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Running title: Infants and toddlers' masticatory performance

Abstract

This study evaluated the feasibility of assessing masticatory performance in infants and toddlers. Four groups of healthy children (n=97, 42 girls and 55 boys) participated in the study: two study groups (SG) followed at 6, 8 and 10 months old (mo) or at 12, 15 and 18 mo, and two control groups (CG) of respectively 10 and 18 mo children. Masticatory performance was determined from children's ability to comminute a model gel during videotaped lab measurements. The gel was inserted in a mesh feeder and offered to the child for a 60s oral processing duration, then gel particles were collected from the feeder and photographed. Resulting gel breakdown was assessed from the characterization of the area and number of formed particles. Children strategy to orally process the gel (sucking vs. biting/chewing) was evaluated from video recordings. Children's compliance (acceptance of the feeder in the mouth for the expected duration) was average (51%) overall. It decreased from 1 year of age and was higher in SG than in CG. The number and area of gel particles formed under oral processing increased significantly with age, demonstrating an increase in children masticatory performance as they grew up. Median particles area was positively associated with sucking behavior and negatively associated with biting/chewing. The association with teeth emergence was not significant. In conclusion, the proposed method is relevant for quantifying the development of early masticatory performance in children who accept to hold the feeder in their mouth.

Keywords: masticatory performance, children, teeth, feeding skills, bolus particles

Practical applications:

In this paper a method to easily quantify masticatory performance in young children aged 6-18 months old was evaluated. The method is based on a feeder and could be used for collecting boluses, as an alternative to the chew-and-spit method when it is unfeasible. Children's compliance to the method and the impact of previous study participation on compliance to the protocol are detailed, giving thus a rationale for an optimal application of this method in future experiments. Finally, the determination of masticatory performance as a function of age thanks to this method could contribute to the understanding of food oral processing and food texture acceptance in childhood in future studies.

Introduction

Mastication is essential for food ingestion. It involves the coordinated action of the teeth, the jaw muscles, the temporomandibular joints, the tongue, the cheeks and the lips. Mastication produces the mechanical breakdown of solid food, bolus formation and thus the transfer of the food into the oesophagus. The masticatory process is adapted to the mechanical properties of the food eaten and is known to vary between individuals (Dan & Kohyama, 2007; Peyron, Lassauzay, & Woda, 2002; Simione et al., 2018; Woda, Foster, Mishellany, & Peyron, 2006). Characterizing mastication is important because it plays a role in food choice, consumption and nutrition, especially during sensitive periods in the lifespan (Lee, Yang, Ho, & Lee, 2014; Schwartz, Vandenberghe-Descamps, Sulmont-Rossé, Tournier, & Feron, 2018).

Chewing emerges during the second half of the first year of life and continues to develop during childhood (Le Reverend, Edelson, & Loret, 2014; Nicklaus, Demonteil, & Tournier, 2015). After birth up to 4-6 mo infants are only fed with liquid (milk) which is ingested by sucking. Then, they are introduced to solid foods and thus have to develop oral–motor skills necessary to ingest these foods. The tongue has to learn to move solids within the oral cavity in preparation to swallowing (Gillian Harris & Coulthard, 2016) and the development of bones, muscles and teeth allow infants to break foods down in particles, which is necessary to form a bolus that can be swallowed (Le Reverend et al., 2014). The first months of complementary feeding are characterized by a transition from sucking to chewing behavior and the further development of masticatory efficiency (Delaney & Arvedson, 2008; Gisel, 1991; Le Reverend et al., 2014; Nicklaus et al., 2015; Stolovitz & Gisel, 1991). In literature, the development of chewing has been studied using video analysis and recordings of muscle activity and of jaw movements in situations where children were eating foods of different nature (da Costa, Remijn, Weenen, Vereijken, & van der Schans, 2017; Gisel, 1991; Green et al., 1997; Remijn, Speyer, Groen, van Limbeek, & Nijhuis-van der Sanden, 2014; Simione et al., 2018; Torola, Lehtihalmes, Yliherva, & Olsen, 2012; Wilson & Green, 2009). However, it is difficult to compare studies as the results depend both on the mechanical properties of the studied food, which are not always described, and on the characterization methods. Studying the development of early mastication with a test food that can be objectively described, repeatedly produced and which is not softened by saliva would be useful to overcome these issues. Moreover, as food oral processing is known to vary between individuals and food bolus properties at swallowing may not be necessarily directly related to chewing behavior, we believe that looking at the results of mastication (i.e. bolus properties) is a relevant approach to objectively characterize the development of the masticatory function.

In adults, the masticatory function is evaluated using so-called masticatory performance or efficiency tests, consisting in evaluating the capacity of an individual to grind a food material/dental polymer in particles (Manly & Braley, 1950; Mowlana & Heath, 1993; Van Der Bilt, 2011; van der Bilt, Mojet, Tekamp, & Abbink, 2010; Woda et al., 2010). Such methods are easy to apply and can be used for evaluating the success of dental treatments. The median particle size values of masticated raw carrots collected at swallowing has been proposed as an indicator for the clinical evaluation of the normality of the masticatory function (Witter, Woda, Bronkhorst, & Creugers, 2013; Woda et al., 2010).

In children, masticatory performance has been assessed with the main objective to investigate the impact of dentition (number of healthy teeth, primary vs. mixed dentition, occlusion class) and to relate performance to other variables (bite force, age, gender, body height and weight). Measurements were performed by chewing dental polymers for a given number of chewing strokes (Barrera, Buschang, Throckmorton, & Roldan, 2011; Consolacao Soares et al., 2017; de Souza Barbosa, de MouraisTureli, Nobre-dos-Santos, Puppin-Rontani, & Duarte Gaviao, 2013; Gavião, Raymundo, & Sobrinho, 2001; Julien, Buschang, Throckmorton, & Dechow, 1996; Kaya, Akyuz, Guclu, Diracoglu, & Yarat, 2017; Marquezin, Kobayashi, Montes, Gaviao, & Castelo, 2013), or using colour-changing chewing-gum chewed for a given duration or number of strokes (Hama et al., 2017; Ohira, Ono, Yano, & Takagi, 2011; Oueis, 2009). These methods are unfeasible in infants and toddlers because they can neither be instructed on how to complete such a task nor spit out boluses on demand, their chewing force is limited and choking is a risk in this population. Therefore, to the best of our knowledge masticatory performance has never been objectively determined before the age of 3 years and especially not in early infancy, when the masticatory function is developing.

Taking into account these limitations we proposed a method based on the evaluation of the breakdown of a sweet gel enclosed in a mesh feeder. We previously validated with an adult panel that oral gel breakdown was not affected by the presence of the mesh (Tournier, Rodrigues, Canon, Salles, & Feron, 2015). The purpose of the current study is to evaluate the feasibility of this method to assess the masticatory performance in young children, the ability to discriminate performance as a function of children's age (from 6 to 18 mo) and to study the potential relationships between masticatory performance and children's other characteristics, such as dentition.

MATERIAL AND METHODS

1. Study design

Participants

The data were collected during a study on the development of food texture acceptance and of oral feeding skills during infancy (Demonteil et al., 2019). Only data on masticatory performance are reported in the present paper.

Ninety-seven parent-child pairs participated in the study between August 2015 and February 2017. Eligibility for participation in the study was checked by an initial phone interview, and participants were included if parents had reached legal majority (18 years old), if children were born full-term (≥ 37 gestational weeks), had a birth weight ≥2500 g, had not been tube-fed since birth, had no chronic diseases or food allergies and were introduced to complementary foods at 5 months±21 days. This study was conducted according to the guidelines established in the Declaration of Helsinki; the protocol was approved by the local ethics committee (Comité de Protection de Personnes Est III Nancy, ID RCB :2015-A00323-46), and by the national food safety authority (ANSM, file A00323-46). Parents received informed consent in which they were explained that this test is a laboratory procedure for determining masticatory performance and that the procedure involved the use an apple jelly contained in a feeder. Written informed consent was obtained from both parents.

Study organization

To evaluate and to monitor chewing skills as a function of age, two follow-up groups were included in the study (SG1 and SG2). SG1 children started the study at 6 mo and were followed at 8 and 10 mo, and SG2 children were included at 12 mo and were followed at 15 and 18 mo (Figure 1). To evaluate if the participation in the different measuring sessions have an influence on both children's willingness and way to perform the task, two control groups (CG1 and CG2) were included. Parents and SG children participated in the lab sessions: 2 sessions at 6 mo and 3 sessions at 8 and 10 mo for SG1; 3 sessions at 12, 15, 18 mo for SG2 infants. Sessions at a given age were performed during the same week. CG children participated to the study only at one session at one age: when they were 10 months old for CG1 and 18 months old for GC2 (Figure 1). Groups were well balanced in terms of gender (% of male varied between 48 and 64 % depending on the group) and mean age of complementary feeding initiation, which varied between 4.6±0.6 and 4.8±0.6 months over the four groups.

(Figure 1 about here)

Sessions took place at meal or afternoon snack time in the Center of Taste and Feeding Behavior (Centre des Sciences du Goût et de l'Alimentation, CSGA, INRA) facilities and the general organization was similar across ages and across groups. Sessions comprised a parent's interview and the evaluation of children's acceptance for 3 trials of a maximum of 5 different solid foods (See Demonteil et al. (2019) for details). Each session ended up with a collection of saliva using a cotton swab and then the determination of children's masticatory performance. During the whole session children were video-recorded. Parent's interview provided information on thumb sucking, use of pacifier, abilities to eat alone with the spoon and with fingers were collected using categorical scales (never/rarely/sometimes/often). Information on current breastfeeding was also collected (yes/no). Finally, throughout the study, teeth emergence (and date of emergence) was tracked at each age using a teeth emergence booklet filled-in by parents.

2. Determination of masticatory performance

Masticatory performance was determined by measuring the ability of children to comminute a test food under free oral processing conditions, using a method specifically developed for them. Briefly, the method is based on the evaluation of the breakdown of a gel enclosed in a feeder (a little mesh bag with a handle). The feeder is offered to the participant and after a given oral processing duration, the feeder is collected, the bolus extracted from the mesh and bolus granulometry is determined (Tournier et al., 2015).

For this study, the model gel was composed of agar-agar (2%; NAT-ALI, Rezé, France-), sucrose (20.2 %, Carrefour, France) and apple juice (77.8%, Intermarché, France). The ingredients were mixed at room temperature. The mixture was boiled for 1 min then poored in a financier silicone baking mold (48 x 25 x 10 mm) using a syringe and kept at 4°C until the following day. The day of the experiment, the gel was introduced in the feeder (1mm mesh netting, Figure 2) and proposed to the child at room temperature.

(Figure 2 about here)

The feeder was preferentially held in the mouth by the child himself/herself but he/she could be helped by the parent or the experimenter, if needed. For children who were not spontaneously putting the feeder in their mouth, it happened that parents asked the child to put it or even they showed him/her how to proceed. So children were only encouraged by imitation. The oral exposure time was set to 60 seconds and was measured by the experimenter during the session. At the end of the oral processing test, the gel particles were manually transferred from the feeder mesh onto a black tissue, so that they were well separated from each other. Granulometry was determined using the same methodology as described in Tournier et al. (2015). Images were acquired using a camera and the granulometry analyzed using IgorPro software (WaveMetrics, USA). The number of bolus particles and their area were determined. Data of particle area in pixels were converted in mm2 after calibration using a test card digitized in similar conditions. The particle areas representing 5 and 50 % of the cumulative distribution (D5 and D50) were computed for each collected bolus (D5 is an indication for the size of the (5%) smallest particles, and D50 for the median size of the particles).

Video recordings collected during measurements were analyzed in order to characterize children behaviors during the test. Information coded were: the number of time required by the child to process the gel (indeed children were mainly processing the gel via several oral sequences rather than during 60s in a row), the exact oral processing duration (i.e. cumulated duration over the different sequences), sucking behavior (backward - forward movements of the jaw), biting/chewing behavior (down-and-up movement of the jaw, the feeder being placed between the front or the posterior teeth) and if the child was self-processing the task (i.e. holding the feeder alone during the measurement or not). Video were coded by two investigators who agreed beforehand on the way to assess behaviors.

3. Data analysis

All analyses were performed using the SAS Software (version 9.3, SAS Institute Inc., Cary, NC, USA).

Data describing children's usual behavior, as reported by their parents (thumb sucking, use of pacifier, eating alone with finger, holding alone a spoon in mouth), were coded as 0 (when parents answered never/rarely) or 1 (sometimes/often). Impact of age on these binary data was evaluated by group using generalized linear mixed model (Proc GLIMMIX, logit link) with age class as fixed effect and subject as random effect. Differences in behaviors between SG and CG children at a given age were assessed using Chi-Square tests. The numbers of teeth were compared across ages within a given group using two-way generalized linear mixed model (GLM with age class and random subject as factors) and between groups (SG vs. CG) at a given age using Student T-tests.

Concerning masticatory performance measurements, we first determined the frequency of children accepting to orally process the feeder (number of bolus collected in total, Table 2) and those really complying with the protocol (i.e. orally processing the gel for the duration fixed by the experimenter (60s)). Indeed, it turned out that some children decided to stop oral processing beforehand, or more rarely did not want to stop processing the gel after 60s. We thus decided that children were compliant with the protocol if they orally processed the gel during 60s ± 10s. The total duration of oral processing was the only criteria for compliance. Evolution of compliance with age was studied using proc GLIMMIX (age class, experimental session, random subjects). Comparisons of compliance between the study groups (SG1 and SG2) and between the study (SG - at the first experimental session) and the control (CG) groups at a given age (10 and 18 mo) were performed using Chi-Square tests.

Only compliant data were kept for the determination of masticatory performance. Thus, data concerning boluses collected for durations shorter than 50s or longer than 70s were discarded (noncompliance). Replicates obtained for each child (different session at a given age) were averaged. Bolus granulometry variables (number of particles, D5 and D50) were analyzed using two-ways GLMs (age class, random subjects) for each study group. LSMEANS post-hoc analyses were used. Children behaviors while performing the test (number of in-mouth sequences, holding feeder alone, sucking, biting/chewing) were analyzed using GLMs (age class, random subjects). Bolus property variables and children behavior (binary data) were compared between study (SG) and control (CG) groups at a given age (10 and 18 mo) using student T-tests and Chi-Square tests, respectively. Only data corresponding to the first experimental session of the SG groups at 10 and 18 mo were considered for these comparisons, in order to avoid any potential exposure effect to the experiment between data collected over the different sessions at a given age in SG children.

Mixed model were performed to test potential association between masticatory performance (bolus median particles area D50) and children behavior during the test (sucking, biting/chewing, numbers of in-mouth movements) and children characteristics (number of teeth, gender, age of complementary feeding, thumb sucking, use of pacifier, current breastfeeding, ability to hold a spoon alone and eat with finger). Associations between masticatory performance and each variable were tested separately and were adjusted for the children age (PROC GLIMMIX, dependent variable, age, random subjects). For all analyses, significance was set at $p<0.05$.

RESULTS

1. **Description of the population**

Children's characteristics reported by parents are presented in Table 1. Increase in age was shown to have a significant impact on teeth emergence both between 6 and 10 mo (SG1, $F(2, 72)=48.6$, $p<0.0001$) and between 12 and 18 mo (SG2 : $F(2, 76)=97.8$, $p<0.0001$). Ability to eat with finger increased between 8 and 10 mo (SG1 : $F(2, 46)=6.3$, $p=0.004$), whereas ability to hold a spoon alone increased from 12 to 15 mo (SG2 group, $F(1, 51)=15.6$, $p<0.001$). Thirty-six percent of SG1 children were breastfed at 6 mo and this proportion slightly decreased with age, despite being not significant ($p>0.05$). Breastfeeding was not practiced in the 12-18 mo group. Pacifier use was a common practice in both groups (> 50% of the children) and was relatively stable over ages ($p>0.05$). Thumb sucking varied between 48 % to 25% between 6 and 10 mo, but the decrease was not significant ($p>0.05$).

Comparison of children from the study and control groups showed that they differed neither with regards to their number of teeth ($p>0.05$) nor to the general behaviors listed in Table 1. Only a trend was observed for eating with fingers at 18 mo: this behavior tended to be less present in the control group than in the study group ($\chi^2(1, N = 48) = 3.5$, $p=0.06$).

(Table 1 about here)

2. **Evaluation of the method feasibility**

The present method was developed specifically for young children but has never been applied so far in this population. To be feasible, the method and more specifically the feeder has to be accepted by the child for the duration fixed by the protocol. Table 2 summarizes the number of collected boluses in total and within a 60±10 s range for oral processing duration by group, age and session and the average compliance (%).

(Table 2 about here)

The feeder was well accepted by SG1 children especially at 8 and 10 mo (acceptance varied between 83 to 91 % depending on the session, Table 2). However, the preset duration of oral processing was not always respected, and compliance was found to vary between only 50 % (6 mo) to 68 % (8 and 10 mo). Compliance tended to increase between 6 and 8 mo but the age effect was marginal ($F(2, 161)=2.9$, $p=0.06$). When comparing study and control groups, compliance was higher in SG1 (15/24; Table 2) than in CG1 (9/22; Table 2) but this effect was not significant ($p>0.05$).

When looking at older children (SG2), we observed that from 1 year on compliance varied between 40 to 57%, which is on average lower than in the younger group of children ($\chi2(1, N = 426) = 12.7$, $p=0.0004$). SG2 children's compliance was significantly higher at 18 mo than at 12 and 15 mo ($F(2, 198)=3.5$, $p=0.031$). At 18 mo, compliance was clearly higher in the study group (SG2 : 14/25; Table 2) than in the control group CG2 (2/23; Table 2) but this could not be tested because of small sample size.

These data showed that compliance depended both on age and on previous exposure to the study. Taking into account the small number of bolus collected in the control group (CG) at 18 mo masticatory performance measured in this group were not included in the analyses in the following sections.

3. Determination of masticatory performance: evolution with age and exposure

3.1. Examples of collected images

Figure 3 shows example of images collected from 4 different children at different ages. It can be observed that the level of gel fragmentation obtained after oral processing increased as children got older and varied between children of a given age. Image analyses were performed to quantify these observations. Results for D5, D50 and the number of particles are presented in Figure 4.

3.2. Analysis of bolus granulometry

(Figure 4 about here)

Impact of age

At 6 mo, some children were able to break down the gel in particles while others were not (Figure 3). As a result, the average number of particles was low (7.8 particles ($SD = 7.4$)) and high inter-individual variability was observed for the area of small particles (D5, Figure 4). Median particles area (D50, Figure 4) was high and relatively homogeneous suggesting that collected boluses were on average poorly broken down. As children aged between 6 and 10 mo, the number of particles increased ($F(2, 53)=11.6$, $p=0.0002$) whereas median particle area decreased ($F(2, 53)=18.9$, $p<0.0001$). The number of formed particles increased to 103.8 ($SD=100.1$) at 8 mo and 159.8 ($SD=124.0$) at 10 mo (Figure 3). Decrease in D50 was significant between 6 and 8 mo. In older children (SG2), the number of particles increased between 12 and 18 mo ($F(2, 44) = 4.16$, $p=0.028$); and the decrease in D50 was not significant ($p>0.05$). Over boluses collected at different ages, we observed that D50 was negatively correlated to the number of particles in SG1 ($r(54)=-0.79$, $p<0.0001$) and in SG2 ($r(45)=-0.59$, $p<0.001$) and positively correlated with D5 in SG1 ($r(54)=0.71$, $p<0.0001$) and in SG2 ($r(45)=0.61$, $p<0.0001$).

Overall, as children get older, the fragmentation of the gel increases, leading to more particles of smaller size. This reveals an increase in the ability to break down a hard gel via oral processing. We also observed a high variability in bolus properties at a given age, especially in younger children (SG1), suggesting that masticatory performance does not depend only on age.

Impact of participation in the experiment

As described earlier, previous participation in our experiment impacted on children compliance to the masticatory performance task. We further investigated whether participation also impacted on the parameter of chewing performance, i.e. on the bolus properties collected after oral processing. In order to do so, the variables D50 and number of particles were compared at 10 mo between children of a control group (CG1, n=9) and those of the study group (SG1 – session 1, n=15). We did not observe a group effect (p>0.05; Figure 4), suggesting that exposure at an earlier age is not a strong factor affecting masticatory performance, in these small groups of children; despite the fact that exposure affected compliance as shown above.

4. Children's oral processing behaviors used during masticatory performance determination

Video analysis was run a posteriori to study in details children behaviors during the measurements. Results are presented in Table 3.

(Table 3 about here)

Between 6 and 10 mo, SG1 and CG1 children required on average 5 to 6 oral processing sequences to reach the 60±10 s of cumulated oral processing duration, whatever the age and the group (p>0.05; Table 3). In the SG2 group, children tends to process the gel through a higher number of oral sequences at 12 mo than at a later age but this effect was marginal (F(2, 44)=3.3, p=0.053). Children's autonomy in performing the task was evaluated by the child's holding the feeder in his/her mouth alone. Frequency varied between 8 to 19 % in 6 to 10 mo-old children and between 40 and 55 % in 12 to 18 mo-old children. No effect of age was observed for any group (p>0.05). Concerning the strategy (sucking vs chewing/biting) used by children to orally process the gel, marked changes were observed with increasing age in younger children (SG1). At 6 mo, 100% of children sucked on the gel and biting /chewing was observed for only 34 % of them. With age, sucking behavior decreased (F(2, 53)=9.9, p=0.0005) and biting/chewing increased (F(2, 53)=15.8, p<0.0001). At 8 months, children used both sucking and chewing behavior to an equal extent (80 %). Then, from 10 mo on chewing became the most performed behavior (95%, Table 3). During the second year (SG2), occurrence of biting/chewing was observed in 87-97% of the children and sucking was still observed in 37 to 42 % of children. These behaviors were stable up to 18 mo (age effect: p>0.05). Concerning the comparison of 10 mo children in CG and SG (first session) data from Table 3 suggests that chewing behavior may be less present in CG children however this could not be tested statistically because of the too small sample size in CG group to apply Chi-square test.

5. Relationship between individual masticatory performance and children oral processing strategy and characteristics

Children's ability to break down a gel by processing it orally was found to increase with age. However, large inter-individual variability was also observed among children of a given age (Figures 3 and 4). In order to better understand this variability, associations between bolus properties and children behavior during the test (self-processing the task, sucking, chewing/biting) and children characteristics (number of teeth, gender, thumb sucking, use of pacifier, age of complementary feeding, current breastfeeding, hold a spoon in mouth alone and eating with finger (Table 1)) were studied independently for both study groups using binary regressions corrected for age. As bolus variables were found to be correlated with each other, regression were performed on D50 values as it is the most common variable characterizing masticatory performance in literature.

In younger children (SG1) D50 was negatively associated with the presence of a biting/chewing behavior (β=-352.9, $F(1, 28)$=6.27, p=0.018) and marginally negatively associated with children number of teeth (β=-68.1, $F(2, 28)$=3.63, p=0.067). In older ones (SG2), D50 was found to be positively associated with the presence of sucking behavior (β=335.2, $F(1, 23)$=10.9, p=0.003) and negatively associated with biting/chewing behavior (β=-597.8, $F(1, 23)$=62, p=0.020) and the number of in-mouth sequences (β=-30.9, $F(1, 23)$=7.0, p=0.014). Thus, at all ages studied, children who processed the gel by chewing/biting produced smaller particles than children processing the gel mainly by sucking. Further analysis of associations with other children characteristics showed that D50 was not related to any other tested variables (gender, thumb sucking, use of pacifier, age of complementary feeding, current breastfeeding, hold a spoon in mouth alone and eating with finger), whatever the study group considered (p>0.05).

DISCUSSION

This study is the first to objectively assess masticatory performance from bolus destructuration during oral processing in children in a period when the masticatory function is rapidly developing. As the methods currently used in literature are not applicable, we proposed a new method specifically developed for infants and toddlers and evaluated its applicability in 6 to 18 mo children. Applicability was studied from children's compliance to the test and through the method's ability to discriminate masticatory performance as a function of age, given the longitudinal nature of the sample. We further examined whether individual children's oral behaviors during the test and children's characteristics predicted inter-individual differences in masticatory performance.

Children's compliance to the method

To be feasible the proposed method had first to be accepted by infants and toddlers.

We quantified children's compliance to the method in order to evaluate its applicability for future studies. Average compliance was 51 % of the measurements and varied between 9 and 68 % over all groups and ages. This may appear low and highly variable and demonstrates that measuring masticatory performance at this age period may be more challenging than after the age of 3 years, which is the earliest age at which chewing performance was determined as reported in literature (Gavião et al., 2001; Oueis, 2009).

We believe that the primary factor affecting compliance is methodological. Indeed, masticatory performance was determined at the end of a c.a. 45-min experimental session (Demonteil et al., 2019), in which children had been eating various textured foods and saliva had been collected. It is possible that tiredness impacted on children's willingness/ability to perform the measurements.

Moreover, we believed that the lower compliance observed in the control groups can also be due to the novelty of participating in the experimental study as a whole.
Compliance depended on children's age: younger children (6-10 mo) were more compliant than older ones (12-18 mo). Within each group, compliance increased with age: a trend was observed between 6 and 8 mo in SG1 and a significant increase was observed between 15 and 18 mo in SG2. Moreover, at 10 and 18 mo the method was better applicable in children of the study group than in children of the control group. At 18 mo, children of the control group barely completed the task. Altogether, this suggests that children may be more compliant if they have been exposed to the method at an earlier age when they would better accept the test. It is likely that low compliance can also be related to the novelty of the used tool (the feeder may be considered as a novel non-food object), as children have probably not been exposed to it at home. Higher compliance observed in 6-10 mo children in our study may be explained by mouthing stage developmental phase which consists in children putting their hands and any object into their mouth for few seconds, as a way to explore their environment (Tulve, Suggs, McCurdy, Cohen Hubal, & Moya, 2002). Mouthing is known to decrease with age and studies reported that object-to-mouth frequency was lower in 12-24 mo children as compared to 6-12 mo children (Tsou et al., 2015; Xue et al., 2010). Inversely, it is also possible that compliance to the task of older children was affected by the development of food neophobia which is known to emerge between 18 and 24 mo (Nicklaus & Monnery-Patris, 2017). We assumed that children of 12 to 18 mo would be physically able to process the task autonomously, however we observed that only 40 to 55 % of them at maximum were actually doing it by themselves; suggesting that the task was unfamiliar to them. Moreover, it is likely that compliance also depended on children's temperament and willingness to participate. As the number of studied children per group was low, it is possible that these parameters varied between the different groups. However, this was not measured in this study. Finally, some collected data were excluded from analysis because the oral exposure duration was outside the acceptable duration range (60 ± 10s). This is especially the case for the 6-10 mo children who largely processed the gel but for whom the oral duration was outside the one fixed by the protocol. More research seems necessary to determine an optimal oral processing duration necessary for comminuting the gel while limiting children tiredness.

Evolution of masticatory performance with age, relationships with oral processing strategy

Another point to consider when evaluating the feasibility of the method is the ability to discriminate bolus properties as function of children age. Indeed, the period between 6 and 18 mo is a sensitive period when children are learning to chew solid food with more and more complex texture (Harris & Mason, 2017; Nicklaus et al., 2015). Therefore, the method should be able to demonstrate an increase in children ability to comminute the test food. Using two groups of children followed during 4 (SG1) and 6 months (SG2) we were able to demonstrate that as children grew up they were better able to break down a brittle gel via oral processing. Therefore, we demonstrated the relevancy of our method to characterize the development of masticatory performance. Masticatory performance was also found to be highly variable among children of a given age. Main factor explaining this variation was the oral strategy (chewing/biting or sucking) used by the child to perform the task. Obviously chewing was found to be more efficient than sucking for breaking down the gel. This strategy was found to evolve with children age and vary between children at a given age.
A clear change in oral processing strategy was observed between 6 and 10 mo. All 6 mo-old- children processed the gel by sucking and chewing behavior was observed in only 34 % of them. At 8 months, most of the children processed the gel by both sucking and chewing and at 10 months all children

chewed/bite on the gel, sucking was less present. This transition in oral processing strategy between 6 and 10 mo is congruent with the strategy naturally adopted by children when eating hard foods. Indeed, we previously studied the same children in eating situations and observed that the probability of eating a biscuit by sucking decreased from 1 to 0.7 between 6 and 10 mo whereas the chewing probability sharply increased from 0.1 to 0.8 during the same period (Demonteil et al., 2019). A transition from sucking to chewing at an early age of complementary feeding was also reported in another study characterizing 6 and 24 mo children in feeding situations (Gisel, 1991; Stolovitz & Gisel, 1991). The authors showed that munching (immature chewing) and suckling (voluntary sucking) behaviors were used depending on children's age and the type of food studied. Chewing behavior in response to solid food (cereal) was found to increase between 6 and 8 months from 4.8 out of 10 trials to 8.5/10 trials (Stolovitz & Gisel, 1991). Authors mentioned that chewing was well established at 8 mo for solid food (Gisel, 1991), which is also observed in our study (81% of children). Comparatively, sucking behavior was observed more often for a viscous food: children up to 10 mo were found to suckle on approximately 5 of the 10 trials and munched on the other half (Gisel, 1991). In our study, chewing was observed for almost all measurements performed in 10 mo children (95%) and sucking was observed in half of them. The dominance of chewing in our study can be explained by the fact that the agar-agar gel is poorly melting at mouth temperature, contrary to the gelatin gel used in Gisel's study (1991). From 10 months on, we found that the ratio of chewing vs sucking behaviors were stable until 18 mo. The gel was processed by chewing/biting by all children and by chewing/biting and sucking for half of them. The frequency of sucking behavior observed is higher than observed with real foods (Demonteil et al., 2019). We believe that this behavior can be either induced by the presence of the mesh or favored by possibility to better taste the sweetness of the gel by sucking.

We observed that oral processing strategy was stable between 12 and 18 mo, however, the number of particles collected in boluses still increased suggesting a development of the ability to comminute the test gel by chewing. Interestingly, our data suggest that exposing children to the method impacted neither the way of processing the gel nor the bolus properties at 10 mo, suggesting that a familiarization with the test through repeated testing did not have drastic impact on the results.

Studies conducted in literature on older children (4-6 and 8-12 years old) demonstrated that masticatory performance is influenced by children dentition (Consolacao Soares et al., 2017; de Souza Barbosa et al., 2013; Hama et al., 2017). In our study conducted in infants and toddlers, the association between the emergence of deciduous teeth and masticatory performance was not significant. Children's masticatory performance was not further explained by the gender of the children, the age of complementary feeding introduction, breastfeeding, thumb/pacifier sucking, or their ability to self-feed. However, this was exploratory and it is difficult to conclude as our sample size does not allow to really studying these effects. The question whether children characteristics and feeding practices play a role in the development of masticatory performance should be addressed in another study with a larger population to come to firm conclusions. Future study should also taking into account the impact of children's previous exposure to food texture, as it is thought to play a role in the development of chewing skills (da Costa et al., 2017; Demonteil et al., 2018; Nicklaus et al., 2015).

The strength of the current study is that, for the first time, masticatory performance was quantified in infants and toddlers. The proposed method was found to be applicable to very young children as they could freely process the gel and bolus was easily collected. The combination of studying children's compliance, oral behavior and bolus granulometry allowed us to determine the optimal age range to apply this method: between 8 and 15 months. At 6 months, children barely adopt chewing/biting behavior, at 18 mo children are not complying anymore to perform the task if not exposed to it at an earlier age.

So far young children's mastication abilities had been characterized though visual evaluations and biomechanical approaches. The former included video evaluations of the time of mastication and the numbers of chews (Gisel, 1991), presence of circumoral movements (Stolovitz & Gisel, 1991) and rating on 4 point-scales of a series of detailed oral behaviors including biting, chewing, swallowing, gagging, etc. (Remijn et al., 2013). The latter included measurements of mandibular control through the kinematics of jaw movements and muscle coordination through electromyographic activity (Green et al., 1997; Simione et al., 2018; Wilson & Green, 2009). Both types of methods provide different pieces of information which are relevant from a developmental perspective: visual observations allow evaluating the development of global chewing behaviour and ability to eat foods with age, whereas biomechanics allow one to assess the development of chewing, motor control and coordination. We propose here another non–invasive approach which can add complementary information coming from the study of bolus destructuration after chewing. Our method has the advantage to be easy to analyze as it does not require specific training and can therefore be easily incorporated in future studies focusing on food oral processing during early childhood.

This study has some limitations that deserve mentioning. Compliance was low for some groups and highly variable across groups but can possibly be improved by changing some methodological parameters, as discussed. Moreover, the number of children was low and did not allow studying in detail the impact of individual characteristics on masticatory performance. Concerning the method, one possible drawback could be that some very small (<1mm^2) chewed particles may have passed through the mesh and may therefore be lost for the granulometry analysis. It is difficult to study this precisely as it would be necessary to collect lost particles via mouth rinsing which is not possible in young children. However, despite this, we demonstrated that the collection of chewed particles inside the mesh can successfully discriminate children masticatory performance as function of their age. Finally, there is a limitation concerning the definition of masticatory performance used in this study. Indeed, it may not be directly comparable to measurements usually performed in literature in older children and adults, for two reasons: i) young children also process the gel by sucking and ii) because the gel is enclosed in a mesh, the tongue movements may less solicited than it would have been if the gel would be freely chewed. However, food oral processing in early childhood is under development and mastication is not mature, and our method allows taking into account these specificities.

Conclusion

For the first time, the development of masticatory performance was quantified in infants and toddlers from bolus destructuration under oral processing. We demonstrated an increase in performance between 6 to 18 mo. Compliance was moderate but can possibly be increased by modifying some methodological parameters (decreasing the experimental session duration and potentially decreasing duration necessary to orally process the gel). Inter-individual differences in masticatory performance at a given age were mainly related to children's oral strategy used to process the gel (sucking vs biting/chewing), this strategy being congruent with those previously observed for real foods. The number of teeth did not seem to play a key role in children masticatory performance in our study. Our method is a valuable tool that can be used in future studies for investigating the evolution of masticatory function and relationship with food texture acceptance at early stage of complementary feeding.

Acknowledgements

The authors thank all the parents and children participating in this study. We also gratefully acknowledge Floée Schultz and Emilie Szleper (INRA) for help in the experimental measurements; and Christophe Martin, Fabienne Bouillot and Françoise Durey (ChemoSens platform, INRA) for their global technical support. The authors thank Hugo Weenen (Danone Nutricia research, Utrecht) for valuable comments and corrections on this article.

This work was supported by a grant from Blédina, S.A., and by the Research and Technology National Association (ANRT), the Conseil Régional Bourgogne, Franche-Comté (PARI grant) and the FEDER (European Funding for Regional Economical Development).

Ethical Statements

The study was approved by the local ethics committee (CPP Est III - ID RCB 2015-A00323-46 and ANSM) and written informed consents were obtained from both parents prior the start of the study.

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TABLES

Table 1: Children characteristics per group (study (SG) or control (CG) groups) and age

	SG1			CG1	SG2			CG2
Age (in months)	**6**	**8**	**10**	**10**	**12**	**15**	**18**	**18**
n	25	24	24	22	27	26	25	23
Number of teeth	0.5±0.8c	1.3±1.4b	2.9±1.9 a A	3.5±2.6 A	5.4±2.9c	8.9±3.8b	11.6±4.3 a A	13.1±4.4 A
Current Breastfeeding (%)	36.0a	29.2a	25.0 a A	13.6 A	0.0	0.0	0.0	0.0
Thumb sucking (%) †	48.0a	37.5a	25.0 a A	45.5 A	29.6a	30.8a	28.0 a A	13.0 A
Use of pacifier (%) †	76.0a	75.0a	75.0 a A	59.1 A	66.7a	61.5a	56.0 a A	65.2 A
Eats with finger (%) †	12.0b	33.3b	62.5 a A	45.5 A	70.4a	80.8a	100 a A	87.0 A
Holds a spoon in mouth alone (%) †	16.0a	25.0a	25.0 a A	22.7 A	22.2b	80.8a	100 a A	91.3 A

† Behaviors observed 'sometimes' or 'often'. For a given group (SG1 or SG2), values with the same lower case letter (a, b, c) are not significantly different (p<0.05). For a given age, group values associated with the same upper case letter (A, B) are not significantly different (p<0.05).

Table 2: Children feeder acceptance and compliance to the masticatory performance measurement: Number of boluses collected per group (SG1, CG1, SG2, CG2), age and session

Group	Age (mo)	Session	Nb children	Children accepting the feeder	Children processing the gel for 60 ± 10s at each session	Average compliance (%) †	Nb children for who of masticatory performance was determined ‡
SG1	**6**	**1st**	**25**	**19**	**13**	**50**	**16**
		2nd	**25**	**17**	**12**		
	8	**1st**	**24**	**22**	**16**	**68**	**20**
		2nd	**24**	**21**	**17**		
		3rd	**24**	**20**	**16**		
	10	**1st**	**24**	**21**	**15**	**67**	**18**
		2nd	**24**	**20**	**17**		
		3rd	**24**	**20**	**16**		
CG1	**10**	**1st**	**22**	**15**	**9**	**41**	**9**
SG2	**12**	**1st**	**27**	**12**	**10**	**41**	**13**
		2nd	**27**	**15**	**11**		
		3rd	**27**	**14**	**12**		
	15	**1st**	**26**	**16**	**12**	**40**	**14**
		2nd	**25**	**16**	**11**		
		3rd	**25**	**13**	**8**		
	18	**1st**	**25**	**19**	**14**	**57**	**18**
		2nd	**25**	**19**	**16**		
		3rd	**25**	**18**	**13**		
CG2	**18**	**1st**	**23**	**7**	**2**	**9**	**2**

Nb: number; † Mean ratio (%) between the number of compliant measures and the initial number measurements performed (all children and session together) in the study for each age ‡ Replicates measurements performed over different sessions at the same age were averaged for each child. These numbers refer to the number of compliant children, i.e. the number of children of a given age and group for whom we measured masticatory performance at least once (in one session).

Table 3: Mean frequencies of behaviors observed during masticatory performance measurements (i.e., among compliant children)

	SG1			CG1	SG2		
Age (mo)	6	8	10	10	12	15	18
Number of children†	16	20	18	9	13	14	18
Number of in-mouth sequences (SD) ‡	5.1 (4.4) a	5.6 (3.5) a	5.7 (3.9) a A	6.3(4.3) A	7.5(4.5) a	5.0(3.3) a	5.2(3.8) a
Performed the task autonomously (%)§	12 a	8 a	19 a	11	40	42	55
Sucking (%)§	100 a	80 b	50 c	67	37 a	37 a	42 a
Chewing (%)§3	34 b	81 a	95 a	67	97 a	98 a	87 a

† *Number of compliant children (see Table 1);* ‡*Number of times the children orally processed the gel to reach a processing duration of 60±10s (a number of in-mouth sequence equal to 2 would mean that the gel has been processed twice during the 60s-period, with a pause in between). (a, b, c : lower case letters : test for the age effect within a given group (SG1 or SG2,) and upper case letters : test between groups (SG vs.CG).Values associated with the same letter are not significantly different (p<0.05)).* §*Frequency of behaviors between SG1 and CG1 at 10 mo could not be compared because sample sizes were too small in some cases (n<5).*

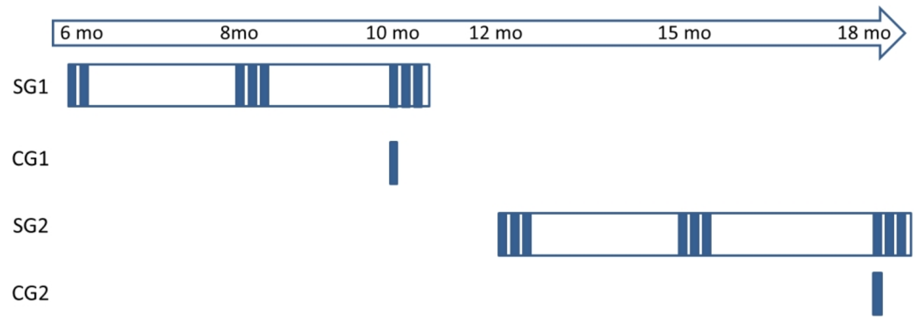

Figure 1: Scheme of the general study set up (each vertical bar corresponds to one assessment day of masticatory performance) (SG : Study Group; CG : Control Group)

Determination of masticatory performance

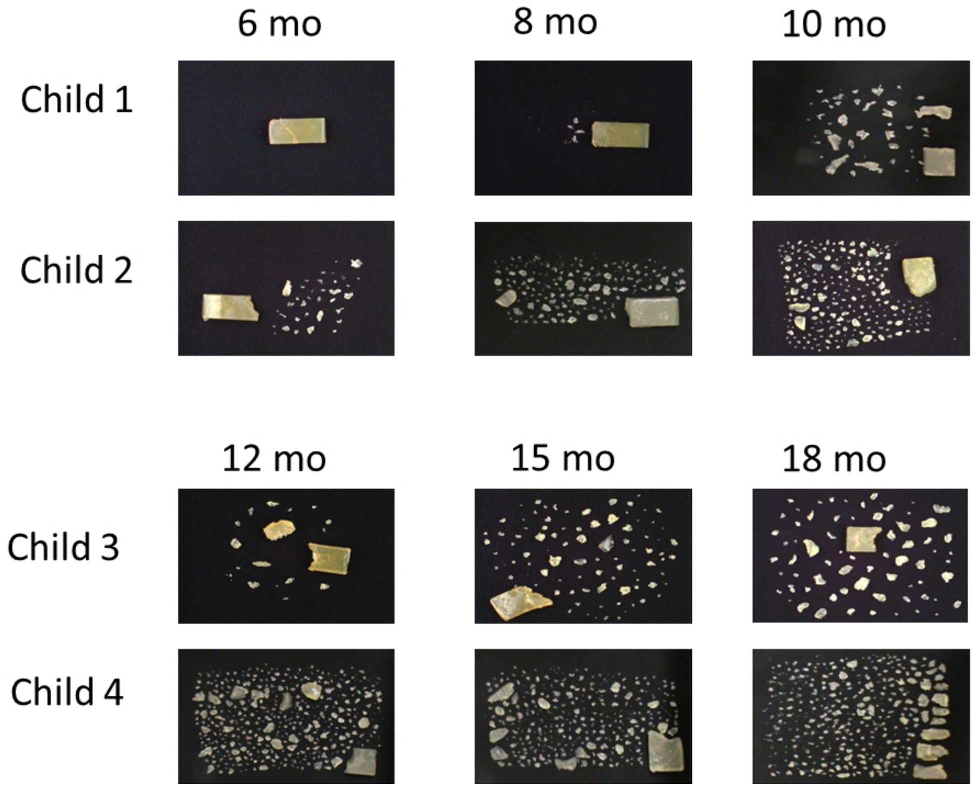

Figure 3 : Examples of images of gel fragments collected from different children at different ages (6, 8, 10 or 12, 15, 18 mo).

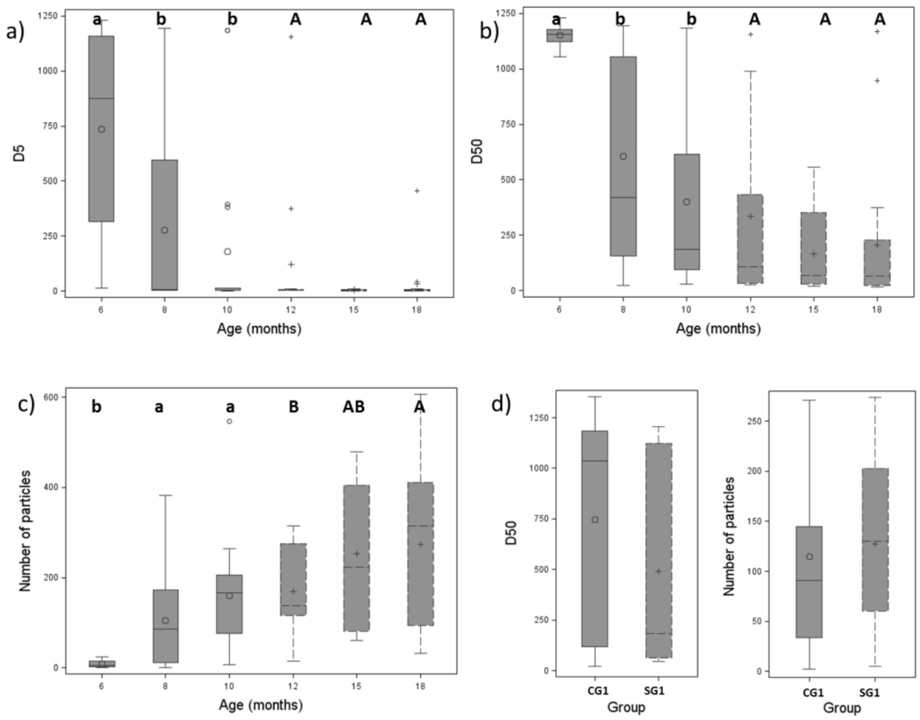

Figure 4 : Box plots for gel bolus properties (D5, D50 and number of particles) collected at different ages (study groups SG1: solid line, SG2: dotted lines, Figure 4a, b and c) and at 10 mo in two different groups (CG1 and SG1-session1, Figure 4d). For each box plot, the bottom and the top of the box are the 25th and 75th percentiles and the line within the box is the median; the circular (○) or the cross (+) signs are the mean. The whiskers extend from the box as far as the data extend, to a distance of at most 1.5 × interquartile range. Extreme values are marked by a small circle or cross (° or +). a, b, c: values associated with the same letter are not significantly different from each other ($p>0.05$). Data were analyzed for SG1 (lower case letter) and for SG2 (upper case letters) independently. Differences between data collected in CG1 and SG1 were not significant (Student T-test, $p<0.05$).