

Oral processing behavior and dynamic sensory perception of composite foods: Toppings assist saliva in bolus formation

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1	Oral processing behavior and dynamic sensory perception of
2	composite foods: Toppings assist saliva in bolus formation
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# 16 Abstract

17 Composite foods consist of combinations of single foods, such as bread with toppings. Single 18 foods can differ considerably in their mechanical and sensory properties. This study aimed to 19 investigate the effect of toppings on oral processing behavior and dynamic sensory perception 20 of carrier foods when consumed as composite foods. Two carriers (bread, crackers) and three 21 toppings (firm cheese, cheese spread, mayonnaise) were selected and six carrier-topping 22 combinations were prepared. Mastication behavior, bolus properties (33, 66 and 100% of total 23 mastication time) and dynamic sensory perception were determined for single carriers and all 24 carrier-topping combinations. Both carriers with cheese spread and mayonnaise were chewed 25 shorter and with fewer chews than single bread and crackers, although twice the mass of food 26 was consumed. These toppings contributed to a faster bolus formation by providing moisture, 27 so that less saliva was incorporated into the bolus during mastication. As a result of the moisture 28 incorporation, carrier boli with toppings were softened and perceived less firm and less dry than 29 carrier boli alone. The largest effects of toppings on oral processing behavior and perception 30 were found for liquid-like mayonnaise, and these effects were more pronounced in dry crackers 31 than in moist bread. We conclude that toppings assist saliva in bolus formation of carriers. 32 Carriers drive oral processing behavior and texture perception whereas toppings drive overall 33 flavor perception. This knowledge contributes to food design tailored for specific consumer 34 segments and future personalized nutrition.

Keywords: composite foods, carrier foods, toppings, oral processing, bolus properties,
 sensory perception

#### 37 Highlights

- Oral processing behavior and texture perception were driven by carriers
  Carriers with cheese spread and mayonnaise were chewed shorter than carriers alone
- 40 Cheese spread and mayonnaise facilitated bolus formation of bread and crackers
- Less saliva was incorporated into bolus of carriers after addition of toppings

# 42 **1** Introduction

43 Many foods that are frequently consumed are composed of combinations of multiple single 44 foods such as bread with cheese, crackers with spreads or yogurt with cereals. Throughout this 45 paper, the term composite foods will refer to foods that are composed of two single foods. The 46 single foods can differ considerably in composition, mechanical properties and sensory 47 characteristics (Scholten, 2017; Szczesniak & Kahn, 1984).

48 Oral processing behavior of composite foods is different from that of single foods. From the 49 start of consumption, two single foods are mixed in the mouth, which results in complex oral 50 processing behavior depending on the properties of both food components. Few studies 51 investigated the impact of composite foods on oral processing behavior. One of those studies was performed by Hutchings et al. (2011), who studied oral processing behavior of gelatin and 52 53 chocolate matrices with embedded peanuts. They found that the mastication behavior (chewing 54 duration, number of chews, chewing frequency) was influenced by the type of matrix, and this 55 was not altered by the type of peanuts (dry or moist) embedded in the matrix. The matrix was 56 shown to influence breakdown of the embedded peanuts, as obvious from a variation in peanut 57 particles size distributions. Larsen, Tang, Ferguson, Morgenstern, and James (2015) found that 58 the release of embedded inclusions from a gel matrix ensured differences in breakdown 59 pathways. While these two studies focused on matrices with embedded components, Devezeaux 60 de Lavergne et al. (2016) investigated bi-layer model gels displaying mechanical contrast by 61 combining two gel layers with different mechanical properties. Oral processing behavior of such gels showed oral processing characteristics between that of the two separate layers 62 63 indicating the importance of the properties of both gel layers present. However, hard gel layers 64 were found to influence oral processing behavior slightly more than soft gel layers. Only one 65 research group investigated mastication behavior of commercially available composite foods (Engelen, Fontijn-Tekamp, & Van Der Bilt, 2005; Gavião, Engelen, & Van Der Bilt, 2004). 66 67 They investigated the effect of spreading butter on different carrier foods, such as bread, cake and toast. They found a significant difference in mastication behavior between carrier foods 68 69 alone and carriers with butter. Addition of butter decreased the number of chewing cycles of 70 the drier carriers, such as toast and cake, but not for the more moist bread (Engelen et al., 2005). 71 The researchers hypothesized that butter facilitated the lubrication and bolus formation of 72 carriers and thereby reducing the number of chewing cycles. However, no information is

currently available on the mechanisms underlying bolus formation in such foods and itsinfluence on mastication behavior.

75 Few studies have attempted to investigate the sensory perception of composite foods. Adding 76 sauces to carrier foods (vegetables with gravy, salmon with culinary sauce) decreased the 77 perceived intensity scores of such carrier foods (Meinert, Frøst, Bejerholm, & Aaslyng, 2011; 78 Paulsen, Ueland, Nilsen, Öström, & Hersleth, 2012). In another study, the impact of different 79 carriers (chicken broth, white rice and grilled chicken) on soy sauce perception was investigated 80 (Cherdchu & Chambers, 2014). They found that the carriers did not strongly affect the 81 classification pattern of soy sauces, and differences between soy sauces were still observed. However, solid white rice and grilled chicken tended to modify the sensory properties of the 82 83 soy sauce more than liquid chicken broth. In another study, dynamic texture perception was 84 shown to increase in complexity (i.e. the number of dominant attributes increased) when model 85 gel matrices contained two or more embedded inclusions (Tang, Larsen, Ferguson, & James, 86 2017). In the case of model bi-layer gels, dynamic sensory perception corresponded to an 87 average of the two single layers they were composed of (Devezeaux de Lavergne et al., 2016), 88 while for breads with a crust, the harder crust dominated the dynamic texture perception (Gao, 89 Ong, Henry, & Zhou, 2017).

90 Oral processing behavior is relevant because it imparts structural changes to the food, thereby 91 impacting dynamic sensory perception, oro-sensory exposure time, satiation and food intake 92 (Campbell, Wagoner, & Foegeding, 2016; Chen, 2009, 2015; Devezeaux de Lavergne, van de 93 Velde, & Stieger, 2017; Forde, 2018; Wang & Chen, 2017). Oral processing behavior and 94 dynamic sensory perception have been extensively studied for model foods and various single 95 foods such as bread (Devezeaux de Lavergne et al., 2017; Gao et al., 2017; Jourdren et al., 96 2016; Koc, Vinyard, Essick, & Foegeding, 2013; Le Bleis, Chaunier, Montigaud, & Della 97 Valle, 2016; Panouillé, Saint-Eve, Déléris, Le Bleis, & Souchon, 2014; Witt & Stokes, 2015), 98 but little is known about oral processing behavior and sensory perception of composite foods, 99 although these are often consumed. A multidisciplinary approach integrating food structure, 100 oral processing behavior and dynamic sensory perception for composite foods may help to gain 101 further understanding of how composite foods are perceived and which factors determine their 102 perception. An understanding of the role of each single food may be used to control mastication 103 behavior, bolus formation, sensory perception or liking of foods. Such insights are of relevance 104 for food design tailored to specific consumer segments such as the increasing elderly population 105 or people with decreased eating capabilities (Laguna & Chen, 2016; Mosca & Chen, 2016).

- 106 In this study, carriers (bread and crackers) and toppings (firm cheese, cheese spread and
- 107 mayonnaise) were used as single foods, and they were combined into composite foods (carrier
- 108 with topping). The aim of this study was to investigate the effect of toppings on oral processing
- 109 behavior and dynamic sensory perception of these composite foods. Both carriers and toppings
- 110 are expected to influence the oral processing behavior and dynamic sensory perception,
- 111 however we hypothesize that carriers are the main determinant of oral processing behavior and
- 112 texture perception since they require oral breakdown before being swallowed safely.

#### 113 2 Materials and Methods

114 2.1 Samples

Carrier foods and toppings were combined to form composite foods. Two commercial carriers 115 were used, namely bread crumb (toast bread, Jacquet<sup>®</sup>, France) and crackers (mini-toast naturel, 116 117 Haust<sup>®</sup>, The Netherlands), and three commercial toppings, namely firm cheese (AH Goudse jong belegen, Albert Heijn, The Netherlands), cheese spread (Kiri<sup>®</sup> mit Sahne, BEL Group, 118 119 France) and low-fat mayonnaise (Licht en Romig, Calvé<sup>®</sup>, Unilever, The Netherlands). These 120 foods were selected based on their similar fat content and difference in mechanical properties. 121 The carriers (2) were combined with the toppings (3) to create 6 combinations. The single 122 carriers and toppings (2 carriers and 3 toppings) were included as a reference, which gave a 123 total of 11 samples.

124 Table 1 presents an overview of the composition and product properties of the single foods. 125 The fat content was taken from the nutritional information on the product label. The moisture 126 content of the single foods was determined gravimetrically  $(16 - 18 \text{ h at } 105^{\circ}\text{C})$  in five 127 replicates. This method was also used for the expectorated boli, and is explained in more detail 128 in section 2.5.2. The water activity of the single foods was determined in three replicates using 129 a LabMaster aw (Novasina®). The mechanical properties first peak force (associated to 130 firmness), adhesiveness and cohesiveness were determined in 9 replicates using two cycle 131 puncture tests with a Texture Analyzer (TA.XT Plus). This method was also used for the 132 expectorated boli, and is explained in more detail in section 2.5.3.

133 The carriers were served at constant weight of 2.1 g, which was based on the weight of the 134 single cracker. In addition, the dimensions of bread (35 x 35 x 8 mm) and crackers (35 x 35 x 135 8 mm) were comparable. A feasibility test was performed with 6 consumers to quantify a natural 136 carrier-topping serving ratio. It was observed that consumers showed a natural eating behavior 137 when carriers and toppings were combined in a 1:1 weight ratio. All three toppings were 138 therefore also served at a constant weight of 2.1 g. This gave a total weight of 4.2 g for carriertopping combinations (2.1 g carrier and 2.1 g topping). The toppings covered the carriers 139 140 completely. Carrier-topping combinations were prepared just before serving in order to prevent 141 moisture transfer from the toppings into the carriers.

142

143 <Table 1 about here>

#### 144 2.2 Subjects

145 48 healthy subjects were initially recruited, out of which 18 subjects (7 male and 11 female) 146 were selected to participate ( $25.6 \pm 2.93$  years, mean  $\pm$  SD). Inclusion criteria were good dental 147 health, no missing teeth and/or molars except wisdom teeth, no dental braces, no piercings in 148 the mouth, no swallowing or mastication disorders and non-smoking habits. Only Caucasian 149 adults were included in our study. Selection criteria assessed during the screening session were 150 mechanically stimulated saliva flow rate, mastication time and liking of the carriers, toppings 151 and composite foods. Subjects with low/high saliva flow rates (top and bottom 10%), fast/slow 152 mastication times (top and bottom 10%) and who disliked the samples strongly (scores below 153 3 on 9 point hedonic scale) were excluded. Subjects gave written informed consent and received 154 financial compensation for participation. The study protocol was submitted to the Medical 155 Ethical Committee of Wageningen University (METC-WU) and exempted from ethical 156 approval. All 18 subjects completed the study.

# 157 2.3 Experimental approach

158 Each subject attended 11 sessions of 45 minutes over a time period of three months. In the first 159 two sessions, mastication behavior was characterized using video recordings. Subjects were 160 instructed to chew each food as they would do naturally. These video recordings were used to 161 determine the total mastication time for each sample by averaging the mastication times of all 162 subjects and all replicates. Averaged total mastication times of bread without topping, with firm 163 cheese, with cheese spread and with mayonnaise corresponded to  $13.8\pm0.6$   $14.2\pm0.6$   $13.5\pm0.6$ 164 and 11.7±0.5 s (mean±SE), respectively. Averaged total mastication times of cracker without 165 topping, with firm cheese, with cheese spread and with mayonnaise corresponded to 20.9±0.6 166 21.1±0.6 19.6±0.5 and 16.5±0.5 s (mean±SE), respectively. The third session was a training 167 session, during which the sensory attributes and the procedures of the following sessions were 168 introduced. Reference foods were used to acquaint the subjects with the different sensory 169 attributes. The remaining eight sessions were used for bolus collection and sensory assessment. 170 During these sessions, subjects were asked to expectorate the bolus for each sample after time 171 points corresponding to 33, 66 and 100% of the averaged total mastication time using a 172 stopwatch. In addition, subjects were asked to evaluate intensity of sensory attributes after one 173 chew and at 33, 66 and 100% of total mastication time using progressive profiling method. Each 174 method is explained in further detail in section 2.4 - 2.6.

For all sessions, samples were presented with three digit codes. Carriers and carrier-topping combinations were served on a plate, whereas the single toppings were served on a spoon. Subjects cleaned their palate after consumption of each sample with water and cucumber.
Cucumber was used as palate cleanser instead of commonly used crackers, since using one of
the samples as palate cleanser was not preferred. Cucumber was chosen because it is a relatively
bland tasting solid food with high moisture content by which it could aid in the removal of
cracker/bread/topping residues.

#### 182 2.4 Characterization of mastication behavior using video recordings

183 Oral processing behavior of all samples by n=18 subjects was characterized in triplicate using 184 video recordings. Four stickers were placed on the subjects face, which were later used as 185 reference points during the video analyses. Two stickers with a defined distance of 5 cm were 186 placed on the forehead, one sticker was placed on the nose and one sticker was placed on the 187 chin. Subjects were seated in a chair with a camera in front of them. They were asked to chew 188 each food as they would do naturally, while being video recorded. The subjects were instructed 189 to maintain their head straight to the camera, and not to block their mouth or face with their 190 hand while eating. Furthermore, they were instructed to indicate the moment of swallowing by 191 raising their hand.

The videos were analyzed using Kinovea software (version 0.8.15), which is a free software that can be used to analyze mastication behavior. Each video was calibrated by setting the distance between the two stickers on the forehead as 5 cm. The path of the nose and chin sticker was tracked by the software in order to determine the chewing movements. All videos were analyzed separately by two of the authors, after which they compared their findings until agreement on the start of consumption and the moment of swallowing was reached for each video.

199 The parameters collected from the videos included total mastication time (s), number of chews, 200 chewing frequency (chews/s), maximum vertical jaw movement (cm) and maximum lateral jaw 201 movement (cm). Total mastication time was defined as the time period between the moment 202 when the lips were closed after placing the food in the mouth and the moment of swallowing. 203 Subjects generally take multiple swallows during mastication, but only the moment of the main 204 swallow was used for analysis. Chewing frequency was calculated by dividing the number of 205 chews by the mastication time. Maximum vertical and lateral jaw movements were defined as 206 the maximum distance between the nose and chin in vertical and lateral directions, respectively. 207 In addition, parameters describing oral processing behavior were also analyzed for tertiles of 208 mastication time (i.e. 0 - 33%, 33 - 66% and 66 - 100% of mastication time) in order to analyze 209 changes throughout mastication.

# 210 2.5 Characterization of bolus properties throughout mastication

211 Different bolus properties were analyzed at 33, 66 and 100% of total mastication time 212 corresponding to early chew down, late chew down and the moment of swallowing, 213 respectively. Boli were collected from n=18 subjects in quadruplicate; one replicate was 214 photographed for illustration purposes, one replicate was used to determine the moisture and 215 saliva content of the boli and two replicates were used to determine the mechanical properties 216 of the boli. Within each replicate, the sample serving order was randomized with respect to 217 sample type and expectoration time point. One replicate was presented in two different sessions, 218 so that half of the samples was analyzed in the first session and the other half in the second 219 session. All boli were analyzed for the different properties immediately after expectoration.

# 220 2.5.1 Images of expectorated boli

Pictures of expectorated boli were taken for all time points and all samples. The boli were photographed on a grey background using a digital camera (Canon IXUS 180). The camera was placed in a tripod at a distance of 40 cm above the bolus. One image was obtained for each bolus. These pictures were taken in order to illustrate differences between samples at different time points. They were not used for further image analysis.

### 226 2.5.2 Moisture and saliva content of expectorated boli

227 Boli were collected in petri dishes covered with lids and analyzed within an hour after expectoration in order to prevent moisture evaporation from the samples. 2 - 3 g of the 228 229 expectorated boli were placed on aluminum dishes, weighed and dried for 16 - 18 h at 105°C 230 in an atmospheric oven (Venti-line, VWR®). After drying, samples were cooled in a desiccator 231 for 30 min and subsequently weighed. Bolus moisture content (MC) on a wet weight basis was 232 calculated using  $MC = (m_0 - m_1)/m_0 \cdot 100\%$ , where m<sub>0</sub> is the weight of the sample before 233 drying and m<sub>1</sub> is the weight after drying. Bolus moisture content on a dry weight basis was 234 calculated using  $MCdb = (m_0 - m_1)/m_1$ , which was subsequently used to calculate the 235 saliva content (SC) per gram dry food by subtracting the moisture content on a dry weight basis 236 of the product from the moisture content on a dry weight basis of the bolus (MCdb). The rate 237 of saliva incorporation (g/min) was calculated by dividing the saliva content (SC) by 238 mastication time. These calculations were based on the assumption that the bolus was fully 239 expectorated. Few samples (8 out of 432 samples) resulted in slightly negative values for the saliva content, most likely due to small measurement errors. These samples were excluded fromthe statistical analyses.

# 242 2.5.3 Mechanical properties of expectorated boli

243 The mechanical properties of the expectorated boli were analyzed in duplicate using two cycle 244 puncture tests with a Texture Analyzer (TA.XT Plus) fitted with a 500 g load cell. A cylindrical 245 probe with a flat surface and a diameter of 4 mm was used. Punctures were performed up to a 246 strain of 50% of the initial bolus height with a constant speed of 5 mm/s. The probe was then 247 retrieved at the same speed of 5 mm/s and a resting time of 5 s was applied before a second 248 puncture was performed. Three measurements, each at a different location of one expectorated 249 bolus, were performed to obtain an averaged value. The mechanical properties of expectorated 250 mayonnaise samples were not analyzed since these samples could not been detected by the 251 Texture Analyzer due to their liquid behavior.

First peak force (associated to firmness), adhesiveness and cohesiveness were determined from the force-time curves as described before by Devezeaux de Lavergne, van de Velde, van Boekel, and Stieger (2015). First peak force was defined as the maximum peak force during the first puncture cycle. Adhesiveness was defined as the area under the negative force-time curve during the first cycle. Cohesiveness was defined as the ratio between the area under the (positive) force-time curve obtained during the second and first puncture cycle.

### 258 2.6 Characterization of dynamic sensory perception using progressive profiling

259 All samples were evaluated in duplicate for four sensory attributes (firmness, stickiness, 260 dryness and flavor intensity) at four different moments of mastication (first chew, and 33, 66 261 and 100% of total mastication time) by n=18 subjects using progressive profiling. The attributes 262 firmness (i.e. force required to push the tongue through the product to the upper palate), 263 stickiness (i.e. degree to which the product adheres to any mouth surface while chewing) and 264 dryness (i.e. dry and rough feeling on the tongue and oral cavity) were selected because they 265 best describe the differences between the carriers and toppings and the expected changes thereof 266 during oral processing. The attribute overall flavor intensity (i.e. the total amount of flavor) was 267 included because the samples assessed differed strongly in flavor quality and to avoid dumping 268 effects.

Subjects were asked to evaluate the sensory attributes immediately after expectoration. An unstructured 100 mm line scale anchored from "not at all" to "extremely" was used (Eye Question software, version 4.5.6). Sensory attributes were assessed according to a balanced design, so that 9 subjects started the sessions with the assessment of the attributes firmness and
stickiness and the other 9 subjects started with the attributes dryness and flavor intensity.

# 274 2.7 Statistical data analyses

Results were reported as mean values with standard error (n=18 subjects). Outliers (Z-275 276 score>3.29) were removed from the data (typically less than 1.6% of all values). Data were 277 checked for normality, and a log transformation was applied for the parameters peak force and 278 adhesiveness in order to obtain normally distributed data. Linear mixed models were performed 279 for all mastication, bolus and sensory parameters for single carriers and all carrier-topping 280 combinations (lmerTest package)(RStudio, version 1.0.143). Significance level of p<0.05 was 281 chosen. For the mastication parameters, carrier and topping were set as fixed effects, and 282 subject and replicate were set as random effects. For the bolus and sensory parameters, carrier, 283 topping and time were set as fixed effects, and subject and replicate were set as random effects. 284 Then, a model including only the bolus and sensory data at 100% of mastication was carried 285 out to study the differences in bolus and sensory properties between carriers with and without 286 toppings at the moment of swallowing. For this analysis, *carrier* and *topping* were set as fixed 287 effect, and subject and replicate were set as random effects. Interaction effects were included 288 in all models, and random effects that were not significant were excluded from all models. The 289 relationships between oral processing parameters throughout mastication of the different 290 carriers with and without toppings were summarized using Principal Component Analysis 291 (PCA) on subject averaged data (The Unscrambler X software, version 10.4.1).

#### **292 3 Results**

293 *3.1 Characterization of mastication behavior* 

294 For both carriers, mastication time and number of chews are summarized in Figure 1. 295 Significant carrier effects (F=638.4, p<0.001; F=584.9, p<0.001), topping effects (F=44.0, 296 p<0.001; F=26.9, p<0.001) and carrier-topping interactions (F=4.0, p=0.008; F=3.6, p=0.013) 297 were found for both mastication time and number of chews (Table 2), indicating that the effects 298 of toppings on carrier foods were not the same for bread and crackers. On average, crackers 299 were processed longer in the mouth and with a higher number of chews compared to bread. For 300 example, single crackers were masticated for 20.9±0.6 s with 27.7±0.8 chews, whereas single 301 bread was masticated for 13.8±0.5 s with 17.8±0.8 chews. Addition of mayonnaise to bread 302 significantly decreased total mastication time from  $13.8\pm0.5$  to  $11.7\pm0.5$  s (p<0.05), whereas 303 no significant effects were found for addition of firm cheese and cheese spread. In the case of 304 crackers, both mayonnaise and cheese spread had a significant effect on mastication time 305 (p<0.05). The mastication time of crackers decreased from  $20.9\pm0.6$  to  $19.6\pm0.5$  s for cheese 306 spread and to 16.5±0.5 s for mayonnaise. Addition of firm cheese to crackers did not 307 significantly affect the total mastication time  $(21.1\pm0.6 \text{ s})$ . Similar results were found for the 308 number of chews required until swallowing. Addition of mayonnaise significantly decreased 309 the number of chews required to swallow both bread and crackers, and cheese spread 310 significantly decreased the number of chews for crackers only. Firm cheese did not lead to 311 changes in number of chews for neither carriers. On average, similar effects of toppings on oral 312 processing behavior of carriers were found for bread and crackers, but the effects were larger 313 in crackers than in bread.

314

316

Chewing frequencies of the carriers with and without toppings varied between  $1.25\pm0.02$  and  $1.36\pm0.03$  chews/s (data not shown). Even though these differences in chewing frequencies were small, significant carrier effects (F=10.9, p=0.001) and topping effects (F=2.9, p=0.034) were found.

Jaw movements were recorded during mastication, and maximum vertical and lateral jaw movements were determined (data not shown). A significant carrier effect was found for both maximum vertical jaw movements (F=16.4, p<0.001) and maximum lateral jaw movements

<sup>315 &</sup>lt;Figure 1 about here>

324 (F=12.7, p < 0.001). The results show that jaw movements in both directions were larger for 325 crackers than for bread. For example, the maximum vertical and lateral jaw movements of 326 single bread were  $19.7\pm0.6$  and  $19.1\pm0.9$  mm, whereas this was  $22.4\pm0.8$  and  $20.3\pm0.7$  mm 327 for single crackers. In case toppings were added, larger maximum vertical jaw movements were 328 observed (F=7.8, p<0.001), but this effect was only significant after addition of firm cheese and 329 cheese spread and not for mayonnaise. A significant topping effect was found for maximum 330 lateral jaw movements (F=3.8, p=0.011), but none of the toppings affected the lateral jaw 331 movements of single carriers significantly.

332 Besides characterization of total mastication behavior, the mastication parameters were also 333 analyzed for tertiles of mastication time, i.e. early chew down (0 - 33%) of total mastication 334 time), middle chew down (33 - 66%) of total mastication time), and late chew down (66 - 100%)335 of total mastication time). Chewing frequency decreased towards the end of mastication. 336 Averaged chewing frequency for all samples was 1.10±0.01 chews/s during late chew down, 337 which was lower than during the earlier stages of mastication (1.28±0.01 chews/s for early chew 338 down, 1.32±0.01 for middle chew down). In addition, maximum vertical jaw movements were 339 largest during early chew down (19.4±0.2 mm), and decreased during middle and late chew 340 down (16.4±0.2 and 16.4±0.2 mm). No differences in maximum lateral jaw movements 341 throughout mastication were observed.

342

- 343 <Table 2 about here>
- 344 <Table 3 about here>
- 345
- 346 3.2 Characterization of bolus properties

## 347 *3.2.1 Moisture content of boli and saliva incorporation*

Figure 2 displays the moisture content as a function of mastication time for bread (Figure 2A) and crackers (Figure 2B). Bolus moisture content increased with mastication time, and more moisture was taken up at the beginning of mastication. More moisture uptake was observed for crackers compared to bread (carrier:time interaction, F=17.1, p<0.001). The moisture content of single crackers increased from  $2.7\pm0.1$  to  $50.4\pm1.9\%$  at the moment of swallowing, whereas for single bread it increased from  $34.4\pm0.5$  to  $55.0\pm1.5\%$ . When toppings were added to the carriers, the initial moisture content was larger than for the carriers alone, and moisture content increased to a lesser extent during mastication (carrier:topping interaction, F=2.3, p=0.033). Moisture contents at the moment of swallowing varied between  $48.1\pm1.2$  and  $57.9\pm1.1$  wt%, and a significant carrier effect (F=55.2, p<0.001) and topping effect (F=22.1, p<0.001) were observed (Table 3). On average, bread samples were swallowed at slightly higher moisture content than crackers. Carriers with firm cheese exhibited the lowest moisture content at the moment of swallowing, whereas this was the highest for carriers with mayonnaise.

361

362 <Figure 2 about here>

363

364 Saliva content increased with increasing mastication time, and more saliva was incorporated at 365 the beginning of mastication. Figure 3 shows the averaged saliva content at the moment of 366 swallowing in bread (Figure 3A) and crackers (Figure 3B). A higher saliva content was found 367 for crackers compared to bread (F=96.4, p<0.001), e.g. 0.97±0.06 g/g dry weight for single 368 crackers and 0.68±0.06 g/g dry weight for single bread. In addition, a significant topping effect 369 was found (F=29.0, p<0.001). All three toppings significantly decreased the amount of saliva 370 incorporated in both bread and crackers. In bread, firm cheese, cheese spread and mayonnaise 371 decreased the saliva content from  $0.68\pm0.06$  to  $0.50\pm0.04$ ,  $0.42\pm0.07$  and  $0.46\pm0.07$  g/g dry 372 weight, respectively. In the case of crackers, the toppings decreased the saliva content from  $0.97\pm0.06$  to  $0.66\pm0.05$  g/g dry weight for firm cheese, to  $0.66\pm0.07$  g/g dry weight for cheese 373 374 spread and to  $0.74\pm0.08$  g/g dry weight for mayonnaise.

375 Significantly higher rates of saliva incorporation were found for crackers than bread samples 376 (F=45.7, p<0.001)(Table 3). As an example, the rate of saliva incorporation was  $6.1\pm0.6$  g/min 377 for single crackers and  $4.5\pm0.5$  g/min for single bread. Furthermore, a significant topping effect 378 was found (F=5.0, p=0.003), and only mayonnaise increased the rate of saliva incorporation of 379 carriers significantly.

380

381 <Figure 3 about here>

### 383 *3.2.2 Mechanical properties of boli*

384 Figure 4 shows the average first peak force (associated to firmness) of the expectorated boli as 385 a function of mastication time. The peak force of all samples decreased over mastication time, 386 in particular during the first 33% of total mastication time, to reach a plateau at around 66% of 387 total mastication time (time effect, F=123.6, p<0.001). A significant carrier:topping effect was 388 found (F=4.6, p=0.004). Both the initial first peak force and its decrease during mastication was 389 higher for crackers than for bread. For example, the peak force of single bread decreased from 390  $0.92\pm0.08$  to  $0.49\pm0.05$  N, while for the crackers a much larger decrease from  $16.92\pm2.89$  to 391 0.67±0.06 N was found. Addition of toppings to carriers decreased the first peak force of both carriers, especially at the early stage of mastication. After 33% of total mastication time, the 392 393 peak force of single bread was 1.04±0.08 N. This decreased to 0.84±0.04, 0.85±0.05, and 394  $0.35\pm0.02$  N after addition of firm cheese, cheese spread and mayonnaise, respectively. In the 395 case of crackers, the toppings decreased peak force at 33% of total mastication from 2.40±0.45 to 2.17±0.35 N for firm cheese, to 1.07±0.09 N for cheese spread, and to 0.93±0.16 N for 396 397 mayonnaise. On average, the addition of mayonnaise (grey square) showed the largest decrease 398 in peak force for both bread and crackers and resulted in the lowest peak forces during all stages 399 of mastication.

400

401 <Figure 4 about here>

402

403 Adhesiveness of boli from bread and cracker increased with increasing mastication time (data 404 not shown). Significant carrier:topping, carrier:time and topping:time interactions were 405 observed (Table 2). At the moment of swallowing, bread and crackers did not differ in 406 adhesiveness, but a significant topping effect (F=27.7, p<0.001) and carrier:topping interaction 407 (F=3.8, p=0.01) were observed (Table 3). In the case of topping addition, the adhesiveness of 408 bread and crackers either increased or decreased. Addition of toppings showed an increase for 409 firm cheese and cheese spread and a decrease for mayonnaise.

410 Small, but statistically significant differences between samples and time points were found for 411 cohesiveness (Table 2). On average, bread boli were slightly more cohesive than cracker boli 412 at all three time points (data not shown). Addition of firm cheese, cheese spread and mayonnaise 413 increased the cohesiveness of single carriers in ascending order. At the moment of swallowing, 414 a significant carrier effect (F=134.8, p<0.001) and topping effect (F=12.5, p<0.001) were

415 observed (Table 3).

# 416 3.3 Characterization of sensory properties

417 Figure 5A and Figure 5B show the sensory scores for dryness perception as a function of the 418 mastication time for bread and crackers. Dryness scores decreased with increasing mastication 419 time for all samples. After one chew, single crackers (90.4±2.1 mm, Figure 5B) were perceived 420 drier than single bread (65.4±3.4 mm, Figure 5A). A larger absolute decrease in dryness 421 perception was observed for crackers than bread (carrier:time interaction, F=15.0, p<0.001). 422 However, a difference in dryness between the carriers was maintained through mastication, and 423 crackers (62.3±4.2 mm) were still perceived drier than bread (45.3±3.2 mm) at the end of 424 mastication. Addition of toppings decreased dryness perception at all four mastication time 425 points for both carriers (F=115.3, p<0.001)(Figure 5A, 5B). Although all three toppings 426 decreased dryness perception, the type of topping added to the carriers determined the degree 427 of the decrease. At the end of mastication, a significant carrier effect (F=52.1, p<0.001) and 428 topping effect (F=25.3, p<0.001) were observed. Dryness scores of bread decreased 429 significantly from 45.3±3.2 to 36.6±3.0 mm after addition of firm cheese, to 28.4±2.8 mm after 430 addition of cheese spread, and to 29.0±3.3 mm after addition of mayonnaise (p<0.05). Similar 431 results were found for the dryness perception of crackers, and dryness scores of crackers 432 significantly decreased from 62.3±4.2 to 48.5±3.8, 44.6±3.5 and 36.7±3.3 mm after addition of 433 firm cheese, cheese spread, and mayonnaise, respectively (p<0.05). Overall, the absolute 434 decrease in dryness was largest after the addition of mayonnaise, followed by cheese spread 435 and then firm cheese for both carriers at all mastication time points.

436 Figure 5C and Figure 5D display the sensory scores for firmness perception as a function of the 437 mastication time for bread and crackers. Firmness scores decreased with increasing mastication 438 time for all samples. After one chew, single crackers (86.6±2.6 mm, Figure 5D) were perceived 439 firmer than single bread (58.3±3.5 mm, Figure 5C). The absolute decrease in firmness through 440 mastication was greater for crackers than for bread, resulting in similar firmness intensities at 441 the moment of swallowing (F=1.9, p=0.166). Addition of toppings did not significantly change 442 the firmness scores after one chew. Addition of toppings to carriers decreased firmness scores 443 throughout mastication (Figure 5C, 5D), but the effect depended on the type of topping. The 444 absolute decrease in firmness perception was greatest after the addition of mayonnaise, followed by cheese spread, and then firm cheese. At the moment of swallowing, a significant 445

446 topping effect was found (F=10.2, p<0.001), and addition of mayonnaise significantly</li>
447 decreased the firmness intensity perception of both carriers (Table 3).

448 Stickiness increased with increasing mastication time, especially at the early stage of 449 mastication (data not shown). At the moment of swallowing, no significant difference in 450 stickiness was observed between bread and crackers (F=0.4, p=0.553), but a significant topping 451 effect was found (F=4.6, p=0.004). Addition of mayonnaise to the carriers significantly 452 decreased stickiness at the moment of swallowing (p<0.05)(Table 3); stickiness of single bread 453 decreased from  $58.8\pm2.7$  to  $48.7\pm3.8$  mm after addition of mayonnaise, whereas the stickiness 454 of cracker decreased from  $59.9\pm3.5$  to  $54.1\pm3.7$  mm.

455 Flavor intensity slightly increased with increasing mastication times (Figure 5E, 5F). Overall, 456 flavor intensity was driven by the presence of toppings rather than carriers. Addition of toppings 457 to carriers increased flavor intensity scores at all four mastication time points. The degree of 458 the increase was determined by the type of topping that was added to the carriers. The absolute 459 increase in flavor intensity of both carriers was largest after the addition of mayonnaise (flavor 460 intensity scores increased by a factor of 3), followed by cheese spread and firm cheese (flavor 461 intensity scores increased by a factor of 2). Furthermore, the absolute increase in flavor intensity 462 was larger when toppings were added to bread than crackers. At the moment of swallowing 463 (Table 3), the flavor intensity of bread without topping, with firm cheese, cheese spread and 464 mayonnaises were 26.7±2.9, 56.8±3.3, 55.6±2.8 and 68.9±2.8 mm, whereas this was 26.0±3.1, 465  $50.4\pm2.7$ ,  $44.2\pm3.4$  and  $60.9\pm2.7$  mm for crackers, respectively.

466

467 <Figure 5 about here>

## 468 **4 Discussion**

We investigated the contribution of toppings to carriers on the oral processing behavior and dynamic sensory perception of composite foods. Carriers (bread and crackers) and toppings (firm cheese, cheese spread and mayonnaise) were used as single foods, and they were combined into composite foods (carrier with topping) with contrasting composition, mechanical properties and sensory characteristics.

474 As was seen in Figure 1, spreading the toppings cheese spread and mayonnaise on carrier foods 475 reduced total mastication time and number of chews until swallowing, and no effect was found 476 for firm cheese. It is interesting to note that the weight of carriers alone was 2.1 g, while the 477 weight of carrier-topping combinations doubled to 4.2 g (i.e. 2.1 g carrier and 2.1 g topping). 478 Thus, even though addition of toppings to carriers doubled the sample weight, the carriers 479 shortened the total mastication time in the case of cheese spread and mayonnaise while it 480 remained similar for firm cheese. This implies that toppings influenced the mastication behavior 481 of carriers. These data are consistent with those of Gavião et al. (2004) and Engelen et al. (2005) 482 who showed that spreading butter on toast decreased the time and number of chewing cycles 483 until swallowing. In those works, it was hypothesized that toppings facilitated saliva in the 484 bolus formation of carriers leading to boli that are broken down and lubricated enough to be 485 safely swallowed after shorter mastication times and less chews. This is indeed confirmed by 486 the results of our current study. Addition of toppings to carriers led to less saliva incorporation 487 (Figure 3) and a faster decrease in first peak force (Figure 4) of carrier boli. Addition of toppings 488 to carriers led to decreased dryness and firmness perception (Figure 5). Due to their dry 489 character, both bread and crackers require an increase in lubrication behavior and reduction of 490 structure before they can be swallowed safely. These results show that toppings moistened and 491 softened the bolus, and consequently less time had to be spent on reducing structure and 492 increasing lubrication to safely swallow earlier. This confirms the hypothesis that toppings 493 assist saliva in bolus formation.

494 Comparing the two different carriers (bread versus cracker), crackers have a lower moisture 495 content and are harder than bread (Table 1). It is known that dry and/or hard foods are processed 496 in the mouth for longer time because they require more saliva and/or need to be softened more 497 (Chen, Khandelwal, Liu, & Funami, 2013; J. B. Hutchings & Lillford, 1988). This is confirmed 498 by the present study, which found longer mastication times and higher number of chews until 499 swallowing for crackers than for bread (Figure 1). Hence, dry and/or hard foods were expected 490 to benefit more from the facilitating effects of toppings than moist and/or soft foods. We indeed 501 found that for crackers, the addition of toppings resulted in a larger absolute decrease in total 502 mastication time and number of chews until swallowing (Figure 1), and this effect was less 503 pronounced in bread. These findings are in line with previous studies. Engelen et al. (2005) 504 reported that adding butter to toast and cake reduced the number of chewing cycles until 505 swallowing, whereas no effect was found for bread (higher initial moisture content, softer). In 506 addition, adding fluids to toast (low moisture content, hard), cake (low moisture content), and 507 peanuts (low moisture content, hard) decreased the number of chews until swallowing, whereas 508 this effect was not found for cheese (soft) and carrots (high moisture content, hard)(Pereira, de 509 Wijk, Gavião, & van der Bilt, 2006; Pereira, Gavião, Engelen, & Van Der Bilt, 2007; Van Der 510 Bilt, Engelen, Abbink, & Pereira, 2007). These studies clearly indicate that mainly dry foods 511 benefit from the facilitating effects of fluids or toppings leading to shortened mastication times, 512 but no data was found on the bolus properties underlying this observation. Our present study 513 highlights that indeed the bolus formation of crackers rather than bread was affected by the 514 presence of toppings. Absolute decrease in saliva incorporation and first peak force after 515 addition of toppings was larger for crackers compared to bread (Figure 3 and 4). Thus, dry and 516 hard crackers with a low moisture content absorbed more moisture from the toppings than soft 517 bread with a higher moisture content.

518 Different toppings (firm cheese, cheese spread and mayonnaise) impacted oral processing 519 behavior and sensory perception of carriers differently. On average, mayonnaise had the largest 520 impact on oral processing behavior of carriers, followed by cheese spread and only a small 521 influence by firm cheese was observed. Similar findings were found for dryness and firmness 522 perception. It appears that the higher the contrast in terms of mechanical properties and/or 523 moisture content between toppings and carriers, the larger the influence of toppings on oral 524 processing behavior and texture perception of carrier foods. These different effects of the three 525 toppings are likely to be caused by variations in their initial composition and product properties. 526 It seems that incorporation of toppings into bread and cracker boli depends on its consistency. 527 Mayonnaise has the highest moisture content and can be characterized as a plastic liquid-like 528 topping (Table 1). Due to its high moisture content and liquid behavior it was easily mixed with 529 the carriers to form a cohesive bolus. Mayonnaise thereby softened the carrier boli most 530 effectively (Figure 4) and consequently decreased dryness and firmness scores largely (Figure 531 5). Cheese spread also decreased mastication times and number of chews of carriers, but to a 532 lesser extent than mayonnaise. This could be attributed to less moisture incorporation into the 533 carrier boli due to its semi-solid texture and slightly lower moisture content. Another possible 534 explanation might be found in differences in adhesiveness between cheese spread and 535 mayonnaise. Increased adhesiveness of foods requires longer cycle duration and increased 536 muscle activities to remove it from oral surfaces and thereby increasing total mastication time. 537 This has been shown for caramels (Cakir et al., 2012). Cheese spread is more adhesive than 538 mayonnaise and was perceived more sticky, which might have contributed to longer mastication 539 time and a higher number of chews. Firm cheese had the lowest impact on both oral processing 540 behavior and sensory perception of carriers, which can be explained by its solid-like consistency 541 and limited ability to incorporate moisture in the boli. The facilitating effects of toppings on 542 bolus formation of bread and crackers depends strongly on the consistency of toppings. Fat 543 content is not likely to have caused the differences between toppings found in the present study, 544 since all three toppings contained similar fat content (Table 1).

545 Figure 6 shows a Principal Component Analysis (PCA) bi-plot of carriers with and without 546 toppings over oral processing parameters throughout mastication to summarize the oral 547 processing pathways of such foods. Mastication behavior parameters were taken at three stages 548 during oral processing (i.e. 0-33, 33-66 and 66-100% of total mastication time) and bolus 549 properties were taken at three time points throughout oral processing (33, 66 and 100% of total 550 mastication time). As can be seen, bread samples are located on the left side of the first the 551 principal component (PC1) and the cracker samples are located on the right side of PC1 (X axis, 552 55%). Oral processing of bread starts near bolus adhesiveness and cohesiveness, whereas oral 553 processing of crackers starts near vertical jaw movements, saliva incorporation rate and bolus 554 first peak force. Interestingly, all foods move towards the left and towards the bottom part of 555 the plot with increasing mastication time, moving towards the parameters moisture content and 556 saliva content. Carriers with toppings are positioned relatively close to the corresponding single 557 carrier, showing that the oral processing pathways of composite foods are relatively close to 558 that of the single carrier. This indicates that the oral processing pathways and oral processing 559 behavior are driven by carriers rather than toppings, which might be due to the dominating 560 texture of the carriers. This is consistent with previous studies that investigated model foods, in 561 which a hard layer was found to dominate the oral processing behavior of bi-layer model gels 562 containing hard and soft layers (Devezeaux de Lavergne et al., 2016). In addition, the hard crust 563 was found to dominate the dynamic texture perception of bread crumb with crust sample 564 throughout consumption (Gao et al., 2017). Although the pathways of carriers with and without 565 toppings were similar, the presence of a topping induced some changes to the oral processing 566 pathways of single carriers (Figure 6). Carriers with firm cheese and cheese spread are 567 positioned closest to its single carrier, whereas carriers with mayonnaise are positioned more 568 towards the left top part of the plot. This illustrates that the addition of mayonnaise had the 569 highest impact on the oral processing pathways of carriers.

570

571 <Figure 6 about here>

572

When carrier foods were combined with toppings, the oral processing pathways and texture perception were influenced by both carriers and toppings, but in different ways. Carriers drove the oral processing behavior and texture perception because these need to be broken down into smaller particles and need to be lubricated before swallowing. Toppings moistened the carrier foods by which they adapted their oral processing pathways and texture perception.

578 Flavor perception is another important aspect of the dynamic sensory perception of composite 579 foods. Flavor intensity increased with increasing mastication time. The addition of toppings to 580 carriers increased flavor intensity scores at all four time points, indicating that flavor perception 581 throughout consumption was driven by the toppings rather than the carriers. This increase in 582 flavor intensity by adding toppings might be one of the factors that explains why bread and 583 crackers are frequently consumed together with toppings. Besides, flavor intensities were lower 584 for cracker-topping combinations than for bread-topping combinations (Figure 5, Table 3). This 585 might be explained by the dry character of crackers, dry crackers might take up the topping to 586 a larger extent than moist bread, leading to a lower flavor intensity perception. Another 587 explanation might be the hard character of the crackers, hard cracker require more oral 588 breakdown than soft bread, which might distract consumers attention from flavor perception. 589 This result is in line with previous studies on single foods, showing that an increase in hardness 590 of gels and candies decreased the perceived flavor intensities (Boland, Delahunty, & van Ruth, 591 2006; Saint-Eve et al., 2011).

The present study has important implications for food design, and these insights allow the tailoring of oral processing behavior and subsequent consumer perception of composite foods. For example, adding toppings facilitated mastication and bolus formation of carrier foods, which could be applied in foods for elderly or people with decreased eating capabilities. Although this study focuses on the oral processing behavior and sensory perception of composite foods, the findings may well have implications for food intake and satiation. 598 Addition of toppings to carrier foods decreased oro-sensory exposure time while it increased 599 the total energy content of the food consumed. This might cause faster consumption of higher

600 energy dense foods and subsequently this may result in increased consumers food intake.

### 601 **5 Conclusion**

602 This study shows that toppings (firm cheese, cheese spread and mayonnaise) impacted the 603 mastication behavior, bolus formation and dynamic sensory perception of carrier foods (bread, 604 crackers). Carriers to which semi-solid cheese spread and liquid-like mayonnaise were added 605 were chewed for a shorter time and with fewer chews than single carriers bread and crackers, 606 although twice the mass of food was orally processed. No effect was found for solid firm cheese. 607 Less saliva incorporation and a faster decrease in bolus peak forces were observed after addition 608 of toppings to carriers. Addition of toppings also decreased dryness and firmness perception, 609 whereas flavor perception was increased at all stages of mastication. We conclude that toppings 610 assist saliva in bolus formation of bread and crackers. In particular, liquid-like mayonnaise and 611 semi-solid cheese spread moistened and softened the carriers, leading to shorter mastication 612 times and a lower number of chewing cycles to break down and lubricate boli enough to be 613 safely swallowed. As the effects were most pronounced after the addition of mayonnaise, 614 followed by cheese spread and firm cheese, the consistency of the toppings seems to play a 615 major role. More liquid-like toppings are more easily mixed with the carriers, leading to faster 616 moisture incorporation into the bolus and a faster softening of the bolus. The effects of toppings 617 were more pronounced in crackers than in bread, indicating that also the dryness of the carriers 618 affects oral processing. Thus, oral processing depends on the mechanical properties of both the 619 carrier and the topping. To develop a full understanding on the role of single foods on the oral 620 processing behavior and sensory perception of composite foods, additional studies investigating 621 carriers and toppings with systematically varied product properties will be required.

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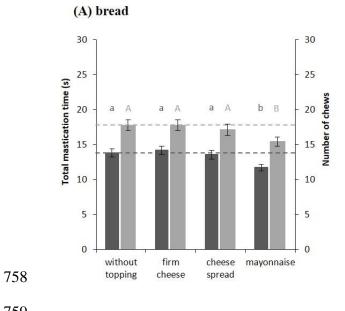
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#### 722 Figures

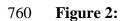
- 723 **Figure 1:** Total mastication time (dark grey) and number of chews (light grey) until swallowing
- for bread (A) and crackers (B) without topping and with different toppings (firm cheese, cheese
- spread and mayonnaise). Error bars represent standard error of the mean. Dashed lines represent
- 726 averaged value of single carriers (n=18 subjects, in triplicate). Different letters indicate
- significant differences between means (p < 0.05).
- Figure 2: Bolus moisture content (wt%) for bread (A) and crackers (B) with and without toppings (n=18 subjects). Time points correspond to 33, 66 and 100% of total mastication time.
- 730 The initial moisture content of samples is presented at t=0s. Error bars represent standard error
- 750 The initial moisture content of samples is presented at 1–65. Error ours represent samare err
- 731 of the mean. Dotted lines are added to guide the eye.
- **Figure 3:** Bolus saliva content at moment of swallowing in bread (A) and crackers (B) with and without toppings. Error bars represent standard error of the mean. Dashed lines represent averaged value of single carriers (n=18 subjects). Different letters indicate significant differences (p<0.05).
- Figure 4: Bolus peak force during first puncture cycle (associated to firmness) for bread (A)
  and crackers (B) with and without toppings. Time points correspond to 33, 66 and 100% of total
  mastication time. Error bars represent standard error of the mean (n=18 subjects, in duplicate).
  Dashed lines are added to guide the eye.
- **Figure 5:** Dryness, firmness and flavor intensity for bread and crackers with and without toppings determined by progressive profiling (n=18 subjects, in duplicate): (A) dryness of bread; (B) dryness of crackers; (C) firmness of bread; (D) firmness of crackers; (E) flavor intensity of bread; and (F) flavor intensity of crackers. Time points correspond to 1 chew and 33, 66 and 100% of total mastication time. Error bars represent standard error of the mean. Dashed lines are added to guide the eye.
- **Figure 6:** Principal component analysis (PCA) representing oral processing pathways of bread and crackers with and without toppings. Mastication behavior parameters (number of chews, chewing frequency, maximum vertical and lateral jaw movements) were taken at three stages during oral processing (i.e. 0-33, 33-66 and 66-100% of total mastication time). Bolus properties (moisture content, saliva content, saliva incorporation rate, first peak force, adhesiveness and cohesiveness) were taken at three time points throughout oral processing (33, 66 and 100% of total mastication time). Bread samples are presented in gray, and cracker

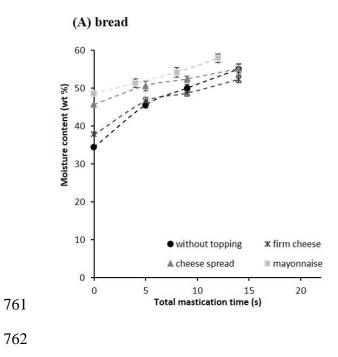
samples in black. Lines were drawn to guide the eye. Pictures of single carriers and carriers
with mayonnaise were presented close to the corresponding time points for illustration
purposes.

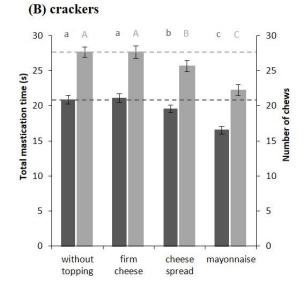
#### 757 Figure 1:

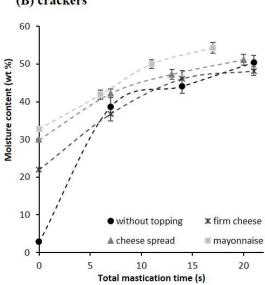






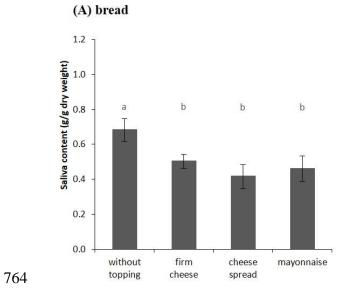




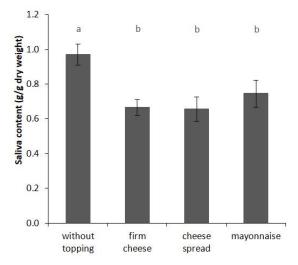


(B) crackers

# 763 **Figure 3**:



(B) crackers



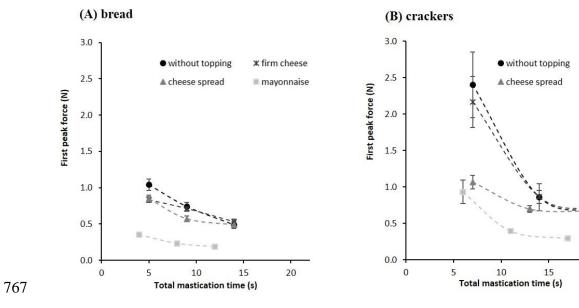
X firm cheese

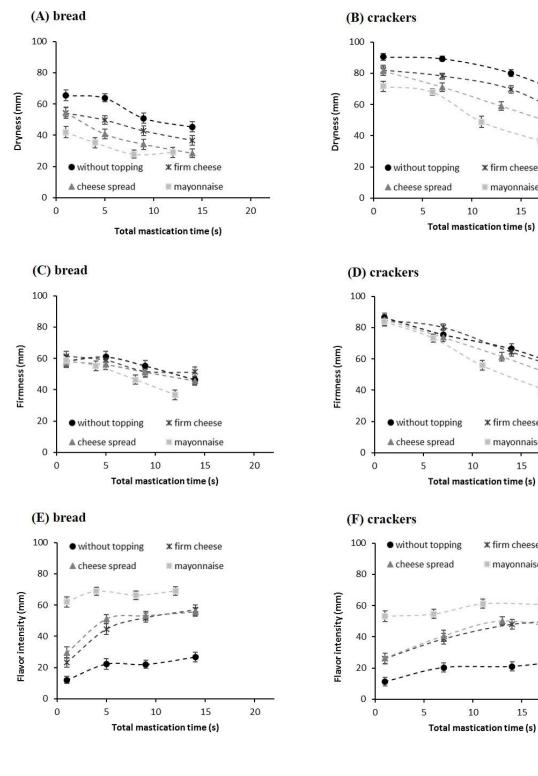
mayonnaise

-

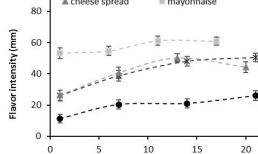
20



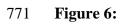


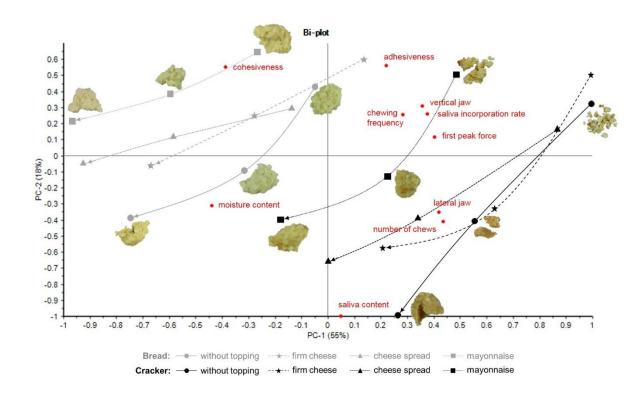


 without topping **x** firm cheese ▲ cheese spread mayonnaise 10 15 20 Total mastication time (s) Ŧ without topping **x** firm cheese cheese spread mayonnaise 10 15 20 Total mastication time (s) • without topping X firm cheese ▲ cheese spread mayonnaise



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# 774 Tables

- 775 **Table 1:** Overview of composition (fat and moisture content), physical-chemical properties
- (water activity, first peak force, adhesiveness, cohesiveness, texture category) and serving size
- of single foods (2 carriers, 3 toppings). Mean values  $\pm$  standard error of the mean are given.
- **Table 2:** Fixed effects of linear mixed models carried out for all mastication parameters, bolus
   properties and sensory properties of single carriers and carrier-topping combinations.
- properties and sensory properties of single carriers and carrier-topping combinations
- 780 Table 3: Fixed effects and descriptives (mean±SE) of bolus properties and sensory
- characteristics at moment of swallowing (t=100%) for bread and crackers with and without
  toppings, derived by linear mixed models.

#### Table 1: 783

Single foods	Fat content* (wt%)	Moisture content (wt%)	Water activity	First peak force** (N)	Adhesiveness** (g·s)	Cohesiveness**	Texture category	Serving size (g)
Carriers								
Bread	4	34.4 ± 1.0	$0.91\pm0.002$	$0.92\pm0.08$	$-1.9 \pm 4.9$	$0.77\pm0.06$	Soft solid	2.1
Cracker	4	$2.7\pm0.2$	$0.21\pm0.015$	$16.92\pm2.89$	$-7.7 \pm 9.4$	$0.06\pm0.03$	Hard solid	2.1
Toppings								
Firm cheese	31	$41.1\pm0.5$	$0.96\pm0.004$	$4.00\pm0.05$	-103.1 ± 21.9	$0.75\pm0.01$	Soft solid	2.1
Cheese spread	30	$57.0\pm0.1$	$0.98 \pm 0.001$	$1.11\pm0.02$	$-221.9 \pm 47.4$	$0.25\pm0.02$	Semi-solid	2.1
Mayonnaise	27	$62.8\pm0.2$	$0.97\pm0.001$	-	-	-	Plastic liquid	2.1

784 785 \* The fat content was taken from the packages.\*\* The mechanical properties of mayonnaise were not analyzed since mayonnaise is a plastic liquid.

#### Table 2: 786

Measurement										
		Carrier		,	Topping		Ca	rrier:To	pping	
(A) Mastication behavior <sup>a</sup>	F	р		F	р		F	р		
Total mastication time (s)	638.4	< 0.001	***	44.0	< 0.001	***	4.0	0.008	} **	
Number of chews until swallowing	584.9	< 0.001	***	26.9	< 0.001	***	3.6	0.013	3 *	
Chewing frequency (chews/s)	10.9	0.001	**	2.9	0.034	*	0.1	0.98	NS	
Maximum vertical jaw movement (mm)	16.3	< 0.001	***	7.8	< 0.001	***	1.7	0.15	3 NS	
Maximum lateral jaw movement (mm)	12.7	< 0.001	***	3.8	0.011	*	0.2	0.90	5 NS	

		Carrier		r	Topping			Time		Car	rier:Top	ping	Ca	rrier:Ti	ne	Т	opping:Ti	ime
(B) Bolus properties <sup>b</sup>	F	р		F	р		F	р		F	р		F	р		F	р	
Moisture content (wt%)	237.0	< 0.001	***	41.3	< 0.001	***	190.2	< 0.001	***	0.2	0.9	NS	17.1	< 0.001	***	2.3	0.033	*
Saliva content (g/g dry weight)	173.9	< 0.001	***	54.6	< 0.001	***	163.2	< 0.001	***	3.1	0.026	*	7.4	< 0.001	***	2.0	0.072	NS
Rate of saliva incorporation (g/min)	92.3	< 0.001	***	4.3	0.006	**	15.8	< 0.001	***	5.2	0.002	**	1.5	0.228	NS	2.5	0.024	*
Peak force (N)	55.7	< 0.001	***	124.3	< 0.001	***	123.6	< 0.001	***	4.6	0.004	**	2.9	0.058	NS	1.1	0.336	NS
Adhesiveness (g·s)	99.0	< 0.001	***	51.0	< 0.001	***	108.6	< 0.001	***	21.7	< 0.001	***	54.8	< 0.001	***	6.9	< 0.001	***
Cohesiveness	298.5	< 0.001	***	8.9	< 0.001	***	3.5	0.03	*	6.4	< 0.001	***	0.3	0.749	NS	2.2	0.037	*
		Carrier		, ,	Topping			Time		Car	rier:Top	ping	Ca	rrier:Ti	ne	Тс	opping:Ti	ime
(C) Sensory characteristics <sup>b</sup>	F	р		F	р		F	р		F	р		F	р		F	р	
Dryness	651.5	< 0.001	***	115.3	< 0.001	***	154.9	< 0.001	***	0.2	0.925	NS	15.0	< 0.001	***	1.9	0.054	NS
Firmness	245.7	< 0.001	***	11.1	< 0.001	***	160.6	< 0.001	***	0.1	0.978	NS	30.9	< 0.001	***	1.4	0.174	NS
Stickiness	21.4	< 0.001	***	3.7	0.011	*	54.4	< 0.001	***	0.8	0.472	NS	3.4	0.017	*	3.5	< 0.001	***
Flavor intensity	30.2	< 0.001	***	328.5	< 0.001	***	75.0	< 0.001	***	3.6	0.013	*	2.0	0.107	NS	6.1	< 0.001	***

787 <sup>a</sup> F-values and p-values are derived from linear mixed models with carrier, topping and the interaction as fixed effect, and subjects and replicates as random effects.

<sup>b</sup> F-values and p-values are derived from linear mixed models with carrier, topping, time and the interactions as fixed effect, and subjects and replicates as random effects.

Significance is presented as NS (non-significant); \* (p<0.05), \*\* (p<0.01), and \*\*\* (p<0.001).

#### Table 3: 790

	Carrier	Topping	Carrier:Topping			
(A) Bolus properties	Fр	F p	F р			
Moisture content (wt%)	55.2 <0.001 ***	21.1 <0.001 ***	0.2 0.865 NS			
Saliva content (g/g dry weight)	96.4 <0.001 ***	29.0 <0.001 ***	1.4 0.258 NS			
Rate of saliva incorporation (g/min)	45.7 <0.001 ***	5.0 0.003 **	2.6 0.057 NS			
Peak force (N)	32.6 <0.001 ***	76.0 <0.001 ***	0.6 0.634 NS			
Adhesiveness (g·s)	0.0 0.835 NS	27.7 <0.001 ***	3.8 0.010 *			
Cohesiveness	134.8 <0.001 ***	12.5 <0.001 ***	1.9 0.125 NS			
	Carrier	Topping	Carrier:Topping			
(B) Sensory characteristics	Fр	Fр	Fр			
Dryness	52.1 <0.001 ***	25.3 <0.001 ***	1.4 0.242 NS			
Firmness	1.9 0.166 NS	10.2 <0.001 ***	0.9 0.419 NS			
Stickiness	0.4 0.553 NS	4.6 0.004 **	0.8 0.508 NS			
Flavor intensity	14.9 <0.001 ***	87.3 <0.001 ***	1.7 0.174 NS			

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		Bread (m	Crackers (mean±SE)						
(A) Bolus properties	without topping	firm cheese	cheese spread	mayonnaise	without topping	firm cheese	cheese spread	mayonnaise	
Moisture content (wt%)	$55.0 \pm 1.5$ b	$52.3 \pm 0.9$ c	$55.1 \pm 1.2$ b	57.9 ± 1.1 a	$50.4 \pm 1.9$ b	$48.1 \pm 1.2$ c	$51.2 \pm 1.4$ b	54.3 ± 1.5 a	
Saliva content (g/g dry weight)	$0.68 \pm 0.06$ a	$0.50~\pm~0.04~b$	$0.42~\pm~0.07~b$	$0.46~\pm~0.08~b$	$0.97 \pm 0.06$ a	$0.66~\pm~0.05~b$	$0.66~\pm~0.07~b$	$0.74~\pm~0.08~b$	
Rate of saliva incorporation (g/min)	$4.5 \pm 0.5$ b	$5.6 \pm 0.4$ ab	$4.2 \pm 0.7$ b	5.1 ± 1.6 a	$6.1 \pm 0.6$ b	$6.2 \pm 0.4$ ab	$5.9 \pm 0.6$ b	$7.6 \pm 0.8$ a	
Peak force (N)	$0.49~\pm~0.05~b$	$0.53 \pm 0.03 \ a$	$0.49~\pm~0.04~\mathrm{ab}$	$0.19~\pm~0.01~c$	$0.67~\pm~0.08~$ b	$0.69 \pm 0.05 \ a$	$0.67~\pm~0.06~\mathrm{ab}$	$0.29~\pm~0.03~\rm{c}$	
Adhesiveness (g·s)	$-22 \pm 2$ b	-26 ± 3 ab	-30 ± 3 a	-8 ± 1 c	-19 ± 2 b	-25 ± 3 a	-32 ± 4 a	$-15 \pm 3$ c	
Cohesiveness	$0.46~\pm~0.01~c$	$0.52~\pm~0.01~b$	$0.53 \pm 0.01$ ab	$0.52 \pm 0.01 \ a$	$0.38 \pm 0.01$ c	$0.39~\pm~0.02~b$	$0.41~\pm~0.02~ab$	$0.45 \pm 0.02$ a	
		Bread (m	Crackers (mean±SE)						
(B) Sensory characteristics	without topping	firm cheese	cheese spread	mayonnaise	without topping	firm cheese	cheese spread	mayonnaise	
Dryness	$45.3 \pm 3.2$ a	$36.6 \pm 3.0$ b	$28.4~\pm~2.8~~c$	$29.0 \pm 3.3$ c	$62.3 \pm 4.2$ a	$48.5 \pm 3.8$ b	$44.6 \pm 3.5$ c	$36.7 \pm 3.3$ c	
Firmness	$46.2 \pm 2.6$ ab	$51.3 \pm 3.4$ a	$45.5 \pm 2.9$ b	$36.7 \pm 3.0$ c	$51.5 \pm 2.7$ ab	$50.3 \pm 2.7$ a	$45.6 \pm 3.2$ b	$41.2 \pm 3.5$ c	
Stickiness	$58.8 \pm 2.7$ a	$62.2 \pm 3.1$ a	$56.9 \pm 3.6$ a	$48.7 \pm 3.8  b$	59.9 ± 3.5 a	59.3 ± 3.5 a	$57.8 \pm 3.6$ a	$54.1 \pm 3.7$ b	
Flavor intensity	$26.7 \pm 2.9$ c	$56.8~\pm~3.3~b$	$55.6~\pm~2.8~~b$	$68.9 \pm 2.8$ a	$26.0 \pm 3.1$ c	$50.4 \pm 2.7$ b	$44.2~\pm~3.4~~b$	$60.9 \pm 2.7$ a	

F-values and p-values are derived from linear mixed models with carrier, topping and the interaction as fixed effect, and subjects and replicates as random effects. Significance is presented as NS (non-significant); \* (p<0.05), \*\* (p<0.01), and \*\*\* (p<0.001). Different letters indicate significant differences between bread samples or cracker samples (p<0.05)