection that progresses gradually in a two-way interaction.

When the number of half-cubes is reduced from 9 to 4, hence reducing the degree of initial saturation of the breakage sites and shortening the phase with an one-way interaction in selection, the initial shape of  $\log(X_{50})-\log(N)$  relationships becomes less convex, even approximately linear in subjects with a large chewing efficiency. However, the  $\log(X_{50})-\log(N)$  relationships have initially again a pronounced convex shape when the particle number of half-cubes is reduced to 2. Without saturation of the breakage sites for such small particle numbers (large selection chance of 0.90), this initial shape is due to an enhanced breakage, hence a larger degree of fragmentation of the selected particles during the first chewing cycle. Offering 2 half-cubes of 9.6 mm yields a larger degree of fragmentation of selected particles during a single chew than offering 4 or 9 half-cubes (unpublished observations), which is probably due to a more optimal placement between antagonistic teeth when large particles are offered in small numbers. Hence, an enhanced breakage of on average 1.8 half-cubes which are selected out of 2 offered half-cubes, will cause an enhanced reduction in  $X_{50}$ , during the first chewing cycle. This enhanced reduction will yield initially a lower  $\log(X_{50})$  level in the  $\log(X_{50})-\log(N)$  relationship than with 4 offered half-cubes (Fig. 6). The degree of fragmentation will be reduced from the second chewing cycle on because more particles are present from the first cycle. Together with some initial one-way interaction in selection, the rate of a further decrease in  $X_{50}$  will therefore be reduced until the transition to a two-way interaction in selection, yielding a convex initial shape of the  $\log(X_{50})-\log(N)$  relationship.



#### 4.3. Considerations on chewing efficiency and chewing performance

The criterion for chewing efficiency is the number of chews needed to attain a value of  $X_{50}$  which is half the initial particle size,  $1/2-X_0$ . The log-value,  $\log(1/2-X_0)$  intersects a  $\log(X_{50})$ - $\log(N)$  relationship at the transition between the initial phase of this relationship with its convex shape and the subsequent phase in which  $\log(X_{50})$  decreases linearly as a function of  $\log(N)$  with gradient  $-d$ . Such an intersection near the origin of the linear part occurs in the  $\log(X_{50})$ - $\log(N)$  relationships from individual subjects (Fig. 8A), and in the group relationships, regardless of the type of particle sample (Fig.6).

Regarding the similar gradient  $-d$  for four types of samples (group relationships), the phase transition in a  $\log(X_{50})$ - $\log(N)$  relationship reflects a transition between a one-way interaction of larger and smaller particles in selection and subsequently a two-way interaction (cf. section 4.2., above). The values of  $w$  (and the values of  $d$ , which approximately equals  $1/w$ ) from various subjects remain constant between non-initial phases of chewing. Hence, although the number of particles of various sizes varies between such chewing phases, the values of  $w$  and  $d$  do not depend on this variation under conditions of a two-way interaction in selection. The gradient  $-d$  in  $\log(X_{50})$ - $\log(N)$  relationships will therefore be nearly identical for various types of particle samples, despite that chewing on these samples yields different particle numbers in a mixture. Previous studies with additionally larger numbers of chews than in the present study (Olthoff et al., 1984; van der Bilt et al., 1987) show gradients  $-d$  which are still constant within subjects following 120-160 chews on Optosil. Values of  $d$  between young adults with a natural dentition, vary within a range of 0.46 to 0.78.

Hence, the initial curvature of  $\log(X_{50})$ - $\log(N)$  relationships from different types of particle samples varies between subjects, but the subsequent linear part is parallel between types of samples, with a subject-specific gradient  $-d$ . Such characteristics have the following consequences for values of chewing efficiency of individual subjects and for inter-subject comparisons. First,  $\log(N(1/2-X_0))$  which occurs near the origin of the linear part of a subject's  $\log(X_{50})$ - $\log(N)$  relationship, will have a particle-sample-specific value. Values of  $\log(N(1/2-X_0))$  from different types of samples have mutually specific log-distances, which reflect constant ratios between the sample-specific values of  $N(1/2-X_0)$  from a subject. An analysis of log-transformed experimental values of chewing efficiency reveals that inter-sample ratios are nearly constant indeed for any subject. Thus, the gradients of regression lines between log-values of chewing efficiency from pairs of sample types are nearly parallel with a coefficient of variation, CV, of 11.2%. Furthermore, a large degree of correlation

occurs between intra-subject log-values of chewing efficiency for various pairs of particle samples ( $r=0.918-0.970$ ).

Because of constant inter-particle-sample ratios for any particular subject, a sample-specific value of  $\log(N(1/2-X_0))$  of another subject will be shifted with a subject-specific log-distance along the  $\log(1/2-X_0)$  level. This log-distance reflects a constant ratio between  $N(1/2-X_0)$  values from two subjects when the same type of particle sample is used. A small degree of inter-sample variation ( $CV=6.8\%$ ) in the inter-subject variation of  $\log(N(1/2-X_0))$  values within samples, together with a large intra-subject correlation between samples ( $r=0.918-0.970$ ), indicate that the inter-subject ratios are similar for the various types of samples.

Inter-subject differences in chewing efficiency can be compared straightforwardly by choosing a range with larger numbers of chews for determining  $N(1/2-X_0)$  when chewing ability is impaired and a range of smaller numbers of chews otherwise. Chewing ability is then compared at the same stage of food comminution. Because of constant inter-sample ratios, it is also possible to use, for example, samples of only two half-cubes for the subjects with a greatly impaired chewing ability and samples of four half-cubes for control subjects (cf. Appendix: section A.2. Some recommendations on procedures for chewing efficiency). Furthermore, constant inter-sample ratios facilitate inter-study comparisons when different types of particle samples have been used.

The second consequence of  $\log(X_{50})-\log(N)$  relationships with initially a convex shape and subsequently a linear one is that if another criterion would be chosen for chewing efficiency, for example, the number of chewing cycles needed to reduce the initial particle size to a quarter, the number of cycles related to chewing efficiency will change.  $N(1/4-X_0)$  will become larger than  $N(1/2-X_0)$  because of a level of  $\log(1/4-X_0)$  which is lower than that of  $\log(1/2-X_0)$  for intersection with the  $\log(X_{50})-\log(N)$  relationship. However, the particle-sample-specific ratios between values of  $N(1/4-X_0)$  will not change within a single subject because of a constant gradient of the  $\log(X_{50})-\log(N)$  relationship, with the  $d$ -value of that subject. On the other hand, the mutual ratios between different subjects will change because the  $d$ -value varies between subjects. It is notable that for  $N(1/2-X_0)$  when using half-cubes of 9.6 mm (large particles with respect to the cross-sectional dimensions of the teeth), the intersection of the  $\log(1/2-X_0)$  level with an  $\log(X_{50})-\log(N)$  relationship always occur at the inter-phase transition from a lower to a higher rate of particle size reduction. Hence, by using  $N(1/2-X_0)$  as a measure for chewing efficiency, different subjects are not only compared at the same stage of particle size reduction but also at a similar transition in rate of reduction.

Regarding consequences of the characteristics of  $\log(X_{50})-\log(N)$  relationships for chewing performance in individuals: constant ratios in inter-subject values of chewing performance,  $X_{50}$  at some reference value of  $N$ , are inherently impossible. Constant inter-subject ratios are prevented because of inter-subject differences in curvature of the  $\log(X_{50})-\log(N)$  relationships even within a single type of particle sample (Fig. 8B), and differences in  $d$ -values between subjects. Furthermore, an inter-subject comparison at an  $N$ -value in the initial chewing phase will be confounded with the influence of type of particle sample on the curvature of the  $\log(X_{50})-\log(N)$  relationship (Fig. 6). All these confounding factors disturb the use of chewing performance. Although the variation in gradients of regression lines between  $\log(X_{50})$ -values of chewing performance from pairs of sample types is larger than the variation from  $\log(N/2-X_0)$  values of chewing efficiency, the gradients from chewing performance are statistically similar in the present study. However, this similarity is due to a lack of statistical power with small numbers of data rather than to really constant inter-particle-sample ratios for chewing performance at  $N=3$  or  $N=7$ . Constant inter-sample ratios for chewing performance are only possible for large  $N$ -values ( $N > 14$  cycles, cf. Fig. 6), in a range where the  $\log(X_{50})-\log(N)$  relationships from different types of samples are parallel with a gradient  $-d$  which is on average constant for a group of subjects.

A comparison between subjects with different chewing ability is less straightforward for chewing performance than for chewing efficiency. In contrast to chewing efficiency, chewing performance does not directly reflect the amount of inter-subject shifts in  $\log(X_{50})-\log(N)$  relationships. The  $\log(X_{50})$  values from chewing performance are related to different stages of food comminution in the various subjects because  $\log(X_{50})$  values from the subjects' mutually shifted  $\log(X_{50})-\log(N)$  relationships along the  $\log(N)$  axis, are compared at a single value of  $N$  (cf. Fig. 8B). The stage of food comminution which is considered in each subject (reflected in values of  $X_{50}$  and  $\log(X_{50})$ ) and the inter-subject differences or ratios between stages depends on an arbitrarily chosen  $N$ -value. Difference or ratio values in chewing performance are therefore arbitrary. In contrast, the inter-subject differences and ratios in chewing efficiency,  $N(1/2-X_0)$ , are related to inter-subject differences in the number of chewing cycles needed to attain special stage conditions of food comminution; values of  $N(1/2-X_0)$  are therefore not arbitrary. Hence, using chewing efficiency is preferable over chewing performance because of a comparison of inter-subject chewing ability at the same stage of food comminution. Furthermore, constant intra-subject ratios occur for chewing efficiency between types of particle samples, and constant inter-subject ratios within types of samples. Using particle samples of two half-cubes, chewing efficiency can be determined

within 10 minutes or less by using three or even two  $N$ -values (cf. Appendix, section A.2. ‘Some recommendations on procedures for chewing efficiency).

Because preparing food for swallowing is a primary aim of mastication, the masticatory normative indicator (MNI) has been introduced with a cut-off point to distinguish between normal and abnormal masticatory function (Woda et al., 2010). MNI is the value of  $X_{50}$  at swallowing threshold. The cut-off value of MNI is 4 mm when a cylindrical tablet of raw carrot (diameter: 20 mm, height: 10 mm) is used as a test food, which like Optosil forms a loose aggregation of particles during chewing rather than a coherent bolus. Because an impaired functioning of oral structures will be reflected in a reduced chewing ability during an entire chewing sequence, chewing efficiency determined in an early phase of chewing may be strongly associated with MNI determined in a late phase. A first evidence for a strong association in chewing ability between an early and a late phase is the finding that chewing efficiency using the criterion  $N(1/2-X_0)$  is highly correlated (Pearson’s  $r=0.977$ ) with chewing efficiency using the criterion  $N(1/4-X_0)$  (unpublished observations; a study using 9 Optosil half-cubes of 9.6 mm and longer chewing sequences than presently). Determining chewing efficiency in an early phase has two advantages: (1) less time needed for sieving or optical scanning because of particle size distributions where large particles predominate, and (2) much less risk on choking in subjects whose chewing ability is largely impaired.

#### 4.4. Conclusions

Two conditions should be met to obtain valid results of  $X_{50}$  and  $b$  across the entire range of chewing cycles: (1) the initial size of the single-sized particles on which chewing is started should have a mid-size of the upper class of sizes, and (2) the value of 1.0 of the upper border size of the upper class should be included in the cumulative size distribution by volume. By determining chewing efficiency,  $N(1/2-X_0)$ , inter-subject chewing ability is compared at the same stage of food comminution and at a similar phase transition in the rate of food comminution. By using only 2 half-cubes of Optosil with a larger edge size of 9.6 mm (sample volume: 0.88 cm<sup>3</sup>) as initial particles, less numbers of chewing cycles are needed to determine  $N(1/2-X_0)$ , and the bite force needed for fracturing is 40% less than traditionally, thus enhancing feasibility of testing for subjects with an impaired chewing ability.

#### Conflict of interest

The authors report no conflict of interest.

## **Ethical approval**

This study was approved by the Ethics Committee of the Zhejiang Gongshang University, Hangzhou, China (ref no. 2017060201).

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## **Appendix**

### *A.1. A counter of chewing cycles*

The observer followed the cycle numbers during chewing of the subject, using a custom-made spreadsheet in Excel<sup>®</sup> as a cycle counter. This spreadsheet displayed the consecutive cycle numbers on a screen behind the subject. For each total cycle number, a specific Excel file was activated at a distance by using a wireless keyboard and mouse. Each spreadsheet included one column with the sequence of cycle numbers up to the final cycle number. The cycle number in each cell of the column was enlarged so that it nearly filled the entire screen for readability by the observer; the position of the cursor determined which cell was depicted. The display started at cycle 0, start of chewing, with a starting display of '0- $N$ ' in which  $N$  is the total number of intended chews to show the observer that the correct Excel file was activated. At each jaw-closing during chewing, the observer moved the cursor to the subsequent column cell (yielding the subsequent cycle number on the screen) by a tap on the space bar of a wireless keyboard. While in general the cycle number was displayed in back-and-white, the screen became red at the final cycle number after which the observer immediately instructed the subject to halt chewing and to spit out the chewing outcome.

### *A.2. Some recommendations on procedures for chewing efficiency*

Individual  $\log(X_{50})-\log(N)$  relationship are, in general, initially non-linear. Therefore, at least 3  $N$ -values are necessary for curve-fitting with a 2<sup>nd</sup> order polynomial function to account for

possible curvature in the range of  $\log(N)$ -values where  $N(1/2-X_0)$  is assessed. A considerable reduction of the procedure for determining  $N(1/2-X_0)$  is conceivable by roughly assessing  $N(1/2-X_0)$  *a priori* and then choose only two  $N$ -values for testing. When the ratio between the two  $N$ -values is sufficiently small, the effect of the curvature of the  $\log(X_{50})-\log(N)$  relationship will become negligible. Following testing, the *a priori* estimation of  $N(1/2-X_0)$  is then improved by linear interpolation between two pairs of values of  $[\log(X_{50}), \log(N)]$ . A rough assessment of  $N(1/2-X_0)$  can be carried out by means of visual inspection of the size patterns of the particles which have been spitted-out in a coffee-filter following a few exercise chewing sequences. Exercise sequences are carried out anyhow to adapt the subject to the test procedure, with a few  $N$ -values which are adapted to the subject's dental state. An *a priori* assessment of  $N(1/2-X_0)$  will be facilitated by comparing the size pattern with that on photographs from an archive of exercise trials which have been retrospectively linked to values of  $N(1/2-X_0)$ . However, for collecting such an archive, preceding tests using 3  $N$ -values are necessary, of which the procedure is outlined below.

The conditions of a solid test food (intrinsic strength, particle shape and number) for an optimal differentiation within populations should be such that chewing on a sample of particles becomes gradually more difficult but remains still feasible, the more impaired a subject's chewing ability is. Using samples of 4 half-cubes, testing 2, 6 and 12 cycles enables the determination of a  $\log(X_{50})-\log(N)$  relationship, in which  $N(1/2-X_0)$  can be determined by interpolation, for virtually all subjects with a non-impaired chewing ability. The value of 6 cycles is a rounded-off mean  $N(1/2-X_0)$  value from the present study, and the values of 2 and 12 cycles are the rounded-off values from the subjects with the smallest  $N(1/2-X_0)$  and the largest one (Table 2; 4 half-cubes). The number of trials needed is 5 ( $N=2$ ), 2 ( $N=6$ ) and 2 ( $N=12$ ). Because the particle loss is maximally 3.9% in the present study, the minimal number of trials per  $N$ -value can be reduced from 3 to 2, for  $N$ -values which are larger than 5 cycles. More trials are needed for smaller  $N$ -values to ensure that fluctuations in the way that a small number of initial particles are handled by the tongue and the teeth, are averaged out sufficiently.

Using samples of 2 half-cubes, determining  $N(1/2-X_0)$  of subjects with non-impaired chewing ability needs testing of  $N=1$  (10 trials),  $N=4$  (4 trials) and  $N=7$  cycles (2 trials). When chewing efficiency is expected to be impaired by a factor 3, the  $N$ -values of testing are scaled by this factor and become  $N=3$  (4 trials),  $N=12$  (2 trials) and 21 (2 trials). When scaled by a factor 4, the  $N$ -values of testing become  $N=4$  (4 trials),  $N=16$  (2 trials) and  $N=28$  (2 trials). For example, wearers of non-fixated full dentures are a category of subjects with a greatly reduced

chewing efficiency, which will be smaller by a factor 3-4 than that of subjects without impairment. In order to reduce the required bite force by 40% with respect to particle samples of 9 half-cubes, testing with samples of 2 half-cubes is most appropriate for full denture wearers, rather than using 4 half-cubes by which the required bite force is reduced only by 12%, in subjects with a natural dentition. It is notable that  $N(I/2-X_0)$  of most if not all full denture wearers can be determined by testing the  $N$ -values 3, 12 and 21 cycles (range for impairment factor 3). If  $N(I/2-X_0)$  would occasionally be located between 21 cycles and 28 cycles (edge  $N$ -values for factor 3 and 4 respectively),  $N(I/2-X_0)$  could be determined by means of a slight extrapolation on the side of large  $N$ -values where such an extrapolation is less critical because of linearity of the  $\log(X_{50})-\log(N)$  relationship there.

The total number of trials determines the time-load of testing. This total number is 9 trials for subjects with a non-impaired chewing ability when tested with samples of 4 half-cubes, and 8 trials for subjects with impairment factor 3-4 who are tested with samples of 2 half-cubes. The duration of testing will then be 8-9 minutes with a duration of 1 minute per trial. When subjects with none impairment of chewing ability are tested with samples of 2 half-cubes, 16 trials are needed, including 10 trials with a single chew. Because testing duration per trial is shorter than 1 minute when a single chew is involved, the total duration of testing will not exceed 10 minutes.

A comparison of values of  $N(I/2-X_0)$  from subjects with a greatly impaired chewing efficiency and controls can be achieved in two ways: (1) by converting  $N(I/2-X_0)$  of controls which were obtained with samples of 4 half-cubes, to values belonging to samples of 2 half-cubes using an inter-sample factor, which is currently estimated as 0.67 (SEM 0.031,  $n=8$ , ratios from data in Table 2), or (2) by carrying out all tests using 2 half-cubes. Although more trials are needed for the control subjects (16 vs. 9), testing with 2 half-cubes is still feasible for controls, and advantageous because of a direct comparison.

Testing 3  $N$ -values per subject yields 3 pooled samples of chewing outcome for sieving. Three pooled outcome samples, hence handling only three different containers with coffee-filters, is advantageous for an observer to carry out testing alone. While pooling of chewing outcome is not critical for sieving, pooling across more, up to 5 sub-samples is needed to keep the sample volume below  $3.54 \text{ cm}^3$  for optical scanning of particle sizes. Such a volume limit is usual for optical scanning (Eberhard et al., 2012, 2015; Kim et al., 2015) as it enables an adequate spreading of particles. Whereas optical scanning takes less time than sieving and, apart from non-fragile Optosil particles, is also suitable for fragile test foods, sieving has the advantage that particle size distributions by weight, hence volume, are

directly obtained. Size distributions by projected dimensions or area from optical scanning need calibration for a mathematical conversion to size distributions by volume (Eberhard et al., 2012; van der Bilt et al., 1993).

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**Colour is used in Fig. 1 A-B, Fig. 2 A-B, Fig. 6 and Fig. 7**

**Figure legends:**

Fig 1. A: Cumulative underweight distribution from 4 pooled trials of chewing for 3 cycles by subject S06, on a sample of 9 half-cubes of Optosil with a larger edge size of 9.6 mm. The data points (diamonds) included an underweight of 1.0 from an empty top sieve with an aperture of 11.3 mm. Curve-fitting was applied using the Rosin-Rammler equation (solid curve), yielding estimates of median particle size,  $X_{50}$  ( $X$ -value at the intersection between the solid curve and the hatched horizontal line at the underweight level of 0.5), and broadness,  $b$ , of the distribution. B: Curve-fitting without including an underweight data point of 1.0 from the sieve with an aperture of 11.3 mm. For further explanation, see text.

Fig 2. A, Left: weights of Optosil particles and fragments on the various sieves of a stack, from 4 pooled trials of chewing for 3 cycles by subject S06, on samples of 8 cubes of Optosil with an edge size of 8 mm. These weights were converted into a cumulative underweight distribution. A, Right: The cumulative underweight distribution (data points: diamonds) with curve-fitting, using the Rosin-Rammler equation (solid curve). B, Left: A simulation of the weight distribution of the particles before chewing is started. The entire weight (29.144 g) from A has been placed on the sieve with aperture 8 mm. A tiny negligible weight (0.0003 g,

1 per mil of the weight on the sieve with aperture 8 mm) has been used as bottom weight to create a step-function as cumulative underweight distribution. B, Right: the step function as cumulative underweight distribution (data points: diamonds) with curve-fitting, using the Rosin-Rammler equation (solid curve). For further explanation, see text.

Fig. 3.  $R^2$  (mean and SEM), a measure of quality of curve-fitting of underweight-sieve aperture relationships for the 4 types of particle samples and 2 chewing stages. Stage 1, an initial chewing stage with a pronounced presence of undamaged particles. Stage 3, a later chewing stage with hardly any undamaged particles left. Particle sample: CU-P8, 8 cubes of 8 mm; HC-P9, HC-P4, and HC-P2, 9, 4 and 2 half-cubes of 9.6 mm respectively. Horizontal bar, significant inter-stage difference (adjusted  $p$ -value; \* ( $p < 0.05$ ) in Bonferroni's multiple comparison tests. Note that the values of  $R^2$  were always large ( $\geq 0.9794$ ; starting level of depicting the bars).

Fig.4. Mean selection chance per single chew,  $S_{(1)}$  (mean, SEM) in the initial stage of chewing, for the 4 types of particle samples, with 8, 9, 4 and 2 particles offered respectively. Horizontal bars, cases of significant inter-sample differences (adjusted  $p$ -values with significance level), in Bonferroni's multiple comparison tests. Significance level: \* ( $p < 0.05$ ); \*\* ( $p < 0.01$ ); \*\*\* ( $p < 0.001$ ); \*\*\*\* ( $p < 0.0001$ ).

Fig. 5. Mean number of selected particles per single chew,  $n_{s(1)}$  (mean, SEM) in the initial stage of chewing, for the 4 types of particle samples, with 8, 9, 4 and 2 particles offered respectively. Horizontal hatched bar, overall significant ( $p < 0.05$ ) inter-sample differences in a one-way ANOVA for paired observations. The gradual decrease of  $n_{s(1)}$  with decreasing particle numbers was significant ( $p < 0.01$ ) in a post-test for linear trend.

Fig. 6. Group relationships between  $\log(X_{50})$  and  $\log(N)$  for the various types of particle samples. Data points, mean across 8 subjects and SEM; the mean values were curve-fitted using a 2<sup>nd</sup> order polynomial function. Horizontal hatched lines,  $\log(X_{50})$  levels at the initial larger edge size of the half-cubes ( $=\log(9.6 \text{ mm})$ ), and at half of the initial size of the half-cubes ( $=\log(4.8 \text{ mm})$ ). Horizontal hatched dotted lines, the same for cubes with an edge size of 8 mm, i.e. levels at  $\log(8 \text{ mm})$  and  $\log(4 \text{ mm})$ . Arrows, the size interval needed to be bridged by food comminution to attain halving of the initial particle size. The log-number of chews

needed to halve the initial particle size, i.e.  $\log(N(1/2-X_0))$  according to a group function, corresponds for each type of particle sample with the value of  $\log(N)$  at the intersection of its group function with the log-level of half of its initial particle size. From the left to the right, the linear values of  $N(1/2-X_0)$  were 3.8, 5.5, 12.5 and 15.9 chewing cycles.

Fig. 7. Group relationships between  $b$  and  $\log(N)$  for the various types of particle samples. Data points, mean across 8 subjects and SEM; the mean values were curve-fitted using a 2<sup>nd</sup> order polynomial function. Intersection of each of the  $b$ - $\log(N)$  functions with a vertical dotted line of the same colour yields the  $b$ -values at  $\log(N(1/2-X_0))$  values from the  $\log(X_{50})$ - $\log(N)$  group functions in Fig. 6. From left to the right, these  $b$ -values were 3.01, 2.71, 2.24 and 2.34.

Fig. 8. Individual relationships between  $\log(X_{50})$  and  $\log(N)$  which differed most for samples of 4 half-cubes of 9.6 mm. The data points (dots, subject S01; squares, subject S06) were curve-fitted using a 2<sup>nd</sup> order polynomial function. A, chewing efficiency: the intersection of the function with the level of half the initial particle size for half-cubes ( $\log(4.8 \text{ mm})$  level, lower hatched line) occurred at a small  $\log(N)$  value for subject S01, ( $N(1/2-X_0) = 2.7$  cycles, the chewing efficiency of subject S01). The intersection point corresponded to a larger value of  $N(1/2-X_0)$  of 10.1 cycles for subject S06 (less chewing efficiency). Note that the  $\log(4.8 \text{ mm})$  level intersected the function from each subject near the origin of the function's linear part. B, chewing performance at  $N=3$  and  $N=7$ :  $X_{50}$  values at  $N=3$  (hatched vertical line, data points) and at  $N=7$  (hatched-dotted vertical line, data points). Chewing performance was better at both  $N$ -values for subject S01 (smaller  $X_{50}$ -values) than for subject S06.

Fig. 9. Examples of relationships between log-values of chewing efficiency (A: CE,  $\log(N(1/2-X_0))$ ) and chewing performance following 7 chewing cycles (B: CP-N7;  $\log(X_{50})$  at  $N=7$ ) respectively, for two pairs of particle samples. HC-P9\_HC-P2, relationship (triangles, n=8 subjects) between samples of 9 half-cubes of 9.6 mm (X-axis) and 2 half-cubes (Y-axis). HC-P4\_HC-P2, relationship (dots) between samples of 4 half-cubes (X-axis) and 2 half-cubes (Y-axis). Note that the range of data points was larger for chewing efficiency than for chewing performance, reflecting a wider range of inter-subject ratios for chewing efficiency.

**Table 1**

Setup of chewing experiments: type of particle sample, and number of cycles and trials

type of particles	larger edge size (mm)	number of particles per trial	number of chewing cycles per trial	number of trials
cube	8.0	8	3	4
			7	3
			14	2
			28	2
half-cube	9.6	9	3	4
			7	3
			14	2
			28	2
half-cube	9.6	4	2	5
			3	4
			7	3
			14	3

half-cube	9.6	2	1	10
			2	5
			3	4
			7	3

**Table 2**  
Individual chewing efficiency for various particle samples

subject code	gender		chewing efficiency ( $N(1/2 X_0)$ )			
	male=m female=f	age (yrs)	C-P8	HC-P9	HC-P4	HC-P2
S01	m	22.6	9.6	6.6	2.7	1.8
S02	f	24.8	17.9	13.0	5.5	3.8
S03	m	22.6	14.2	12.4	6.1	3.1
S04	m	23.2	27.7	16.8	8.0	6.3
S05	f	22.7	19.9	16.6	6.4	4.5
S06	f	24.3	26.4	17.3	10.1	7.3
S07	f	22.4	19.3	12.2	5.5	3.7
S08	m	25.9	6.8	5.1	3.1	1.8
<i>non-transformed values:</i>						
mean		23.6	17.7	12.5	5.9	4.0
SD		1.3	7.4	4.6	2.4	2.0
			*	*		
			**		**	
			**			**
				**	**	
				***		***
					***	***
<i>log-transformed values:</i>						
mean			1.209	1.063	0.738	0.558



SD **0.211 0.197 0.193 0.223**

Chewing efficiency, number of chews needed to half the initial particle size,  $N(1/2-X_0)$ . Particle samples: CU-P8, 8 cubes of 8 mm; HC-P9, 9 half-cubes of 9.6 mm; HC-P4, 4 half-cubes; HC-P2, 2 half-cubes. Horizontal bars and asterisks: significant differences between pairs of types of particle samples in Bonferroni's multiple comparison tests; adjusted level of significance: \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ . The SD values of the log-transformed values (bold) are a measure of the variation in inter-subject log-ratio values of chewing efficiency for the various particle samples. For further explanation, see text.

**Table 3**

Individual chewing performance at two numbers of chewing cycles for various particle samples

subject code	chewing performance $X_{50}$ at $N=3$ (mm)				chewing performance $X_{50}$ at $N=7$ (mm)			
	CU-P8	HC-P9	HC-P4	HC-P2	CU-P8	HC-P9	HC-P4	HC-P2
S01	6.04	6.33	4.73	4.04	4.79	4.89	2.80	2.45
S02	8.01	8.42	6.45	5.08	6.63	6.43	4.16	3.12
S03	7.94	8.68	5.86	4.86	5.54	6.67	4.70	2.93
S04	8.04	9.27	7.22	6.80	7.23	7.63	5.37	4.51
S05	7.64	8.47	7.69	7.63	7.12	7.26	4.43	3.05
S06	8.37	9.09	8.72	6.62	7.22	7.34	6.18	4.90
S07	7.62	8.52	7.40	5.11	6.38	6.46	3.91	2.58
S08	6.84	6.27	5.13	3.33	4.17	4.21	2.82	1.54
<i>non-transformed values:</i>								
mean	7.56	8.13	6.65	5.43	6.14	6.36	4.30	3.14
SD	0.76	1.17	1.36	1.46	1.18	1.21	1.16	1.09

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<i>log-transformed values:</i>								
mean	0.877	0.906	0.815	0.721	0.780	0.796	0.618	0.472
SD	<b>0.046</b>	<b>0.067</b>	<b>0.092</b>	<b>0.121</b>	<b>0.090</b>	<b>0.091</b>	<b>0.122</b>	<b>0.157</b>

Chewing performance, the median particle size,  $X_{50}$  (mm), following 3 chewing cycles (N=3) and 7 cycles (N=7) respectively, for the various types of particle samples. Horizontal bars and asterisks: significant differences between pairs of types of particle samples in Bonferroni's multiple comparison tests; adjusted level of significance: \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ; \*\*\*\*,  $p < 0.0001$ . For further explanation, see Table 2.

**Table 4**  
Variation in inter-subject log-values of measures of chewing ability within particle samples

particle sample	measure of chewing ability		
	CE	CP, N=3	CP, N=7
CU-8P	0.211	0.046	0.090
HC-9P	0.197	0.067	0.091
HC-4P	0.193	0.092	0.122
HC-2P	0.223	0.121	0.157
mean	0.206	0.082	0.115
SD	0.014	0.032	0.032
CV	0.068	0.393	0.274

SD-values of log-transformed values from Tables 2 and 3 (bottom, bold) as a measure of the variation in inter-subject log-values of chewing ability for the various types of particle samples. CE, chewing efficiency. CP, chewing performance following 3 and 7 chewing cycles respectively. Horizontal bars

and asterisks: significant ( $p < 0.05$ ) differences between pairs of measures of chewing ability in Bonferroni's multiple comparison tests.

**Table 5**

Degree of correlation between intra-subject log-values of measures of chewing ability for various pairs of particle samples

types of particle samples	measure of chewing ability		
	CE	CP, N=3	CP, N=7
CU-P8 vs. HC-P9	0.964	0.922	0.953
CU-P8 vs. HC-P4	0.918	0.816	0.855
CU-P8 vs. HC-P2	0.964	0.679	0.865
HC-P9 vs. HC-P4	0.934	0.849	0.919
HC-P9 vs. HC-P2	0.937	0.832	0.881
HC-P4 vs. HX-P2	0.970	0.862	0.906
mean	0.948	0.827	0.897
SD	0.021	0.081	0.037
	*	*	
	*		*

Correlation coefficients (Pearson's  $r$ ;  $n=8$  subjects) from inter-sample log-values of measures of chewing ability. CE, chewing efficiency. CP, chewing performance at  $N=3$ . CP at  $N=7$ . Horizontal bars and asterisks: significant ( $p<0.05$ ) differences between pairs of measures of chewing ability in Bonferroni's multiple comparison tests.

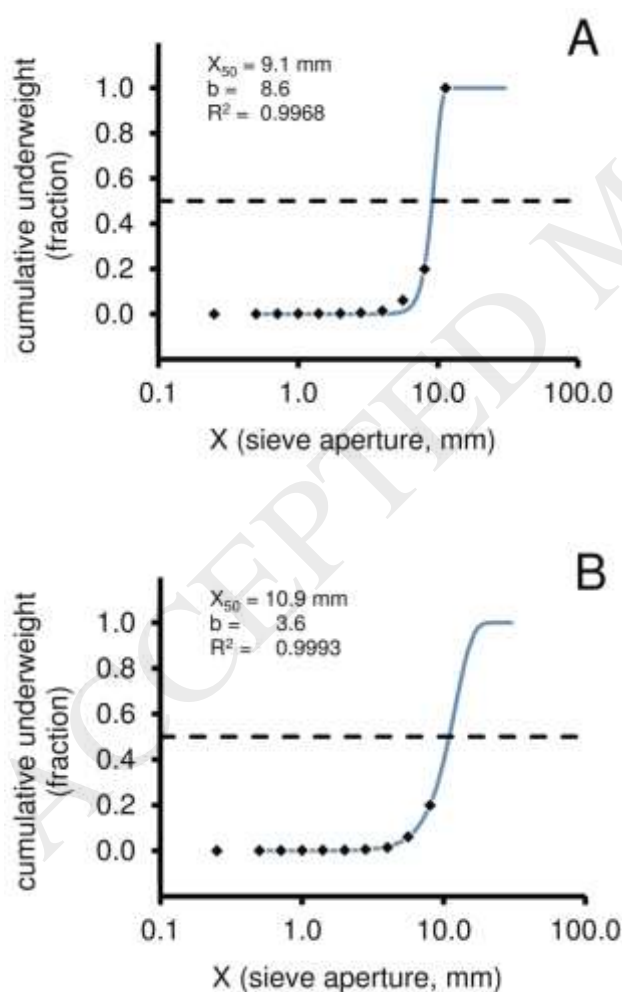
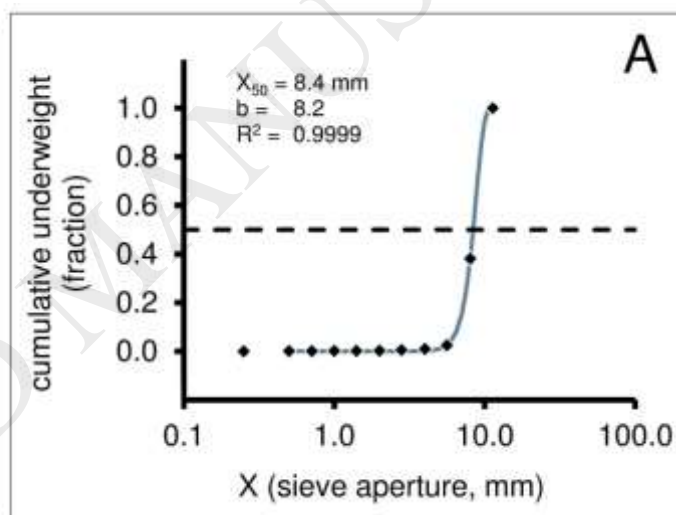


Fig 1. A: Cumulative underweight distribution from 4 pooled trials of chewing for 3 cycles by subject S06, on a sample of 9 half-cubes of Optosil with a larger edge size of 9.6 mm. The data points

(diamonds) included an underweight of 1.0 from an empty top sieve with an aperture of 11.3 mm. Curve-fitting was applied using the Rosin-Rammler equation (solid curve), yielding estimates of median particle size,  $X_{50}$  ( $X$ -value at the intersection between the solid curve and the hatched horizontal line at the underweight level of 0.5), and broadness,  $b$ , of the distribution. B: Curve-fitting without including an underweight data point of 1.0 from the sieve with an aperture of 11.3 mm. For further explanation, see text.

sieve aperture (mm)	weight (g)	weight (fraction)	cumulative underweight (fraction)
11.3	0.000	0.0000	1.0000
8.00	18.048	0.6193	0.3807
5.60	10.379	0.3561	0.0246
4.00	0.443	0.0152	0.0094
2.80	0.128	0.0044	0.0050
2.00	0.063	0.0022	0.0028
1.40	0.030	0.0010	0.0018
1.00	0.018	0.0006	0.0012
0.71	0.009	0.0003	0.0009
0.50	0.013	0.0004	0.0004
0.25	0.012	0.0004	0.0000
0.00	0.001	0.0000	0.0000
sum	29.144		



sieve aperture (mm)	weight (g)	weight (fraction)	cumulative underweight (fraction)
11.3	0.0000	0.00000	1.00000
8.00	29.1440	0.99999	0.00001
5.60	0.0000	0.00000	0.00001
4.00	0.0000	0.00000	0.00001
2.80	0.0000	0.00000	0.00001
2.00	0.0000	0.00000	0.00001
1.40	0.0000	0.00000	0.00001
1.00	0.0000	0.00000	0.00001
0.71	0.0000	0.00000	0.00001
0.50	0.0000	0.00000	0.00001
0.25	0.0000	0.00000	0.00001
0.00	0.0003	0.00001	0.00000
sum	29.1443		

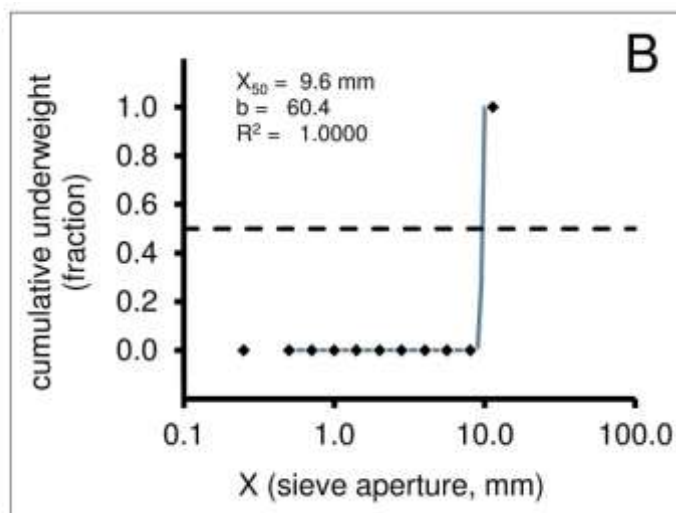


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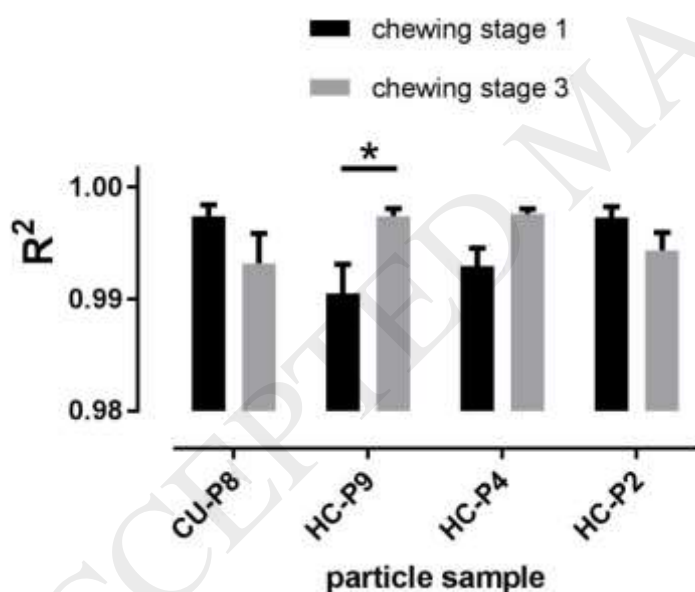


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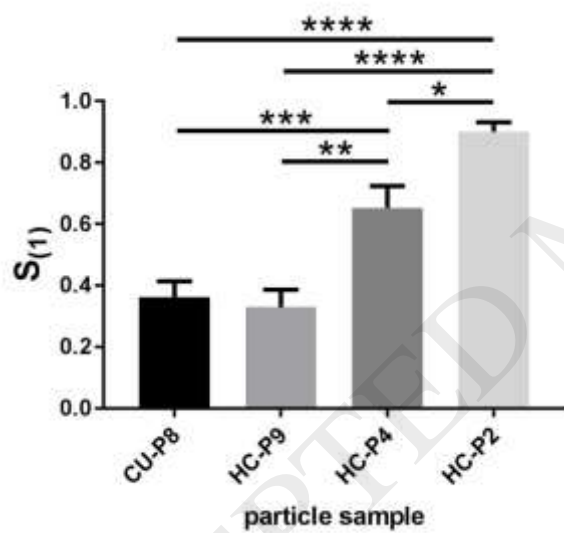


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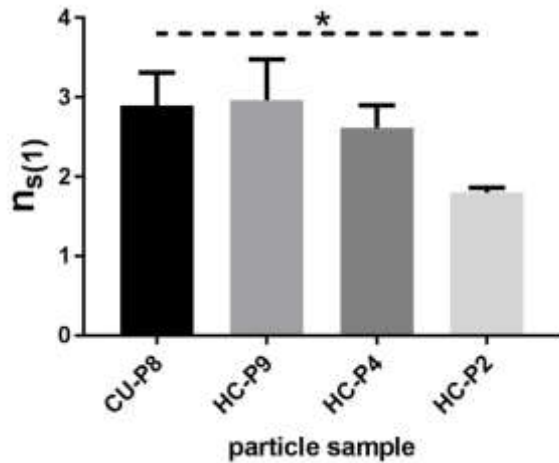


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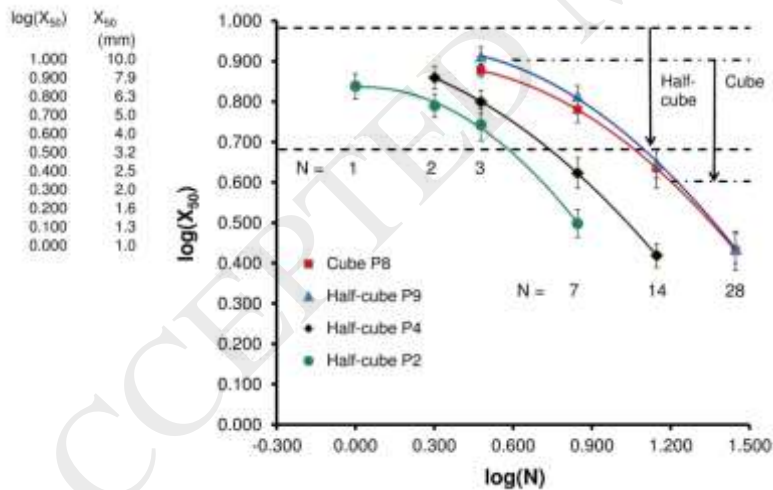


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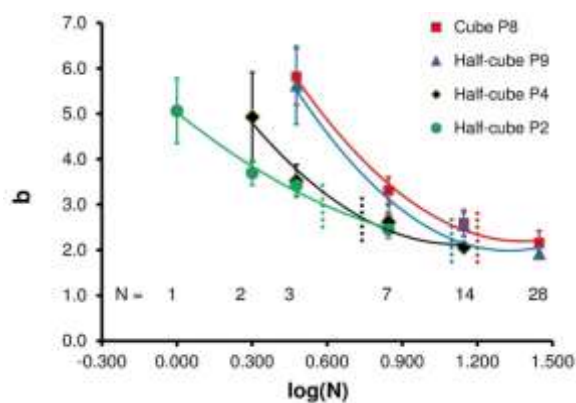


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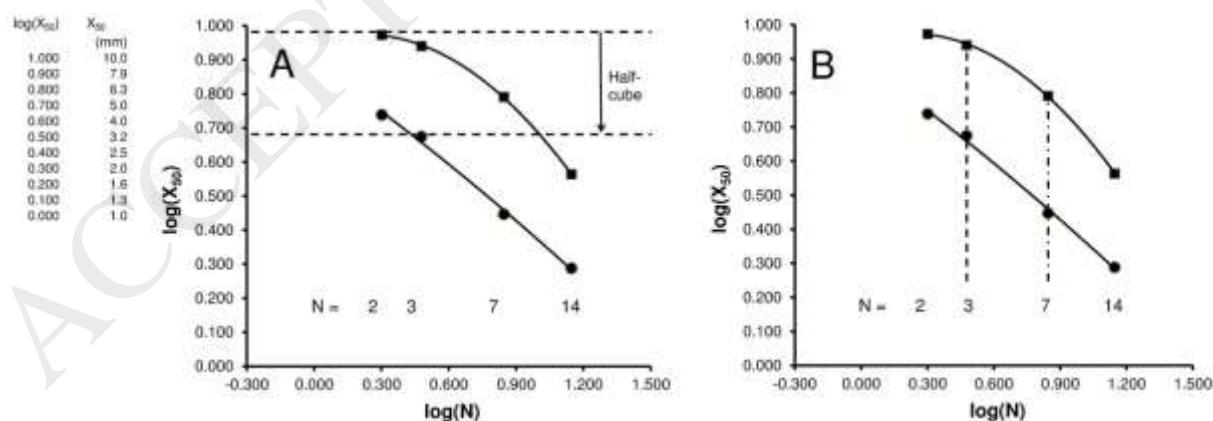
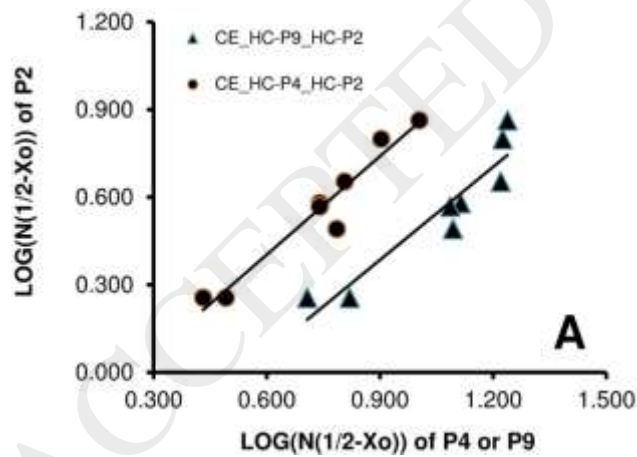


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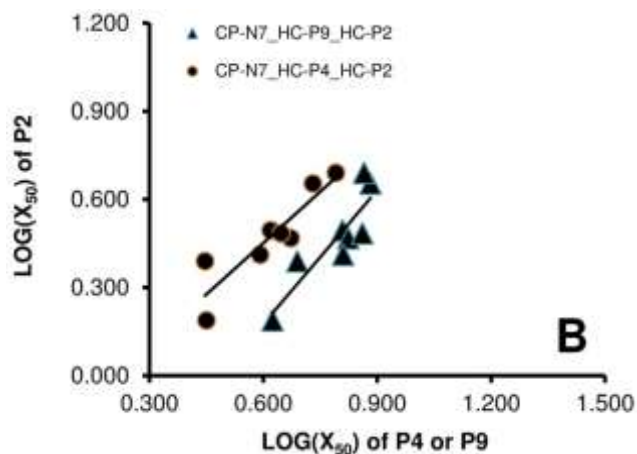


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