Food Research International 137 (2020) 109692

Contents lists available at ScienceDirect

Food Research International

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

The effect of a bread matrix on mastication of hazelnuts

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ARTICLE INFO

Keywords:
Hazelnuts
Oral processing behaviour
Composite foods
Food matrix
Bolus properties

ABSTRACT

The effect of the degree of nut mastication on bioavailability of their nutrients has been established previously. In contrast, the effect of incorporation of nuts into composite food matrixes on oral processing behaviour and structural breakdown has been studied scarcely. This study aimed to investigate the effect of incorporation of hazelnuts into bread matrixes in comparison with plain hazelnuts on bolus properties and chewing behaviour. Amount of plain hazelnuts was varied to investigate the effect of portion size on bolus properties and chewing behaviour. Bolus particle size distribution was obtained by image analysis of expectorated boli by n = 20 participants. Median bolus particle diameter (d_{50}) and broadness of particle size distribution (b) were quantified by fitting the cumulative area distribution curve with a modified Rosin-Rammler function. Oral processing behaviour (number of chews, chewing time, chewing frequency) was quantified by means of a stopwatch. Mastication of two hazelnuts resulted in smaller d_{50} than mastication of six hazelnuts or mastication of two hazelnuts in white bread or baguette. Chewing time of two hazelnuts was significantly shorter than chewing time of six hazelnuts or chewing time of two hazelnuts in white bread or baguette, while chewing frequency did not differ between foods. d_{50} of six hazelnuts did not significantly differ from d_{50} of two hazelnuts in either bread matrix. Broadness b of the particle size distribution was significantly smaller for six hazelnuts compared to the other foods. We conclude that d_{50} was affected by bite size or bite volume rather than by incorporation of hazelnuts into bread. We suggest that incorporation of hazelnuts into bread matrixes has a relatively small impact on size of hazelnut bolus particles produced upon mastication.

1. Introduction

Hazelnuts (*Corylus avellana* L.*)* are the fruits of plants from the *Corylus* genus. They can be consumed raw but are commonly roasted before consumption in order to obtain characteristic sensory or texture features (Amaral, Casal, Seabra, & Oliveira, 2006). As their organoleptic properties are desirable in a variety of foods, they are often used as an ingredient in the food industry (Ozdemir & Akinci, 2004). Nuts are energy dense foods (Ros, 2010). However, their macronutrients are enclosed within cell walls. Cell walls are constituted of dietary fibre which is, by definition, resistant to digestive enzymes in the human small intestine (Jones, 2014). When cellular integrity of plant-based foods is retained at the moment of swallowing, macronutrients are entrapped within cell walls which reduces their digestibility and absorption in the small intestine (Capuano, 2017; Grundy, Wilde, Butterworth, Gray, & Ellis, 2015). Previous studies have shown that the metabolizable energy from walnuts, pistachios and almonds is overestimated because of persistence of intact nut cells during digestion (Baer, Gebauer, & Novotny, 2012, 2016; Novotny, Gebauer, & Baer, 2012). More recently, the same effect has been shown for cashew nuts (Baer & Novotny, 2019). Possibly, this effect is generalizable to all nuts, notwithstanding individual differences in nut structure and texture.

Oral processing is the first step of food digestion by which solid foods are broken down, mixed with saliva and converted into a bolus that can be safely swallowed. Mastication plays a crucial role in the full utilization of nutrients in nuts, as incomplete mastication may limit cell wall breakage and thus access of enzymes to the nutrients (Grassby et al., 2014; Grundy et al., 2015; Parada & Aguilera, 2007; Suzuki et al., 2005). Generally, the amount of intracellular nutrients that are actually digested and absorbed from plant foods consumed intact are greatly influenced by bolus properties such as particle size and number of broken and intact cells.

Several studies have been reported on oral processing of nuts and the corresponding effect on bolus properties and nutrients utilization (Cassady, Hollis, Fulford, Considine, & Mattes, 2009; Jalabert-Malbos, Mishellany-Dutour, Woda, & Peyron, 2007; Olthoff, Van der Bilt, Bosman, & Kleizen, 1984; Peyron, Mishellany, & Woda, 2004). These studies

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https://doi.org/10.1016/j.foodres.2020.109692
Received 10 April 2020; Received in revised form 7 September 2020; Accepted 7 September 2020
Available online 16 September 2020

generally indicate that thorough mastication enhances disruption of the food matrix and thus the amount of nutrients that can be digested and absorbed. However, these studies have focused on mastication of plain nuts. Despite plain nuts are commonly consumed as snacks, very often they are incorporated into a food matrix or are part of composite foods. Little is known about the impact of the food matrix surrounding the nuts on oral processing behaviour, bolus properties and nutrient bioavailability. We hypothesize that oral processing of composite foods such as nuts embedded in bread matrixes may alter oral processing behaviour and consequently the size distribution of nut bolus particles. This may have an impact on the actual utilization of nut nutrients, but utilization of nut nutrients is outside the scope of the current study. Hutchings et al. (2011) studied the mastication of peanuts in two different matrixes, a gelatine matrix and a chocolate matrix (Hutchings et al., 2011). They found differences in particle size distribution of the resulting boli but no differences in the mean particle size. More recently, McArthur, Considine, and Mattes (2018) studied the particle size distribution of almonds, pistachios and walnuts incorporated into yoghurt matrixes (McArthur et al., 2018). They found that incorporation of nuts into liquid and semi-liquid matrixes increased mean particle size of the nut boli. To the best of our knowledge, the effect of incorporation of nuts into solid food matrixes on oral processing behaviour and bolus properties has not been studied yet.

The aim of this study was to investigate the effect of bread matrixes on size distribution of hazelnut bolus particles and oral processing behaviour. We hypothesize that the incorporation of hazelnuts into a bread matrix changes hazelnut particle size distribution of the bolus. Additionally, we hypothesize that the type of bread matrix influences particle size distribution of hazelnuts in the bolus. We speculate that differences in hazelnut bite size lead to differences in bolus properties and oral processing behaviour.

2. Materials and methods

2.1. Materials

Deshelled, deskinned, roasted hazelnuts were obtained from Demir Nut Company B.V. (Wateringen, The Netherlands). White croque-monsieur bread ('Tosti sneetjes wit', called 'White bread' hereafter) was obtained from Bakkersland B.V. (Hedel, The Netherlands), and white baguettes (called baguette thereafter) were obtained from a local supermarket in Wageningen (Jumbo Group Holding B.V., Veghel, The Netherlands). Both types of bread were stored in the freezer and fresh breads were purchased every 4 weeks. During the mastication sessions tap water was used. Pancreatin was obtained from Merck KgaA (Darmstadt, Germany) and sourced from porcine pancreas. A mixture of different pancreatin batches with an average trypsin activity of 2.98 Umg (tosyl-L-arginine methyl ester, TAME-units) was used.

2.2. Test food preparation

The average weight of one hazelnut was determined as 1.1 g by weighing 100 hazelnuts of average-looking size. Odd-sized and oddly-shaped hazelnuts were not used in any of the experiments. As bread crusts were expected to influence mastication behaviour, both amount of crust and total weight of the bread pieces were standardized. This was achieved by using white bread and baguette cutting procedures as shown in Supplementary material. Before cutting, the bread was thawed at 20 °C for one hour. The test food volumes were calculated based on hazelnut density, hazelnut weight and bread piece dimensions. Baguette volume was assumed equal to the white bread volume (despite slightly different dimensions) because of the curvature in the baguette crust. Average density of hazelnuts was calculated to be 715.2 kg/m^3 based on previous results (Ozdemir & Akinci, 2004).

This cutting procedure resulted in white bread pieces of approximately 50 × 20 × 12 mm (length × width × thickness) and baguette pieces of approximately 60 × 20 × 12 mm. The width and length of the baguette pieces were measured at the widest and longest parts, respectively.

Six test foods were prepared as shown in Table 1. From preliminary trials, we observed that a typical bite size of a bread with hazelnuts corresponds to approximately 2 nuts (around 2 g) and 4 g of bread. Since one hazelnut weighs around 1 g, we considered a bite size of around 6 g for the test foods 2H + W and 2H + B. These test foods were prepared by inserting hazelnuts into one crumb piece of either white bread (W) or baguette (B). The test foods 2H and 6H were selected as controls having the same amount of nuts or the same weight/volume as the 2H + W and 2 W + B, respectively.

By comparing foods 2H, 2H + W and 2H + B, the effect of the bread matrix on the size distribution of masticated hazelnuts particles and oral processing behaviour was studied. Test foods 2H + W and 2H + B were compared to study the influence of type of bread on hazelnuts bolus particle size distribution and chewing behaviour whereas test food 6H was used as a control, to study the influence of number of hazelnuts on particle size and chewing behaviour. Test foods W (white bread without hazelnuts) and B (baguette without hazelnuts) served as negative controls to be compared with test foods 2H + W and 2H + B, respectively.

After preparation of the bread samples, the pieces for 2H + W and 2H + B were put into 50 mL Cellstar tubes with a cap (Greiner Bio-One, Kremsmünster, Austria), while the pieces for test foods W and B were put into Gosselin straight sample containers (Fisher Scientific, Pittsburgh, PA, USA), and then frozen at −20 °C until the day of the mastication sessions.

Hazelnuts for 2H and 6H were counted and put into 50 mL Cellstar tubes with caps. The tubes with hazelnuts were then stored at 20 °C until the day of the mastication sessions. The hazelnuts for 2H + W and 2H + B were stored at 20 °C in the plastic bag in which they were originally supplied. These storage conditions were based on previous studies (Giacosa et al., 2016).

2.3. Texture analysis of bread crumbs

Texture profile analysis (TPA) was performed on the bread crumb of baguette and white bread using the TA-XT plus texture analyzer (Stable Micro Systems, UK) according to Boukid et al. (2018). Two slices (12 × 24 × 24 mm; thickness × width × length) were stacked together for each test. A double compression test with a test speed of 5 mm/s to a maximum strain of 40% with a rest time of 5 s between compressions was performed using a cylinder probe (P/75). Hardness, springiness, cohesiveness, chewiness, and resilience of the bread crumb were determined. Hardness was determined as the peak force of the first compression. Springiness was calculated by dividing the time of the second compression by the time of the first compression. Cohesiveness was calculated by dividing the area under the force-strain curve of the second compression by the area under the force-strain curve of the first compression. Resilience was calculated by dividing the area under the force-strain curve of the downstroke of the first compression by the area of the upstroke of the first compression. TPA analysis of the bread crumbs were carried out on three different days on two breads (baguette and white bread) with nine replicate measures. Averages and standard deviations were calculated.

Table 1
Test foods used in the present study, their composition and total weight. H denotes hazelnut, W denotes white bread and B represents baguette.

Test food code	Test food description (total weight)
2H	2 hazelnuts (≈2.2 g)
2H + W	2 hazelnuts (≈2.2 g) + 1 piece of white bread crumb (≈4.0 g)
2H + B	2 hazelnuts (≈2.2 g) + 1 piece of baguette crumb (≈4.0 g)
6H	6 hazelnuts (≈6.6 g)
W	1 piece of white bread crumb (≈4.0 g); no hazelnuts
B	1 piece of baguette crumb (≈4.0 g); no hazelnuts

2.4. Mastication procedure and characterization of oral processing behaviour

The research of this study does not fall within the remit of the 'Medical Research Involving Human Subjects Act'. The study was conducted in agreement with the ethical principles regarding human experimentation outlined in the Declaration of Helsinki. All subjects gave written informed consent.

Twenty volunteers participated in the mastication sessions. Two criteria were taken into account when selecting the volunteers. First, volunteers did not show any adverse reactions to the allergens present in breads or nuts. Secondly, volunteers did not have any dental problems that affect mastication. These problems included missing teeth or strongly irregular teeth, as well as pain or difficulties experienced upon chewing. Volunteers had an average age of 26.8 ± 10.6 (mean ± SD) years. Thirteen of the volunteers were male and seven females.

At least one hour prior to the start of the mastication session, the test foods were taken out of the freezer and allow to thaw at 20 °C. To limit physical changes in the bread, thawed bread was never kept>4 h at room temperature before starting the mastication session. Volunteers were given brief instructions that the number of chews and chewing time would be recorded. The first two test foods were samples W and B. Volunteers were instructed to chew and swallow the test foods as close as possible to the way they normally consume them. Volunteers were allowed to swallow the bolus in multiple smaller portions, but they were not allowed to masticate the food anymore after swallowing the first part. All food samples were put in the mouth at once. The number of chews was determined by counting chews and the chewing time by means of a stopwatch. For these first two samples (W and B), volunteers were not instructed to expectorate the boli.

The third and fourth test food to be masticated were 2H + W and 2H + B, respectively. Volunteers were given a cup of water to clean their mouths of any bread pieces before the session continued. This time, they were asked to take out the piece of bread from the tube, after which they were given two hazelnuts. They were then asked to fold the bread around the hazelnuts. In this way a hazelnut bread with the hazelnuts being incorporated into the crumb was mimicked using our model foods. Volunteers were asked to put the entire test food in their mouth at once, chew the food as they would normally do, and expectorate the bolus back into the Cellstar tube at the moment the food would normally be swallowed. They were instructed not to swallow any hazelnut particles. After expectoration, they rinsed their mouth with water, which was also expectorated into the tube. The volunteers were supplied with enough water and were asked to keep rinsing until they sensed no more nut particles in the oral cavity. At that moment, the cap was twisted onto the tube and the session continued with the next test food.

The fifth and sixth test foods that were masticated were 2H and 6H. As for the previous test foods, the volunteers were given a cup of water to clean their mouth and they were also given a small instruction again. The rest of the mastication procedure for these test foods was identical to the procedure for test foods 2H + W and 2H + B.

The tubes with expectorated boli and water were stored in the freezer at −20 °C within five minutes after the mastication of the last test food.

2.5. Hazelnut particle isolation

Hazelnut particle isolation started after all mastication sessions were completed. Before starting the isolation, the Cellstar tubes were taken out of the freezer and thawed at 20 °C for 4 h.

Afterwards, 1 g of pancreatin was added to every tube to remove the bread, including the boli from the mastication of plain hazelnuts. After the pancreatin addition, the tubes were shaken vigorously for 5 s before putting them in a 40 °C Julabo SW23 shaking water bath (Julabo GmbH, Seelbach, Germany) for 18 h. Treatment with pancreatin removed any trace of bread from the masticated boli and resulted in an equal treatment across all boli.

After removal from the water bath, the contents of the tubes were passed through a 0.355 mm sieve (Metaalgaas Twente BV, Hengelo, The Netherlands). Particles were cleaned while in the sieve by flushing them under running tap water for approximately 30 s. Afterwards, particles were briefly flushed with 70% ethanol. This assisted in the breakdown of particle aggregates with minimal influence on particle size (Hutchings et al., 2011).

After sieving, particles were transferred into a Fisherbrand aluminium dish (Fisher Scientific, Pittsburgh, PA, USA) using a spatula. Hazelnut particles that resulted from test food 6H were divided over three aluminium dishes to compensate for the bigger number of particles and ensure equal treatment across all samples. This resulted in the weights of all dishes being comparable. To all dishes, 15 mL of 70% ethanol was added to assist in particle spreading. Afterwards, dishes were put in a Venti-line oven (VWR International, Radnor, PA, USA) at 100 °C for 24 h, to finalise hazelnut particle isolation.

2.6. Particle size analysis

Particle size analysis was adapted from previous works (Hutchings et al., 2011; Van der Bilt, Van der Glas, Mowlana, & Heath, 1993). Before starting the image analysis, dry hazelnut particles were sieved once again over a 0.355 mm sieve and the particles that passed the sieve were discarded. Weight loss was determined at this stage, comparing the dry weight of what remained on the sieves with the initial dry matter of the hazelnuts used in the mastication experiments.

A CanoScan 9000F Mark II flatbed scanner (Canon Inc., Tokyo, Japan) was used to obtain images of the expectorated boli. Scans were made in greyscale and at 600 dpi. Particles were distributed as much as possible over a square 120 × 120 mm plastic petri dish (Greiner Bio-One, Kremsmünster, Austria). A test scan was then made to identify any particles that were still touching. After separating those, the final scan was made. After re-distribution of the particles using a brush, the entire scanning procedure was repeated once more. This increased the overall accuracy of the scanning results by redistributing the particles on the petri dish.

Black and white paper were used to improve quality of scanning results. Black paper covered the top part of the scanner and served as a background. This helped to identify hazelnut particles on the scan. White paper covered the glass scanning surface and contained a hole in the middle, in which the petri dish was placed. This made the edges of the petri dish clearly distinguishable. It also resulted in the petri dish being positioned in exactly the same place for every scan, simplifying further analysis.

An ImageJ (National Institutes of Health, Bethesda, MD, USA) macro was used to calculate particle area. A scale of 23.1074 pixels/mm was used, together with a dark-background threshold (45–255) and thresholds for particle area (0.1-infinite mm^2) and circularity (0.15–1.00). For the samples from test food 6H, which were distributed over three scans, the three sets of area calculations were added together and treated as one measurement from here on. The areas of the particles were ordered from low to high. Based on their area and assuming that all particles were spherical, the theoretical diameter of every single particle was calculated. These diameters were then used to create the cumulative area distribution of the particles. In order to visually assess differences between the test foods, one cumulative area distribution was also produced per test food. This was done by combining the data from all participants into one set of data in Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA).

The cumulative area distribution was fitted to an adapted Rosin-Rammler function (Eq. (1)) as previously reported (Hutchings et al., 2011)

$$Q = 1 - e^{-\left(\frac{x-d}{d_{50}-d}\right)^b * \ln(2)} \quad (1)$$

Where:

Q = area fraction of particles with a diameter smaller than or equal to × (dimensionless).

x = particle diameter (in mm).

d = baseline constant (in mm).

d_{50} = theoretical sieve size through which 50% of the 2-dimensional particle area fall (in mm).

b = broadness of the cumulative curve (dimensionless).

The fitting procedure resulted in estimated values for d_{50} and b for every scan. These values were then averaged between duplicate scans, to result in one final value for each parameter for each sample (subject/test food). The fitting was based on minimizing the residual sum of squares and was performed using the solver add-in in a Microsoft Excel application.

d_{50} and b parameters were used instead of mean and standard deviation, as they describe the distribution well and using them is common practice in literature that describes particle size distributions (Alderliesten, 2013; Hutchings et al., 2011; Lucas & Luke, 1984; Olthoff et al., 1984). This makes the results of the present study better comparable to previous reports. d_{50} is an approximation of the median of the particle size distribution whereas b is a measure for the slope of the cumulative area distribution, in such a way that an increasing value for b corresponds to a narrower particle size distribution (Hutchings et al., 2011). A baseline constant of 0.355 mm was chosen, as this was the minimum diameter of the particles that could be detected by the software.

2.7. Statistical data analysis

Statistical data analysis was performed in SPSS statistics (version 23, IBM Statistics Inc., Armonk, NY, USA). TPA parameters of breads were analysed by means of a *t*-test ($\alpha = 0.05$). d_{50} and data was tested for normality using a Shapiro-Wilk test in combination with visual assessment of Q-Q plots. Although not all parameters were entirely normally distributed ($p < 0.05$), a repeated measures analysis of variances (ANOVA, $\alpha = 0.05$) was used to compare the means of the number of chews, chewing time, chewing frequency, d_{50} and b between test foods. This choice was justified by the relatively large sample size, the central limit theorem and the robustness of a repeated measures ANOVA against violations of normality (Abbott, 2016). Based on the results of a Mauchly's test for sphericity ($\alpha = 0.05$), the Greenhouse-Geisser correction was applied during the analysis of number of chews, chewing time and chewing frequency. A pairwise comparisons post-hoc test with Bonferroni correction was performed to further analyse the differences between the test foods.

Correlations between the parameters food weight, food volume, chewing time, number of chews, chewing frequency, d_{50} and b were calculated by means of a bivariate Pearson correlation analysis (2-tailed, $\alpha = 0.05$). The correlation between d_{50} and b and between d_{50} and chewing parameters values for each independent test food was also tested by means of bivariate Pearson correlation analysis (2-tailed, $\alpha = 0.05$). Homogeneity of variance for d_{50} and b values distributions across the same test food was tested by a Levene test ($\alpha = 0.05$).

3. Results and discussion

3.1. Effect of incorporation of hazelnuts into bread matrixes on hazelnut bolus properties

Nuts are often consumed in conjunction with other foods within a meal or they are formulated into a continuous liquid or solid matrix (e.g. yoghurt or energy bars). This may modify the particle size distribution of masticated nuts. In this study we selected bread as matrix because hazelnuts are sometimes incorporated in specialty breads. However, we decided not to use commercially available nut breads because of the variability in the degree of processing and thus mechanical properties of different breads. By starting from our own nuts and breads and following the preparation procedure described in Section 2.3, we could standardize the effect of processing. Furthermore, by using the model foods of our study, we could systematically vary the amount of nuts incorporated into the bread matrices. We could also vary the bread crumb matrix without changing the properties of the hazelnuts. To test the effect of the textural properties of bread on particle size distribution of incorporated nuts, we selected two types of breads. The textural properties of the two types of bread are reported in Table 2. It can be noted that the crumb of bread W was significantly harder (absolute difference in hardness between W and B: 0.31 N; relative difference: 24%), more springy (absolute difference in springiness between W and B: 0.03 (−); relative difference: 3%) and more resilient (absolute difference in resilience between W and B: 0.03 (−); relative difference: 10%) than the crumb of bread B whereas no significant difference in cohesiveness was found.

Cumulative area distributions of hazelnut bolus particles of all test foods are shown in Fig. 1. Differences in particle size distributions between test foods were very small. It appears that the cumulative curve for the two hazelnuts slightly tends towards smaller particle diameters whereas the cumulative curve of the six hazelnuts slightly tends towards larger particle diameters, while the cumulative curves for the other two test foods containing two hazelnuts and different breads were very similar.

Oral processing parameters (number of chews, chewing time and chewing frequency) and bolus particle size parameters (d_{50} and b) are summarized in Table 3. Significant differences ($p < 0.05$) in d_{50}, b, number of chews, chewing time and chewing frequency were found between test foods (Table 3). Table 3 shows that the average d_{50} for the mastication of two hazelnuts was 1.44 mm. d_{50} for the mastication of two hazelnuts and the b value for the mastication of six hazelnuts significantly differed from the other d_{50}- and b-values ($p < 0.05$). These differences in median particle diameter and distribution broadness were also apparent in Fig. 1. As for the mastication parameters, a significant ($p < 0.05$) lower number of chews and chewing time were observed for the plain two hazelnuts compared to all other test foods, but there were no significant differences in chewing time between the other test foods. The number of chews was significantly ($p < 0.05$) higher for test food 2H + B compared to all other test foods with the exception of six hazelnuts. Chewing frequency was significantly ($p < 0.05$) lower for both breads (W and B) compared to the corresponding breads containing hazelnuts (2H + W and 2H + B).

In addition to the parameters of the Rosin-Rammler model, mean hazelnut bolus particle sizes were calculated for each test food from the cumulative data (Fig. 1). The mean hazelnut bolus particle sizes were 0.89 ± 0.52 mm for 2H, 0.93 ± 0.56 mm for 2H + W, 0.94 ± 0.55 mm for 2H + B and 0.92 ± 0.56 mm for 6H.

We reported here for the first time information about the particle size distribution of plain hazelnuts boli. The median size is in line with what has been reported for almonds, walnuts and pistachios (McArthur et al., 2018), for peanuts, almonds and pistachios (Peyron et al., 2004) and for almonds (Cassady et al., 2009) but considerable larger than for peanuts (Jalabert-Malbos et al., 2007). Taken together the results of our study show that the incorporation of hazelnuts into bread crumb matrixes has a limited effect on the size distribution of bolus hazelnut particles. Type of bread in which hazelnuts are embedded had no effect on hazelnut bolus properties. Albeit statistically significant, the observed differences

Table 2
Textural properties of white bread (W) and baguette (B). Results expressed as mean ± SD of n = 9 determinations. Different letters in the same column indicate significant differences ($p < 0.05$).

	Hardness (N)	Springiness (–)	Cohesiveness (–)	Resilience (–)
White bread	1.27 ± 0.11^{a}	0.95 ± 0.01^{a}	0.86 ± 0.01^{a}	0.31 ± 0.01^{a}
Baguette	0.96 ± 0.19^{b}	0.92 ± 0.02^{b}	0.85 ± 0.02^{a}	0.28 ± 0.02^{b}

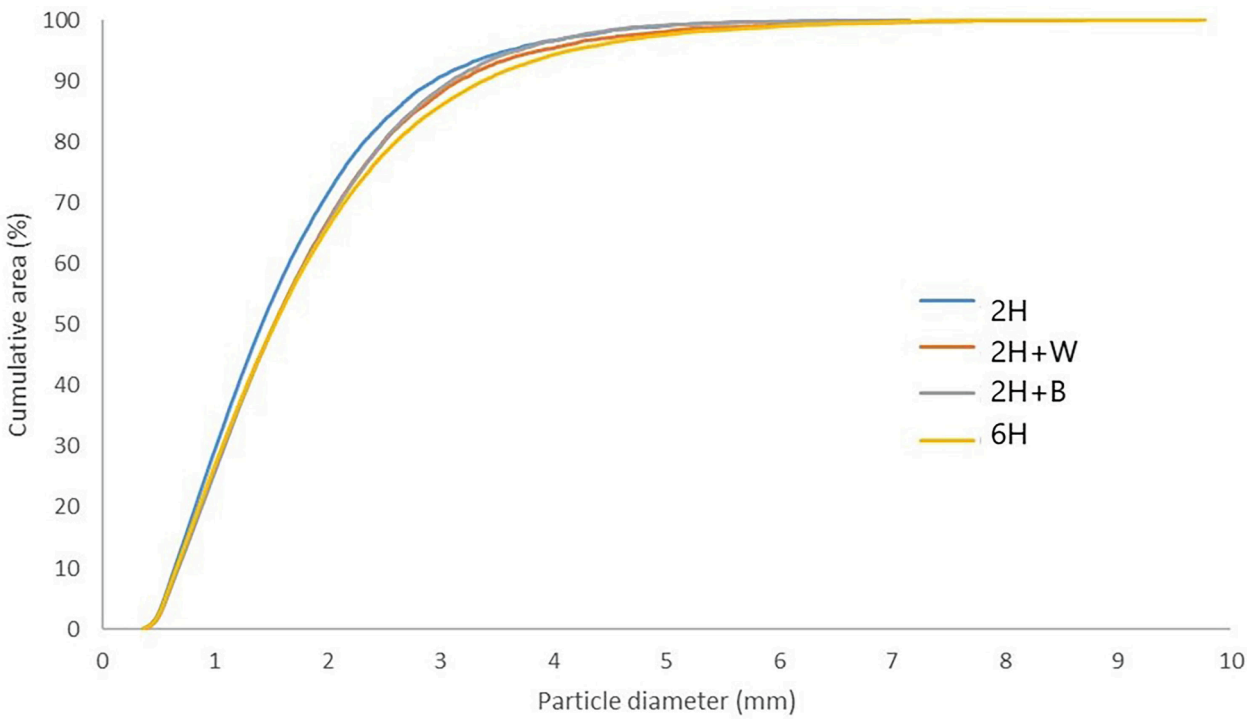

Fig. 1. Cumulative area distribution curves for all four test foods (n = 20 subjects).

Table 3
Comparison of oral processing parameters and particle size parameters between test foods (Mean ± SD). Means within one column that have the same letter were not significantly different ($p > 0.05$). 2H = 2 hazelnuts; 2H + W = 2 hazelnuts with white bread; 2H + B = 2 hazelnuts with baguette; 6H = 6 hazelnuts; W = white bread; B = baguette.

Test food	Mass (g)	Volume (10^{-5} m^3)	d_{50} (mm)	b	Number of chews (–)	Chewing time (s)	Chewing frequency (s^{-1})
2H	2.2	0.3	$1.44^A \pm 0.20$	$1.44^A \pm 0.07$	$19.8^A \pm 7.0$	$14.2^A \pm 4.2$	$1.37^{AB} \pm 0.21$
2H + W	6.2	1.5	$1.54^B \pm 0.21$	$1.44^A \pm 0.07$	$38.3^B \pm 10.0$	$27.5^B \pm 8.0$	$1.42^A \pm 0.18$
2H + B	6.2	1.5	$1.54^B \pm 0.18$	$1.45^A \pm 0.04$	$41.5^C \pm 9.6$	$28.7^B \pm 7.4$	$1.46^A \pm 0.17$
6H	6.6	0.9	$1.55^B \pm 0.20$	$1.38^B \pm 0.06$	$37.8^{BC} \pm 10.4$	$25.8^B \pm 7.2$	$1.47^A \pm 0.18$
W	4.0	1.2	–	–	$30.8^{BD} \pm 9.4$	$26.1^B \pm 8.4$	$1.20^C \pm 0.17$
B	4.0	1.2	–	–	$29.4^D \pm 8.2$	$23.5^B \pm 6.6$	$1.25^{BC} \pm 0.13$
df	–	–	3	3	5	5	5
F	–	–	8.1	17.8	35.8	22.3	17.8
p-value	–	–	<0.001	<0.001	<0.001	<0.001	<0.001

in d_{50} of the boli from two hazelnuts and six hazelnuts and two hazelnuts incorporated into bread were very small (<10% relative difference). The physiological relevance of such a small decrease in bolus hazelnut particle size in terms of potential lipid release and bioavailability is difficult to predict without directly quantifying it, but we expect the effect to be small or maybe even negligible.

Our results indicate that hazelnuts are reduced to a similar size during oral processing, regardless of whether they are consumed plain or incorporated into bread matrixes. This result is coherent with previous findings (Hutchings et al., 2011) reporting no differences in d_{50} of masticated peanuts within either chocolate or gelatine matrixes. However, no comparison between plain nuts and incorporated nuts was carried out in that previous study. On the contrary, a very recent study showed that adding yogurt to almonds, walnuts and pistachios decreased number of chews and increased particle size of nut boli (McArthur et al., 2018).

The finding that hazelnuts are reduced to a similar bolus particle size whether consumed plain or incorporated into bread matrixes differs from our hypothesis. During mastication, the largest bolus particles in the mouth are selected and moved towards the molars using the tongue (Chen, 2009). It could have been expected that the bread matrix would make this selection of large particles more difficult. This would have led to a larger particle size in the bolus masticated in the presence of a bread matrix. Embedded in a semi-solid matrix, soft- and round-shaped particles are perceived to be smaller in the mouth than harder particles of the same size range (Engelen, Fontijn-Tekamp, & Bilt, 2005) which may lower the number of chews. On the other hand, participants masticated the foods until both the matrix and nuts were ready for swallowing, which required a larger number of chews compared to masticating only the nuts. The extra chews would then have led to smaller nut particles in the bolus. Furthermore, swallowing only takes place when the particles in the mouth are both small enough and sufficiently lubricated with saliva (Hutchings & Lillford, 1988). As the bread absorbed part of the saliva, more thorough mastication may have been required before the point of sufficient lubrication was achieved.

We acknowledge that we did not quantify the amount of saliva being incorporated into the different test foods during mastication. If the different breads would absorb different amounts of saliva, then the lubrication threshold would be reached after different chewing times, leading to less or more mastication and thereby to differences in bolus particle size. This effect needs to be determined in future studies (Jourdren et al., 2016). Differences in structure may also have led to easier or harder selection of the bigger hazelnut particles, leading to a smaller or bigger overall particle size, respectively. However, we did not

observe differences in the chewing time or chewing frequency between the two types of bread nor did we find differences in d_{50} of masticated boli between the two breads with incorporated hazelnuts despite the differences in textural properties of the selected bread.

Very limited differences were observed among b values of different test foods, which describes the shape of the particle size distribution. According to Hutchings et al. (2011), the way how particle surfaces interact with food matrix could affect adhesion of particles to the food matrix, and thus influence the breakage function of the particles. The only difference we observed was a smaller b value in the six hazelnuts compared to the other test foods. Larger particles are more likely to be selected over smaller particles in the six hazelnuts test food compared to two hazelnuts and hazelnuts incorporated into bread, resulting in a broadening of the particle size distribution (more spread, lower b value, see below discussion on correlation between d_{50} and b parameters).

A summary of the correlation analysis between mass, volume, mastication parameters and particle size parameters can be found in Table 4. Table 4 shows that there is a highly significant positive correlation between chewing time and number of chews ($r = 0.89$, $p < 0.001$), as expected. Furthermore, a weak but significant negative correlation was found between chewing frequency and d_{50} ($r = -0.28$, $p < 0.05$) and a weak and significant positive correlation between both number of chews and chewing time and b values, but not between chewing frequency and b values. A strong negative correlation was found between d_{50} and b value ($r = -0.66$, $p < 0.001$).

Interestingly, the correlation matrixes between bolus particle size distribution parameters and oral processing parameters showed only a weak correlation between chewing frequency and d_{50}. It has been previously reported that increasing the number of chewing cycles, a more extensive rupture of nuts in small particles occurs (Cassady et al., 2009; Peyron et al., 2004). For instance, we found that the chewing time and number of chews of plain hazelnuts were significantly shorter and smaller than the chewing time and number of chews of the other test foods but produced the smallest average d_{50}. This seems counter-intuitive, as more chewing was expected to decrease the median size of particles. However, when the correlation between chewing cycles and d_{50} was calculated within each test food, significant negative correlations were observed for 2H + W and 6H (-0.60, $p = 0.003$; -0.51, $p = 0.021$ respectively) and a trend for a negative correlation was observed for 2H + B (-0.44, $p = 0.055$, not significant). In general, our correlation data show that variability in chewing performances explains only part of the variability in the particle size distribution, i.e. that individual differences in oral anatomy, physiology and behaviour might contribute to the level of disintegration of nuts more than the number of chewing cycles. It is also clear that the presence of the bread, which represents the bulk of the bolus, interferes with the correlation between number of chews and particle size. The hypothesis that would best explain this is that the selection of hazelnut particles in any bread matrix (regardless of type of bread) was more difficult than the selection of hazelnut particles without the bread matrix. However, this hypothesis does not explain why d_{50} of test foods containing six hazelnuts was significantly larger than d_{50} of the test food containing two hazelnuts. Other food properties that might explain this difference and that were previously linked to mastication are food weight and food volume (Duncan, Bacon, & Weinsier, 1983; Lucas & Luke, 1984) which might explain the higher d_{50} found after mastication of six compared to two hazelnuts. In particular, it has been found that the median bolus peanut particle size swallowed was larger for 5 and 12 g of peanuts compared to 1 g of peanuts. Number of chews before swallowing increased linearly with peanut portion size but the differences between 2 g and 5 g were not significant (Lucas & Luke, 1984). However, the correlation analysis revealed no significant correlations between d_{50} and either food volume or food weight. We speculate that food weight or volume rather than the presence or absence of the bread matrix affected d_{50}.

A significant correlation between d_{50} and b values was found. This correlation is expected based on the selection function and breakage function proposed elsewhere (Epstein, 1947; Lucas, Prinz, Agrawal, & Bruce, 2002; Van der Glas, Van der Bilt, Olthoff, & Bosman, 1987). These respectively describe the likelihood of a particle to be in contact with the teeth and the degree of size reduction when a particle breaks. Since the selection function follows a second power correlation with particle size (Lucas et al., 2002; Van der Glas et al., 1987), larger particles are more likely to be broken down than smaller particles. Combining this with the suggestion that the maximum degree of fragmentation lies around a particle size of 4 mm (Chen, 2009), smaller particles are less likely to be broken down. This might explain why smaller particles would be expected to produce a narrower particle size distribution than larger particles.

Fig. 2 shows box and whiskers plots for the individual d_{50} and b values for all test foods. Fig. 2 gives an impression of the inter-individual differences in hazelnut bolus particle size distribution. Such differences are rather large with the highest d_{50} value being almost 50% higher compared to the lowest d_{50} value within the test food 2H. Addition of bread to hazelnuts or increasing the bite size of hazelnuts did not change the ranking of chewers in terms of d_{50} values (Pearson correlation > 0.75, $p < 0.05$), nor change the distribution of d_{50} values (Levene test, $p = 0.69$). Relatively less inter-individual variability was observed for the b values. Addition of bread to hazelnuts or increasing the bite size of hazelnuts did not change the ranking of chewers in terms of b values (Pearson correlation > 0.8, $p < 0.05$) but changed the distribution of b values (Levene test, $p = 0.03$).

Whereas incorporation of hazelnuts into matrixes has a limited effect on median particle size of masticated hazelnuts boli, we found a relatively large inter-individual variability in the median particle size of masticated boli as previously reported (Hutchings et al., 2011; Jalabert-Malbos et al., 2007) with relative SD ranging between 12 and 14% in the four test foods. We found that the highest d_{50} was 50% higher than the smallest d_{50} value for all the test foods. This difference is rather substantial, i.e. can be translated in substantial difference in the amount of lipids absorbed from nuts. This observation *per se* is not surprising but its implication can be potentially rather vast. Under normal physiological conditions the level of pancreatic lipase is typically not limiting the rate of lipid hydrolysis in the small intestine (Carrière et al., 2005). The fraction of broken cells is not supposed to change due to peristaltic movements. We speculate that individual variability in oral processing behaviour may represent the single most important factor contributing to individual variability in the utilization of lipids from nuts.

Table 4
Pearson correlation coefficients between test food properties, particle size parameters and mastication parameters.

	Mass	Volume[A]	d_{50}	b	Number of chews	Chewing time
Mass	–					
Volume[A]	0.70***	–				
d_{50}	n.s.	n.s.	–			
b	n.s.	n.s.	−0.66***	–		
Number of chews	0.61***	0.52***	n.s.	0.25*	–	
Chewing time	0.48***	0.53***	n.s.	0.24*	0.89***	–
Chewing frequency	0.32***	n.s.	−0.28*	n.s.	0.34***	n.s.

n.s. = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. [A]: Food volume was estimated.

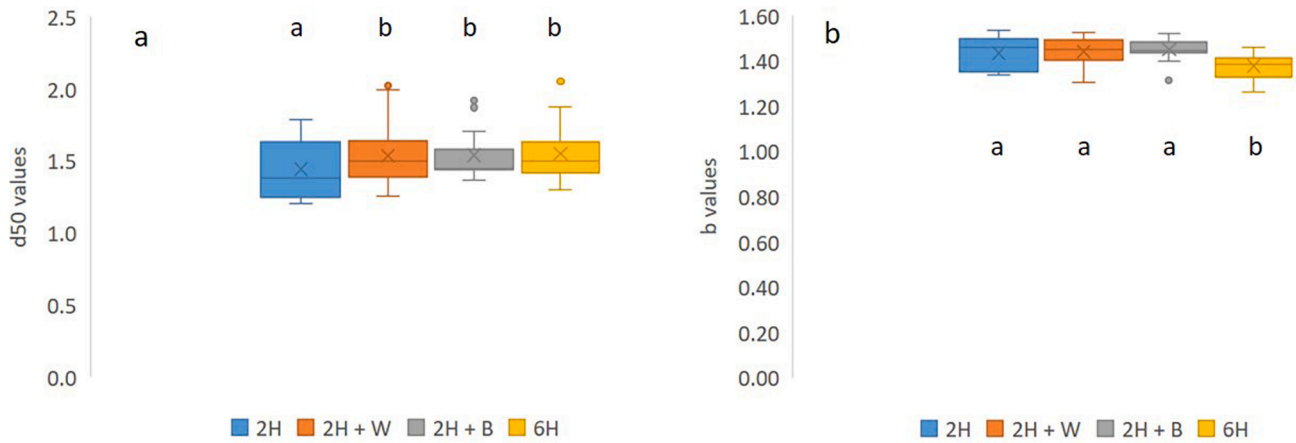

Fig. 2. Box and whiskers plot of the individual d_{50} values (panel a) and b values (panel b) for each of the test foods. Edge of the box represent the upper and lower quartile and the whiskers represent the extreme values; dots represent outliers. 2H = 2 hazelnuts; 2H + W = 2 hazelnuts with white bread; 2H + B = 2 hazelnuts with baguette; 6H = 6 hazelnuts. Different letters indicate differences in d_{50} values (panel a) or b values (panel b) among treatments.

3.2. Experimental setup and methodology

We have chosen to use image analysis to characterize the size distribution of the hazelnut particles in masticated boli as frequently done previously (Chen, Khandelwal, Liu, & Funami, 2013; Hutchings et al., 2011; Mishellany, Woda, Labas, & Peyron, 2006). Compared with sieving, a higher fraction of smaller particles can be detected with image analysis (Van der Bilt et al., 1993) and the particle size distribution can be expressed as a continuous cumulative distribution curve (Fig. 1) from which proper distribution parameters can be obtained using a fitting procedure (Bornhorst, Kostlan, & Singh, 2013; Chen et al., 2013; Hutchings et al., 2011) rather than discrete classes of sizes. Image analysis also has some limitations. Image analysis requires a pre-sieving procedure to select particles bigger than a certain size (d value in Eq. (1)). This size was 0.355 mm in the present study which means that particles with a Feret diameter smaller than 0.355 mm were excluded during the image analysis which may have led to an overestimation of d_{50} (Chen, 2009). The removal of these particles implied a weight loss, which was measured in two random samples to be around 5% of the total particle weight. In order to be detected as individual particles during image analysis, bolus particles need to be separated by at least 0.3 mm from each other (Flynn, 2012). The very large number of particles in a bolus makes it impossible to separate all individual particles from each other. Additional measurement constraints such as circularity or dark background threshold values must be defined to identify the masticated particles and the number of identified particle is sensitive to these constraints. As shown in Fig. 3, there were still some bolus particles touching each other and forming clusters, although they were separated manually as described in Section 2.4. Some of the touching particles were neglected based on the selected constraints, since the circularity of the clusters is usually low, while some of the clusters were detected as one large particles.

It is worth to mention that the removal of bread particles during the washing steps might not have been complete. This was observed upon drying the particles, when the samples that originally contained bread showed a little more browning compared to the other samples (data not shown). However, it is unlikely that this has affected the accuracy of the particle size distribution, because the bread particles were considerably smaller than the hazelnuts particles.

Moreover, it was expected that part of the hazelnut weight was lost due to swallowing (Peyron et al., 2004). We found an average loss of 22% for the boli from mastication of two hazelnuts and < 25% for the mastication of six hazelnuts. Chen (2009) reported that smaller particles are moved to the back of the mouth in order to be swallowed (Chen, 2009). It was therefore assumed that the bulk of the swallowed particles was small. This means that the d_{50} is likely to be an overestimation

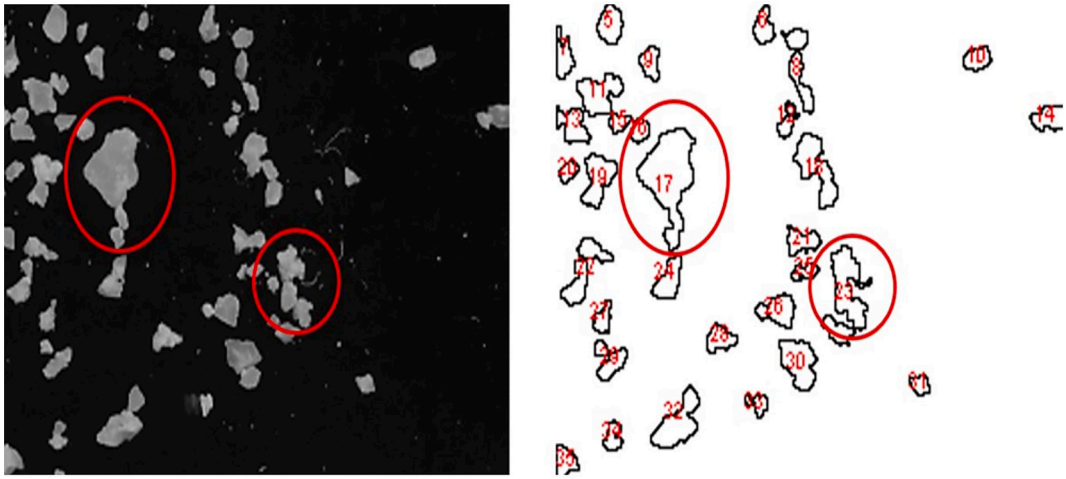

Fig. 3. Example of images taken of masticated bolus and image analysis to identify individual particles. Red circles highlight two cases were neighbouring particles are detected by the software as one single particle. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

compared to what d_{50} would be if the swallowed particles had been included. For the same reason, the values for b were likely overestimated.

Finally, a potential limitation of the present study is the lack of randomization of the treatments which might have led to a bias. Each subject received the samples in the same order. This may give rise to a systematic bias given that the presentation order of samples was fixed and not randomized. It is evident that, given the nature of the present study, randomization would have not helped to conceal the test foods or blind participants towards the test foods, but would have reduced the bias stemming from familiarization and fatigue. It has to be noted though that the first two samples to be given to the volunteers were the bread samples. It should also be noted that chewing foods is an activity done multiple times per day. We believe that familiarization and fatigue are limited in this study, since all participants were familiar with the test foods (hazelnuts, white bread and baguette). Participants chewed per test session<30 g of test foods, so considerably less compared to consuming a meal. While we acknowledge the limitation of non-randomization, we believe that the potential bias is small.

4. Conclusions

This study shows that the incorporation of hazelnuts into bread matrixes has a significant but relatively small effect on the hazelnut bolus particle size distribution. We speculate that the effect on hazelnut bolus particle size is too small to potentially effect lipid release and lipid utilization. Future studies should explore further how individual variation in mastication behaviour may explain variability in nutrient utilization. The effect of co-ingestion of nuts with other food matrixes can be explored in the future as well as the physiological and anatomical factors that explain individual differences in bolus properties such as saliva incorporation and rheological properties of the bolus.

Authors contribution

EC wrote the first draft of the manuscript, participated in the study design and supervision of the project. RvB contributed to the first draft of the manuscript, participated in the study design and performed all the experiments; NP and MS participated in the study design, the supervision of the project and the revision of the draft.

CRediT authorship contribution statement

Edoardo Capuano: Writing - original draft, Conceptualization, Formal analysis, Visualization, Supervision. **Nicoletta Pellegrini:** Conceptualization, Supervision, Writing - review & editing. **Ruben Bommel:** Writing - original draft, Conceptualization, Formal analysis, Visualization, Investigation. **Markus Stieger:** Conceptualization, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare no competing interests.

Acknowledgements

We acknowledge Marianna Tagliasco for the execution of the TPA analysis on bread samples.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2020.109692.

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