Impact of composition and texture of protein-added yogurts on oral activity

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Abstract

Understanding how oral processing is altered in response to changes in composition and mechanical properties of food provides useful information to design food with improved satiating capacity which is largely influenced by oral exposure. In turn, this information deepens the knowledge about the physiology of texture perception. Six yogurts were formulated with different amounts of protein and protein sources and addition of apple cubes: control (C), extra skimmed milk powder-added (MP), whey protein isolate-added (WPI), and whey protein microgels-added (WPM). In addition, MP was also added with maltodextrin (MPMD) and with fresh apple cubes (MPF). Activities of masseter, anterior temporalis and anterior digastric muscles during oral processing of each sample were recorded (electromyography), and jaw movement amplitudes in three dimensions were collected (jaw tracking system).

The jaw muscle activities were highly dependent on type of yogurt. Addition of apple cubes (MPF) almost doubled oral processing time, number of chews, and muscle activity of all samples. MP and MPMD required similar but lower values of oral processing than MPF attributed to their reinforced network of milk protein. The lowest values were found for WPI, C and WPM, indicating a weaker, more fluid material. These behavioral results, which clearly differentiate the samples, are discussed in connection to rheological and sensory properties of the yogurts.

This study suggests that adding apple cubes significantly alters the oral processing pattern, such as they may be a more effective way of increasing oral processing time (time exposure) than more subtle changes in protein amount or source. Nevertheless changes in protein amount and source also affected, although to a lesser extent, the behavioral, rheological, and sensory properties of yogurt.

Keywords: yogurt, protein-added yogurt, whey protein isolate, whey protein microgels, electromyography, 3D jaw tracking, flash profiling.

38 **1. Introduction**

39 Food oral processing collectively accounts for all the physiological processes and
40 sensations occurring from the moment a food enters the mouth to when it is swallowed.
41 The signals collected during oral processing are involved in determine likes and
42 dislikes, the cephalic phase of digestion, and the start of the satiety cascade.¹⁻³

43 One aspect of oral processing is the oro-sensory exposure time, simply the time from
44 placement in the mouth to swallowing. According to a recent systematic literature
45 review and meta-analysis, prolonged oro-sensory exposure of food can enhance the
46 processes that promote satiation.⁴ Food oral exposure is affected by many food
47 characteristics,^{5,6} such as viscosity,^{5,7} bite size, oral processing rate, or chewing
48 frequency,⁸⁻¹⁰ among others.

49 Altering food matrix^{11,12} or increasing food texture complexity¹³ to prolong oral
50 processing are associated with decreased food consumption.² While an increased oro-
51 sensory exposure due to a need for longer oral processing to form a bolus, would
52 contribute to controlling further energy intake,¹⁴ the influence of oral processing
53 physiological processes is unknown.

54 It has been shown that modifications in food composition and processing that produced
55 changes in sensory texture change oral processing parameters of muscle activity and
56 jaw movement. Evaluating the alterations in chewing physiology (and thereby signals
57 going to the brain) in response to changes in food matrix would contribute to an
58 understanding of the dynamics of texture perception during oral food processing.¹⁵

59 Once placed in the mouth, food is subjected to a complex series of manipulations by
60 the tongue, teeth, lips and cheeks, during which it is converted into a form suitable for
61 swallowing.¹⁶ Sensory assessments of food are gathered during each phase of oral
62 food processing. Sensory signals have an early onset during eating and primarily
63 influence satiation. Initial perceptions occurring at low shear rates include attributes
64 related to touch (e.g. *perceived homogeneity* based on the presence, size, and shape
65 of particles), and those requiring only small deformations (*elasticity*, *stickiness to the*
66 *palate*, and *viscous behavior*).¹⁷ Next, the food structure is broken down during the first
67 chews (solid food) and the food mixed with saliva to form a coherent bolus (solid and
68 semi-solid food).¹⁸ As more saliva is added to the bolus, attributes are perceived that
69 relate to the physical structure (e.g. *smoothness*, *lumpiness*, and *pastiness*),
70 consistency (e.g. *creaminess* and *wateriness*), and adhesion to the palate (e.g.
71 *stickiness*).¹⁶ The sensory feedback taken from the food bolus leads to continuous
72 modification of mastication, including muscular forces, velocity and amplitudes of jaw
73 movements, duration of chewing, and the number of cycles until swallowing.¹⁹
74 Therefore, analyzing the modulation of chewing physiology in response to food kind of

matrix could be related to the dynamics of texture perception during food oral processing.²⁰

Recordings of electromyographic (EMG) activities of muscles involved in chewing and movements of the jaw in three dimensions (JT-3D) are the two techniques commonly used to obtain *in vivo* information on masticatory physiology in humans.^{21–25} Kinematic measurements provide information on the jaw movement amplitudes and velocities during the opening and closing phases of chewing cycles,²⁶ while EMG recordings measure muscle activity.^{27,28}

Yogurt contains high levels of protein, which is recognized as the macronutrient with the highest satiating capacity. Consequently, yogurt could be an excellent basis for designing a satiating product that offers the pleasure and satisfaction associated with low-energy/healthier versions of foods.²⁹

Increasing protein (of different sources) content and increasing oral processing activities are the two approaches addressed in the present study. Yogurt was selected as the model system to test the impact of composition on rheological, sensory and behavioral properties.

The hypothesis of the present study is that variation in the amount and source of protein and presence of apple cubes alter the oral processing activities (muscle activity and jaw movement) and the rheology of yogurt which in turn might be translated into differences in texture perception. To understand these relationships, the current study developed model yogurts with a) similar mechanical properties but increased protein due to adding whey protein powder or particles after heating the yogurt mix; b) increased protein and altered mechanical properties by increasing the gel network density, c) addition of maltodextrin (an ingredient that contribute or reproduce fat like mouthfeel) on top of increased gel network density and d) addition of oral processing and texture complexity by addition of apple cubes. The effects of these yogurt matrix alterations on rheology, oral processing parameters and sensory texture were evaluated.

2. Materials and methods

2.1 Ingredients

The protein ingredients used in the preparation of the yogurts were skimmed milk powder (33.77% protein, dry basis, nitrogen x 6.38), kindly supplied by Darigold, Chealis, WA, USA, and whey protein isolate (WPI) Bipro™ (92.83% protein, dry basis, nitrogen x 6.38), obtained from Davisco Foods International, Inc. Le Sueur, MN, USA. Maltodextrin was from Ingredion Incorporated, Bridgewater, NJ, USA, and Granny Smith apples were purchased from a local grocery store (Food Lion, Raleigh, NC).

Freeze-dried lactic culture Yo-Fast 20 was from Chr. Hansen S/A, Hørsholm, Denmark. All solutions were prepared with deionized water.

2.2 Sample preparation

Six different yogurts were formulated (Table 1): control (C), added skimmed milk powder (MP), added whey protein isolate (WPI), and added whey protein microgels (WPM). In addition, two MP formulations were prepared with additional ingredients of 2% of maltodextrin (MPMD) or fresh apple cubes (11g/100g wet basis; 5 mm-side) (MPF). The total amount of protein resulting in each treatment it is presented in Table 1.

Skimmed milk powder and distilled water were placed in glass beakers (1L) and heated in a water bath (Polyscience 28L-M Water bath, Niles, IL, USA) at 82-85°C for 30 min;³⁰ when required, whey proteins, whey protein microgels, maltodextrin or apple cubes added when the dispersions were cooling (50°C). The samples were cooled to the incubation temperature recommended for the culture used (42–43°C) and inoculated with the lactic culture at 0.5 g/100 g of milk. The samples were placed in glass yogurt jars (200 mL) and then positioned in a yogurt-maker (Vonshef digital yogurt maker, Designer Habitat Ltd., Deva Centre, Salford, United Kingdom). After a period of 7 h, samples reached pH values of 4.5-4.6 (Accumet excel XL50, Fisher Scientific, Pittsburgh, PA, USA). Jars were individually covered and stored at 4-5°C for 48h.

2.3 Preparation of the whey protein microgel dispersion

The method of Schmitt *et al.*³¹ was used to produce microgels with some modifications. A 50 L dispersion of whey protein isolate was prepared, WPI (BiPRO, Davisco, Le Sueur, MN, USA) at 4 % protein in water at pH 5.9 ± 0.05 adjusting the pH with 1 M HCl. The WPI dispersion was thermal processed using a using a Microthermics pilot plant thermal processing system (Model 25HV; Microthermics Inc., Raleigh, NC, USA), equipped with a Microthermics clean fill hood and Niro-Soavi homogenizer (Model NS2006H; Niro-Soavi, Hudson, WI, USA). The pressure was set at 0 psi total pressure and the heat was set at 85 °C for 15 min. Following, the solution was cooled to 10 °C and filled in a 60-L stainless steel kettle (model 601BP, Polar Ware Company, Kiel, WI, USA).

After cooling, the solution was concentrated to 25 % using an ultrafiltration system (model Pellicon 2, Millipore Inc., Billerica, MA, USA) using 11 cartridges of polyethersulfone membrane filters (model P2B010V05, nominal separation cutoff = 10,000 kDa, surface area = 0.5 m²) and the backpressure set at 20 psi. The pump used to circulate the product was a variable-speed peristaltic pump (model 77410-10, Cole

Palmer, Vernon Hills, IL, USA) equipped with model 77601-00 pumpheads with silicone tubing (model 96440-73, Cole Palmer). Percentage solids were analyzed using a Smart System 5 moisture/solids analyzer (CEM Corp., Matthews, NC). The retentate were dried in a spray dryer (model Lab 1, Anhydro Inc., Soeborg, Denmark) with a feeding rate of 100 mL/min, an inlet temperature of 160°C and outlet temperature of 90°C. The dried powder was collected in Mylar bags (TF-4000, Impak Corp., Central City, SD). A 2-kg batch of WPM powder was produced. The WPM powder was 95.06% protein. They exhibited a hydrodynamic radius of 141±3 nm determined by dynamic light scattering; the measurement was made at ambient temperature (23±1 °C) and samples diluted to a protein concentration of 1 mg/mL to reduce multiple scattering effects using a Wyatt Möbiuz light scattering instrument (Wyatt Technology, Santa Barbara, CA, USA).

161

162 **2.4 Rheology**

163 *2.4.1 Yield stress*

164 The vane method has been adopted by researchers in food science to measure the
165 yield stresses of various concentrated suspensions, such as ketchup, apple sauce, and
166 baby food^{32,33} as well as of more complex food matrices, such as ice cream.³⁴ In the
167 present study the vane method was used to determine the yield stress of the
168 yogurts.^{35,36} The general consensus is that the vane method is a reliable and easy
169 method to measure yield stress. Yield stress is calculated as a function of the
170 maximum torque value recorded when shearing a sample at a constant shear rate.

171 A four-bladed vane (14 mm diameter, 30 mm height) attached to a rheometer (Haake
172 VT-550 viscometer, ThermoFisher Scientific, Waltham, MA) was used for all
173 measurements. Measurements were made on yogurt in glass container (depth of 60
174 mm and diameter of 50 mm) used for making yogurt to prevent any structural damage.
175 The vane was lowered into the yogurt and rotated at 0.3 rpm for all measurements.
176 Three measurements were obtained from fixed points in each yogurt jar, and 3
177 replications were performed. To account for any potential changes in the yield stress of
178 the yogurt over the measurement period, the order of measurement at the fixed
179 positions was systematically changed with each replication.

180 Values for torque and displacement were recorded and the torque values used for
181 calculating the yield stress (σ_0) according to the formula.³⁷

182

$$183 \sigma_0 = M_0 / ([h/d + 1/6](\pi d^3/2)) \quad \text{equation (1)}$$

184

185 where h and d are the height and diameter of the vane respectively and M_0 is the
186 maximum torque (N·m) recorded by the viscometer.

187

188 2.4.2 Flow behavior

189 A controlled stress rheometer (Anton Paar MCR 302, Graz, Austria) equipped with 25-
190 mm diameter parallel plates (1-mm gap) was used to conduct rheological analyses at
191 room temperature (22 ± 1 °C).

192 Sample flow was measured by recording the shear stress values when shearing the
193 samples with a linearly increasing shear rate from 1 to 200 s^{-1} for a period of 90 s, in
194 reverse sequence for the same time, and finally again an increasing sequence from 1 to
195 200 s^{-1} . The data from the decreasing segment of the shear cycle were fitted to the
196 Ostwald-de Waele model (equation 2):

197

$$198 \sigma = K \dot{\gamma}^n \quad (\text{equation 2})$$

199

200 where σ (Pa) is the shear stress, K (Pa s^n) is the consistency index, $\dot{\gamma}$ is the shear rate
201 and n is the flow behavior index.

202

203 2.5 Sensory analysis

204 Flash profiling — a combination of free-choice term selection with a ranking method —
205 was applied. The simultaneous presentation of the complete set of samples allowed
206 direct sensory comparison.³⁸

207

208 2.5.1 *Panel*. Flash profiling was carried out by 15 assessors (14 women and 1 man,
209 aged 21-45 years) recruited among employees of the North Carolina State University
210 (NC, United States) who had experience in sensory description of food products and
211 were regular consumers of dairy products (IRB #9395). They were asked to describe
212 all the (non-hedonic) differences they perceived in the yogurt samples. No restrictions
213 about attribute modalities were introduced. A total of 111 sensory terms were
214 generated, 19 of which were semantically different. Between 5 and 9 attributes were
215 generated by each panelist.

216

217 2.5.2 *Procedure*. Flash profiling does not demand a specific participant training stage.
218 The single session was divided into two stages. In the first, prior to evaluation, the
219 researcher explained the procedure to each panelist and gave him/ her a printed
220 example of a descriptive ranking of apples according to various attributes that did not
221 apply to yogurt (*crispy*, *hard*, and *mealy*). In the second or evaluation stage, the

complete set of samples was presented simultaneously. After tasting the samples, each panelist generated his/ her own list of attributes to describe the differences among the four yogurts for texture characteristics of each sample.³⁹ No indication was given regarding the number of attributes that should be proposed. The panelists then ranked all samples on an ordinal scale for each attribute they had individually proposed (ties were allowed). The panelists were asked to focus on descriptive terms. Their evaluation was individual. The samples (30 mL) were served at consumption temperature (10 ± 0.5 °C). Since it was not important to assess their color, the samples were served in small plastic cups coded with random three-digit numbers. Mineral water was provided for rinsing the mouth between samples.

2.6 Oral processing measurements

2.6.1 Subject selection. Twelve subjects (10 females and 2 males) participated in the study. All subjects were recruited and screened for oral health and complete dentition (except for third molars or wisdom teeth); with class I type molar occlusion. Participants did not have a history of gum or periodontal disease and had no major dental treatments, including braces, surgery, dental extraction or restoration, for six months prior to the study. No participants exhibited pain or sound (i.e. grinding, popping, or clicking) in their temporomandibular joints during chewing. No participants were trained in food textural evaluation. Subjects were selected based on strict dental criteria as described in Çakir *et al.*²⁵ All subjects gave voluntary informed consent to take part in the study. The study was conducted in accordance with the North Carolina State University Institutional Review Board for the Protection of Human Subjects in Research guidelines (IRB #9395).

Each subject attended a preliminary session to become familiar with the recording system, the environment, and the model food used in the study. Electromyographic muscle activities were recorded during resting, clenching the teeth, and during swallowing. In order to confirm subjects had unrestricted jaw movements, maximum limits of their jaw motions were measured in vertical, anterior-posterior, and lateral dimensions. Maximum limits of jaw motions were measured to confirm that subjects had normal jaw mobility. Electromyographic data and jaw movements of subjects were recorded while chewing gum serving as a control session, but the data collected in this preliminary session were not analyzed.

2.6.2 Electromyography. Muscle activity was recorded in tandem with jaw movements. Electromyographic activities were recorded from left and right sides of the superficial masseter (MM), anterior temporalis (AT), and anterior digastric (AD) muscles using the

259 BioEMG II electromyograph (BioResearch Inc., Milwaukee, WI). During mastication,
260 digastric muscles contribute to jaw opening, while temporalis and masseter muscles
261 act to close the jaw and occlude the teeth.²⁷ Surface electrodes (4.5x2 cm) (BioFlex,
262 BioResearch Inc., WI) were placed on the skin along the length of each muscle with an
263 additional ground electrode on the shoulder to eliminate electrical background noise. A
264 sampling rate of 5000 Hz was applied for each electrode. A detailed description of
265 electrode placement is given in Çakir *et al.*¹⁵

266

267 *2.6.3 Jaw tracking.* A JT-3D Jaw Tracker (JT-3D, BioResearch Inc., Milwaukee, WI)
268 was used to record mandibular movements. A rectangular magnet (13.5x2x2 mm) was
269 attached to the lower frontal incisors with a stomahesive (ConvaTec, Bristol-Myers
270 Squibb Company, NJ) to allow for recording jaw movement. Movement of the magnet
271 was tracked by an array of sensors fit as a unit on the head to record vertical, anterior-
272 posterior and lateral components of jaw movement during mastication. It uses a
273 lightweight headset with dual magnetic sensor arrays positioned several centimeters
274 from each side of the subject's face.

275 The BioResearch Associates JT-3D Jaw Tracker was employed to track jaw
276 movement. The standard commercial unit connects via a PCMCIA card to a laptop
277 computer with dedicated software, which produces a calibrated display of movements
278 in each plane.²⁵

279

280 *2.6.4 Data collection.* Each subject participated in an hour-long session and all
281 recordings were performed during the single session to avoid replacement of the EMG
282 electrodes. The subject was seated in a comfortable upright position and asked to
283 refrain from talking or making unnecessary movements during recordings. Subjects
284 were video recorded during consumption of the test samples. Subjects were presented
285 8 g of sample within 60 ml soufflé cups consumed in quadruplicate. Jaw movement and
286 jaw-muscle electromyography were recorded at the same time in duplicate. The subject
287 was instructed to place the sample onto their tongue and then bring their teeth together
288 to create the zero point of the vertical position for jaw tracker analysis. Upon a signal
289 from the experimenter, the subject started chewing in a habitual manner and
290 recordings were made until the bolus was swallowed.

291

292 *2.6.5 Data analysis.* Data analysis was conducted in LabView graphical programming
293 software (National Instruments, Austin, TX). To minimize the variations arising from
294 different electrodes and electrode positioning across multiple subjects, scaled values
295 were calculated for muscle activities. The scaled muscle activities represent relative

estimates of muscle recruitment during the chewing cycle. A full description of the data analysis and scaling of the EMG data were previously given by Çakır *et al.*¹⁵ Calibration and aggregate data analysis were performed using MATLAB graphical programming software (MathWorks, Natick, MA). Each recording was input into MATLAB and the data set was cropped first at the trigger (time 0) and at the end of the recording. Digastric muscle activity was used as an endpoint of oral processing because it was indicative of movements related to swallowing.⁴⁰ For electromyography, complete sequence analysis involved parameters that characterize the whole chewing sequence including muscle activities of AT, MM, and DA. Oral processing recordings were output as aggregate summaries of peak, average, and total values for muscle activity and jaw movement (Table 2).

2.7 Statistical data analysis

Statistical analysis of treatment means (aggregate data) was performed using JMP 12.0 (SAS, Cary, NC). Significant differences among test samples were determined using a one-way analysis of variance (ANOVA) with Tukey's honestly significant differences at an experiment-wise error rate of $\alpha=0.05$. A repeated measures experimental design was used, with random effects included for subject and subject-by-treatment interactions. Principal Component Analysis was applied separately to each set of oral parameters (EMG and JT-3D). Multifactorial Analysis was performed on the FP ranking data to generate the factorial map used to evaluate the overall sensory positioning of the samples according to the participants' perception.⁴¹

3. Results and Discussion

3.1 Rheological properties

Measurements of the rheological properties were not carried out for MPF and WPM samples because, at rest, they presented a heterogeneous distribution of the apple cubes and the whey protein microgels and needed to be stirred before consumption to produce a homogeneous distribution.

Viscosity results in the downward curve are shown in in Figure 1 in log-log form and fit to an Ostwald-de Waele model as previously outlined (Figure 2).⁴² An analysis of the results for log (viscosity) versus log (shear rate) demonstrated that the flow curves were linear and mostly parallels with a good level of fit to Ostwald-de Waele model (r^2 from 0.989 to 0.997).

333 The analysis of variance showed that rheological values significantly ($p < 0.05$) varied
334 among yogurts, indicating matrices with different mechanical properties. The yield
335 stress (σ_0), consistency index (K), and apparent viscosity at a shear rate of 50 s^{-1} (η_{50})
336 values for the MP yogurt were the highest (Table 3), in particular σ_0 was three times
337 higher than C. This shows that the addition of extra milk protein reinforced the gel
338 network.

339 The MPMD yogurt had the same milk protein level of MP but with added maltodextrin;
340 while σ_0 was still greater than C, the consistency index K was significantly lower and n
341 values higher. It has been suggested that there is a reinforcing effect of the
342 maltodextrin acting as a filler in the milk protein gel that is not observed for other
343 proteins like gelatin.⁴³ However, in the present case, maltodextrin didn't contribute any
344 detectable rheological reinforcement. Indeed, the value of n for MPMD was the highest,
345 indicating the highest Newtonian like behavior.

346 The lower amount of casein in the whey protein-containing samples appeared to disturb
347 the formation of the gel network and produced a weak matrix. The yogurt with added
348 WPI had yield stress, K index and η_{50} values that were significantly lower ($p < 0.05$) than
349 all other yogurt including C (Table 3). Guzmán-González *et al.*⁴⁴ and Cho *et al.*⁴⁵
350 showed higher viscosities when enriching yogurts with skimmed milk powder than whey
351 protein; in these studies, the levels of whey protein or skimmed milk powder added
352 were such that the final protein level was the same, as in the present study. The lower
353 casein content (lower amount of skimmed milk powder, Table 1) and possible
354 interference of whey protein isolate in network formation could have contributed to a
355 weaker matrix than the control (C) yogurt. It is a common practice to add whey protein
356 to increase firmness in yogurt-making industry but this is done before heating the milk.
357 In the present study, it was added after heating to serve as a control for the microgel
358 treatment. The protein content of the MP sample and whey protein added samples
359 (WPI and WPM) had equal total protein (Table 1), but different rheological properties.

360 The comparison of the hysteresis loop areas showed (Table 3) that C and MP needed
361 significantly higher energy levels to break their structure compared to MPMD and WPI.
362 This suggests the added compounds produced weaker connections or fewer
363 interactions in the MPMD and WPI yogurt networks, decreasing the amount of energy
364 needed to disrupt the yogurt structure. Tatdao and Frank⁴⁶ showed that inulin-
365 containing yogurts, in comparison with the control without inulin, were characterized by
366 lower values of firmness, apparent viscosity, yield stress and hysteresis loop area
367 values. Addition of WPI after the heat treatment could have produce the same effect.

368

369 **3.2 Sensory analysis**

Figures 3A and 3B show the biplots obtained by Multifactorial Analysis from the sensory flash profiling data. The first three dimensions of the plot (accounting for 79.80% of total variability) were considered. To help the interpretation of the dimensions, the sensory terms explained by each dimension (correlation greater than 0.6) were summarized in a list.⁴⁷ The first dimension (37.72% of total variability) separated samples MP and MPMD on the left side of the biplot, and C and WPM on the right side of the biplot (Figure 3).

The MP and MPMD yogurts were located quite close together at the very left side of the plot and both were perceived as being more *viscous* and *cohesive*, *creamier*, *denser*, and *thicker* than the rest of samples. These two yogurts had the highest σ_0 values (Table 3), that is, they need higher shear stress to initiate flow.

It is suggested that flow in the mouth is a combination of shear and elongational flow,⁴⁸ and apparent viscosity at a shear rate of 50 s^{-1} has practical utility as a possible instrumental index of perceived thickness in semisolid foods. The MP yogurt had the significantly highest ($p < 0.05$) apparent viscosity (η_{50}) values (Table 3), followed by MPMD indicating that doubling skimmed milk powder in the formulation of a yogurt may be a good way to increase perceived thickness in the mouth.

On the right side of the plot, C and WPM (although not so close together) were differentiated as being more *fluid*, *astringent*, *rougher*, and *grittier*. Sample C had lower values of σ_0 and η_{50} than MP and MPMD (Table 3) that could be related to the perception of a more fluid and melting-away yogurt. On the other hand rough and gritty perceptions were attributable to the WPM yogurt; as commented above, it had a heterogeneous distribution of particles that could have been detected in mouth. Regarding astringency, yogurt WPM but also WPI are placed in the right side of the biplot; it is well known that whey proteins are perceived as astringent at low pH^{49,50}.

The first dimension seemed to oppose textural attributes *fluidity*, and *melts away*, on the right half of the map, and *creaminess*, *viscosity*, *cohesiveness*, *thickness*, and *mouth-coating* on the left half of the map (other attributes were also mentioned in the second dimension of the biplot). In a study evaluating several types of plain yogurts,⁵¹ it was found that consumers mentioned *too fluid*, *gummy texture*, *gritty texture*, *rough* as sensory defects that were drivers of disliking and of negative intention to try, whereas *creamy*, *thick*, and *soft* were mentioned as the sensory characteristics of the tried yogurts. De Wijk *et al.*⁵² working with custards, have also shown the existence of a *rough/creamy* axis (in the present case running from positive to negative values along the X axis); however, in their study custards along this axis were related to the presence of different thickeners and fat levels.

407 The attribute *creaminess* is found in 3 quarters of the plot; this would be explained
408 because *creaminess* is a complex percept for non-trained assessors. Several texture
409 characteristics such as *smoothness*, *softness* or *thickness* would be involved in
410 evaluation of *creaminess*.⁵³

411 The second dimension (22.80% of total variability) placed MPF and WPI in the upper
412 part of the map and separated from the rest of the samples indicating that they were
413 perceived different. These two yogurts were likely to have detectable particles in the
414 mouth (apple cubes and whey protein microgel particles, respectively). While
415 dimension 2 is difficult to interpret, dimension 3 (Figure 3B), that explains a percentage
416 of variability similar to dimension 2 (19.29%), separated MPF from WPI yogurts, being
417 MPF the only yogurt perceived as *grainy*. It should be remembered that flash profile the
418 terms proposed by each participant are used and the resultant biplots are interpreted
419 as a consensus response to the entire set of data. Finally, the third dimension placed C
420 sample as the only yogurt that not described as *creamy* but was perceived as *smooth*.

421 It is noteworthy that the addition of whey protein microgels particles did not contribute
422 the perception of thickness of sample WPM as it was perceived as fluid and melts
423 away and the presence of particles conferred sensations of *astringency*, *grittiness*, and
424 *roughness*.

425 It is unlikely that the grittiness comes from in mouth particle detection considering the
426 size of WPM particles used in the present work. In previous studies, it was shown that
427 WPM introduce lubrication effect via ball-bearing mechanism.^{54–56} One possibility is that
428 the WPM particles were aggregated; it was reported that WPM particles showed a
429 strong tendency for aggregation when the pH was lowered from pH 7 to 4.⁵⁷
430 Consequently, it is likely that it is the final size of the aggregates formed during the
431 fermentation of the milk rather than the initial size of the microparticles that has
432 influenced *grittiness* perception of WPM yogurt.⁵⁸ Another possibility is that microgel
433 particles were not fully rehydrated during its addition to the milk base so dried microgel
434 powdered would has conferred a sensation of *roughness*. In any case sample WPM is
435 closer to C than any other sample in the perceptual map indicating that “particle
436 perception” is not as strong as in samples MPF and WPI (Figure 3A).

437 Sensory and mechanical properties of protein-added yogurt are controlled and
438 modulated by the composition and processing parameters and conditions. A specific
439 adjustment of the heat treatment load applied to the yoghurt milk base seem to be
440 critical for tailoring rheological, and sensory properties of protein-added yogurts.⁵⁹

441

442 Considered as a whole, the perceptual map indicated that, in general, using milk
443 protein for protein enrichment in yogurt resulted in *creamy*, *smooth* and *thick* products

compared to C (not enriched) that was *fluid* and *melt away*. Perception of particles in the mouth contributed several sensations as *roughness*, *graininess*, and *grittiness*.

3.3 Muscle activity and jaw movement

Overall, the DA muscles associated with jaw opening and swallowing were more active than jaw closing muscles (TA and MM) (Table 4). Peak and total muscle activity followed similar trends.

Prominently, the MPF yogurts values for both muscle activity and jaw movement were 2-4 times larger than the rest of samples, followed by MP and MPMD yogurts. The C and whey protein added (WPI and WPM) yogurts were similar and required the least overall muscle activity and jaw motion (Table 5).

The MPF yogurts had the significantly highest ($p < 0.05$) eating time before swallow, peak and total muscle activities, number of chews, and jaw movements (Tables 4 and 5) due to the apple cubes. Despite MPF has the same yogurt base as MP, all oral processing parameter values for MPF were higher. This fruit-containing yogurts required the highest peak values ($p < 0.05$) and total activity values ($p < 0.05$) for the three muscles groups. It also required 4-5 more chews than the rest of the samples and significantly higher jaw ranges and total movements reflected by almost all parameter values. More movement was required to process the apple cubes into a bolus. Increasing food hardness (fracture stress) of solid foods has also been shown to increase muscle activity, oral processing time, number of chews, and jaw movements across biopolymer gels, cheeses, confections, meats, fruits, and vegetables.^{22,24,60–63} Regarding the velocity of jaw movements, MPF had the highest maximum and average velocity values ($p < 0.05$) (Table 5). Rhythmic chews and shearing to break the apple cubes into smaller and softer pieces led to increases in jaw velocities for these samples. Gathering these widely distributed small pieces into a swallowable bolus were likely the driver of increases in jaw movements. The sensory results also “separated” the yogurt MPF from the rest of yogurts (Figure 3B) being the only described as *grainy*. These results indicate that adding fruit pieces to yogurt is a reliable way to add oral exposure time probably regardless (or at least less importantly than) the type of yogurt matrix because the fruit cubes “dominate” oral activity; this has the potential consequences of triggering satiating effects.

MP and MPMD show the second higher values of oral processing showing no significant differences between them in almost all jaw muscle activity or movement. The MP and MPMD treatment is significantly higher ($p < 0.05$) than C for all oral

processing parameters (Tables 4 and 5) with the exception of maximum and average vertical velocity. Lateral maximum and average velocities showed higher values for yogurts made with MP and MPMD than WPI, WPM and C ($p < 0.05$), probably because the reinforced network formed by skimmed milk powder addition needed higher lateral velocity values to process these samples in the mouth (Table 5). Rheological data confirmed the highest values of σ_0 (Table 3) for MP and MPMD. In sensory terms indeed they were described as *creamy*, *dense*, *viscous*, *cohesive* and *thick*.

Yogurt C has the shortest oral processing time and the lowest values for all oral processing parameters measured (Table 4 and 5) although similar to those for WPI and WPM yogurts. All these samples were placed on the right side of the sensory biplot (Figure 3A and B) and described principally as *fluid* with intermediate/ low rheological parameters values (Table 3).

The WPM yogurt had significantly ($p < 0.05$) higher DA peak and total activity values than C and WPI yogurts, indicating that the presence of small particles required more activity of DA muscles probably because swallowing movements are more complex when managing the fluid with suspended particles. A movement that is trying to agglomerate all the particles together into a cohesive bolus that can be swallowed easily is reflected in the DA peak and total activity values.⁶⁴ The masticatory apparatus adjusts to changes in food matrix by applying relatively more or less muscle activity to process the sample.^{65–67}

As stated above, it is commonly observed that increases in oral processing time (exposure) are associated with increases in satiation and satiety or decreases in food intake.^{4,68–72} Addition of apple cubes almost doubled oral processing time, number of chews, and muscle activity and jaw movements. For semisolid foods with a “bolus-like” texture, it appears that addition of particles that require chewing is a more reliable way to extend oral processing time to potentially increase satiation or satiety than more subtle changes in composition.

Figure 4 graphically illustrates the relative differences in oral processing ranges, totals and total activities normalized for eating time among the six samples. Normalization for eating time was performed by dividing total activity or movement by average eating time for each yogurt type to obtain a value in s^{-1} (muscle) or mm/s (jaw).

Essentially, three groups were observed; the first being C, WPI and WPM yogurts. Adding whey protein increased protein content (compositional benefit) but no behavioral benefit as the eating time and number of chews are the same. Trends observed in Figure 4 strongly reinforce those discussed above: no differences were observed between muscle or jaw efforts required to consume C and whey protein added yogurts, with the exception of the DA muscles activity of WPM particles. The second group was MP and MPMD yogurts that had over twice the amount of casein as the C yogurt and increased eating time and oral processing, and the third being MPF yogurt with the same “protein boost” of MP but the additional mechanical/oral processing benefit of the apple cubes. Figure 4 shows that adding fruit pieces had the largest effect, but statistically significant increases for adding extra milk protein (MP and MPMD are greater than C) were also observed (Tables 4 and 5). As mentioned above, Figure 4A showed that the DA muscles, associated with jaw opening and swallowing, were more active than jaw closing muscles (TA and MM). The EMG parameters group all treatments together but the MPF yogurt. The MPF yogurt appears near the position of muscle activity parameters due to the noteworthy difference between samples with and without chewable fruit cubes. Trends in jaw movement parameters were similar to those observed for muscle activity, the added apple cubes required sizeable increases in both activity ranges and total activities. The MPF treatment is at the right half of the map near to all the measured parameters and C, WPI, and WPM at the other end. The MP and MPMD treatments were halfway between the two groups of samples. Increases in jaw ranges indicate that more space within the mouth was required (or utilized) to prepare the apple cubes bolus for swallowing. In a recent study conducted by Mosca *et al.*,⁷³ six iso-caloric combinations of 2 yogurt viscosities with added granola pieces were used. Both yogurt viscosity and granola size significantly affected oral processing behavior and intake. An increase in yogurt viscosity was sufficient to decrease eating rate, number of spoons, swallows and intake. Medium granola pieces (6-mm, similar to our apple-cubes size) had the lowest eating rate and highest number of chews. They conclude that small but perceivable variations in texture properties of yogurt are sufficient to change oral processing behavior and intake. In a recent study where oral processing behavior parameters were extracted from video recordings of participants it was suggested that consumers adapted their oral processing behavior with respect to consumption time, and eating rate, among others parameters to the rheological and mechanical properties of liquid, and semi-solid foods.⁷⁴

554 In the present study, the addition of apple cubes to the yogurts was the major driver of
555 significant differences in oral processing behaviors among yogurts. Multiple research
556 groups have reported that changes in chewing energy, chewing pattern and chewing
557 rate impact directly dynamic sensory perception.^{16,75–77} Also, individual oral processing
558 patterns may be guided by hedonic preferences for specific sensations or sensation
559 intensities.^{16,77,78}

560

561 **4. Conclusions**

562 Increasing protein content by adding whey protein isolate directly as an ingredient or in
563 microgel form did not increase oral processing time or viscosity and produced sensory
564 sensations of *roughness* and *grittiness*. On the other hand, increasing protein content
565 by doubling the amount of skimmed milk powder used to make yogurt caused an
566 increased yield stress and viscosity in the yogurt, which was reflected in an increased
567 in oral processing time and a slightly different oral processing pattern. Skimmed milk
568 powder added yogurts were describes as *creamy*, *dense* and *thick*.

569 Above all, the biggest change was seen when apple cubes were added to the yogurt
570 made with double the skimmed milk powder. Total oral processing time and intensity
571 (time normalized) were increased and the oral processing pattern was altered.

572 In summary the results show that reinforcing the gel network (increased milk powder
573 amount) and adding chewable, high oral processing requiring elements increased
574 notably oral processing time. This protein rich matrix with increased oral exposure
575 would serve as a model system framework for future research on satiating food
576 development.

577

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588

589 **Conflicts of interest**

590 The authors declare that there are no conflicts of interest regarding the publication of
591 this article.

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594 References

- 595 1 L. Chambers, K. McCrickerd and M. R. Yeomans, *Trends Food Sci. Technol.*,
596 2015, **41**, 149–160.
- 597 2 C. de Graaf, *Physiol. Behav.*, 2012, **107**, 496–501.
- 598 3 M. L. Power and J. Schulkin, *Appetite*, 2008, **50**, 194–206.
- 599 4 E. M. Krop, M. M. Hetherington, C. Nekitsing, S. Miquel, L. Postelnicu and A.
600 Sarkar, *Appetite*, 2018, **125**, 253–269.
- 601 5 R. A. de Wijk, N. Zijlstra, M. Mars, C. de Graaf and J. F. Prinz, *Physiol. Behav.*,
602 2008, **95**, 527–532.
- 603 6 S. C. Hutchings, J. E. Bronlund, R. G. Lentle, K. D. Foster, J. R. Jones and M. P.
604 Morgenstern, *Food Qual. Prefer.*, 2009, **20**, 456–460.
- 605 7 M. Viskaal-van Dongen, F. J. Kok and C. de Graaf, *Appetite*, 2011, **56**, 25–31.
- 606 8 D. P. Bolhuis, C. M. M. Lakemond, R. A. de Wijk, P. A. Luning and C. de Graaf,
607 *J. Nutr.*, 2011, **141**, 2242–2248.
- 608 9 N. Zijlstra, R. A. De Wijk, M. Mars, A. Stafleu and C. De Graaf, *Am. J. Clin. Nutr.*,
609 2009, **90**, 269–275.
- 610 10 J. Tang, D. S. Larsen, L. R. Ferguson and B. J. James, *Physiol. Behav.*, 2016,
611 **163**, 17–24.
- 612 11 Y. Pascua, H. Koç and E. A. Foegeding, *Curr. Opin. Colloid Interface Sci.*, 2013,
613 **18**, 324–333.
- 614 12 K. Mccrickerd, C. M. H. Lim, C. Leong, E. M. Chia and C. G. Forde, , *J. Nutr.*,
615 2017, **147**, 1208–1217
- 616 13 J. Marcano, D. Morales, J. F. Vélez-Ruiz and S. Fiszman, *Food Res. Int.*, 2015,
617 **75**, 225–232.
- 618 14 K. McCrickerd, L. Chambers, J. M. Brunstrom and M. R. Yeomans, *Flavour*,
619 2012, **1**, 20.
- 620 15 E. Çakir, H. Koç, C. J. Vinyard, G. Essick, C. R. Daubert, M. Drake and E. A.
621 Foegeding, *J. Texture Stud.*, 2012, **43**, 257–267.
- 622 16 R. A. de Wijk, L. Engelen and J. F. Prinz, *Appetite*, 2003, **40**, 1–7.
- 623 17 A. J. Rosenthal, 1999.
- 624 18 J. F. Prinz and P. W. Lucas, *Proceedings. Biol. Sci.*, 1997, **264**, 1715–21.
- 625 19 K. Hiimae, M. R. Heath, G. Heath, E. Kazazoglu, J. Murray, D. Sapper and K.
626 Hamblett, *Arch. Oral Biol.*, 1996, **41**, 175–189.

- 627 20 J. Chen, *Food Hydrocoll.*, 2009, **23**, 1–25.
- 628 21 W. E. Brown, D. Eves, M. Ellison and D. Braxton, *J. Texture Stud.*, 1997, **29**,
629 145–167.
- 630 22 K. D. Foster, A. Woda and M.-A. Peyron, *J. Neurophysiol.*, 2006, **95**, 3469–
631 3479.
- 632 23 C. H. Gibbs, in *HC and Gibbs, CH, Advances in occlusion*, 1982, vol. 232.
- 633 24 L. Mioche, P. Bourdiol, J.-F. Martin and Y. Noël, *Arch. Oral Biol.*, 1999, **44**,
634 1005–1012.
- 635 25 E. Çakir, C. J. Vinyard, G. Essick, C. R. Daubert, M. A. Drake and E. A.
636 Foegeding, *Food Hydrocoll.*, 2012, **29**, 234–245.
- 637 26 M.-A. Peyron, L. Mioche, P. Renon and S. Abouelkaram, *Food Qual. Prefer.*,
638 1996, **7**, 229–237.
- 639 27 W. L. Hylander, 2006.
- 640 28 A. Woda, K. Foster, A. Mishellany and M. A. Peyron, *Physiol. Behav.*, 2006, **89**,
641 28–35.
- 642 29 P. Morell, B. Piqueras-Fiszman, I. Hernando and S. Fiszman, *Food Res. Int.*,
643 2015, **78**, 141–147.
- 644 30 P. Morell, I. Hernando, E. Llorca and S. Fiszman, *Food Res. Int.*, 2015, **70**, 64–
645 73.
- 646 31 C. Schmitt, C. Moitzi, C. Bovay, M. Rouvet, L. Bovetto, L. Donato, M. E. Leser,
647 P. Schurtenberger and A. Stradner, *Soft Matter*, 2010, **6**, 4876.
- 648 32 B. Yoo and M. A. Rao, *J. Food Sci.*, 1995, **60**, 777–779.
- 649 33 B. Yoo, M. A. Rao and J. F. Steffe, *J. Texture Stud.*, 1995, **26**, 1–10.
- 650 34 J. L. Briggs, J. F. Steffe and Z. Ustunol, *J. Dairy Sci.*, 1996, **79**, 527–531.
- 651 35 N. Q. Dzuy and D. V. Boger, *J. Rheol. (N. Y. N. Y.)*, 1985, **29**, 335–347.
- 652 36 C. W. Pernell, E. A. Foegeding and C. R. Daubert, *Food Eng. Phys. Prop.*, 2000,
653 **65**, 110–114.
- 654 37 J. F. Steffe, 1996.
- 655 38 V. Dairou and J.-M. Sieffermann, *J. Food Sci.*, 2002, **67**, 826–834.
- 656 39 F. A. De Santos, *Univ. Illinois Urbana-Champaign*.
- 657 40 H. Inokuchi, M. González-Fernández, K. Matsuo, M. B. Brodsky, M. Yoda, H.
658 Taniguchi, H. Okazaki, T. Hiraoka and J. B. Palmer, *Dysphagia*, 2014, **29**, 713–
659 721.
- 660 41 J. Delarue, in *Novel techniques in sensory characterization and consumer*
661 *profiling*, eds. P. Varela and G. Ares, CRC Press, Boca Ratón, FL, 2014, pp.
662 175–205.
- 663 42 F. Butler and P. McNulty, *J. Food Eng.*, 1995, **25**, 569–580.

- 664 43 I. S. Chronakis, *Crit. Rev. Food Sci. Nutr.*, 1998, **38**, 599–637.
- 665 44 M. Guzmán-González, F. Morais, M. Ramos and L. Amigo, *J. Sci. Food Agric.*,
666 1999, **79**, 1117–1122.
- 667 45 Y. H. Cho, J. A. Lucey and H. Singh, *Int. Dairy J.*, 1999, **9**, 537–545.
- 668 46 P. Tatdao, S. D. M. and S. Frank, *J. Texture Stud.*, 2008, **39**, 617–634.
- 669 47 A. Tárrega and P. Tarancón, in *Novel techniques in sensory characterization*
670 *and consumer profiling*, eds. P. Varela and G. Ares, CRC Press, Boca Ratón,
671 FL, 2014, pp. 157–172.
- 672 48 T. van Vliet, *Food Qual. Prefer.*, 2002, **13**, 227–236.
- 673 49 J. W. Beecher, M. A. Drake, P. J. Luck and E. A. Foegeding, *J. Dairy Sci.*, 2008,
674 **91**, 2553–2560.
- 675 50 P. Morell, J. Chen and S. Fiszman, *Food Funct.*, 2017, **8**, 545–553.
- 676 51 G. Ares, A. Giménez and A. Gámbaro, *Food Qual. Prefer.*, 2008, **19**, 636–643.
- 677 52 R. A. de Wijk, L. J. van Gemert, M. E. J. Terpstra and C. L. Wilkinson, *Food*
678 *Qual. Prefer.*, 2003, **14**, 305–317.
- 679 53 G. Antmann, G. Ares, P. Varela, A. Salvador, B. Coste and S. M. Fiszman, *J.*
680 *Texture Stud.*, 2011, **42**, 50–60.
- 681 54 K. Liu, Y. Tian, M. Stieger, E. van der Linden and F. van de Velde, *Food*
682 *Hydrocoll.*, 2016, **52**, 403–414.
- 683 55 A. Sarkar, F. Kanti, A. Gulotta, B. S. Murray and S. Zhang, *Langmuir*, 2017, **33**,
684 14699–14708.
- 685 56 L. Laguna, G. Farrell, M. Bryant, A. Morina and A. Sarkar, *Food Funct.*, 2017, **8**,
686 563–573.
- 687 57 B. S. Murray and N. Phisarnchananan, *Food Hydrocoll.*, 2016, **56**, 161–169.
- 688 58 I. C. Torres, T. Janhøj, B. Ø. Mikkelsen and R. Ipsen, *Int. Dairy J.*, 2011, **21**,
689 645–655.
- 690 59 C. E. Jørgensen, R. K. Abrahamsen, E.-O. Rukke, T. K. Hoffmann, A.-G.
691 Johansen and S. B. Skeie, *Int. Dairy J.*, In Press.
- 692 60 K. Agrawal, P. Lucas and ... I. B., *J. Dent. Res.*, 1998, **77**, 1931–1938.
- 693 61 S. Ishihara, M. Nakauma, T. Funami, T. Tanaka, K. Nishinari and K. Kohyama,
694 *J. Texture Stud.*, 2011, **42**, 254–267.
- 695 62 H. Koç, E. Çakir, C. J. Vinyard, G. Essick, C. R. Daubert, M. A. Drake, J.
696 Osborne and E. A. Foegeding, *J. Texture Stud.*, 2014, **45**, 47–61.
- 697 63 M. A. Peyron, C. Lassauzay and A. Woda, in *Journal of Dental Research*, 1998,
698 vol. 77, p. 875.
- 699 64 J. F. Prinz and P. W. Lucas, *Arch. Oral Biol.*, 1995, **40**, 401–403.
- 700 65 M. R. Heath and J. F. Prinz, *Food texture. Meas. Percept.*, 1999, 18–29.

- 701 66 K. Hiimeae, *J. Texture Stud.*, 2004, **35**, 171–200.
- 702 67 P. W. Lucas, J. F. Prinz, K. R. Agrawal and I. C. Bruce, *J. Texture Stud.*, 2004,
703 **35**, 159–170.
- 704 68 H. J. Leidy, J. W. Apolzan, R. D. Mattes and W. W. Campbell, *Obesity*, 2010, **18**,
705 293–299.
- 706 69 R. D. Mattes and W. W. Campbell, *J. Am. Diet. Assoc.*, 2009, **109**, 430–437.
- 707 70 R. D. Mattes and R. V. Considine, *Physiol. Behav.*, 2013, **120**, 173–181.
- 708 71 N. Zijlstra, M. Mars, R. A. de Wijk, M. S. Westerterp-Plantenga and C. de Graaf,
709 *Int. J. Obes.*, 2008, **32**, 676–683.
- 710 72 N. Zijlstra, M. Mars, R. A. de Wijk, M. S. Westerterp-Plantenga, J. J. Holst and
711 C. de Graaf, *Physiol. Behav.*, 2009, **97**, 68–75.
- 712 73 C. Mosca, C. De Graaf, J. A. McEwan and M. Stieger, in *Proceedings of 5th*
713 *International Conference on Food Oral Processing*, The University of
714 Nottingham, Nottingham, 2018, p. 62.
- 715 74 M. G. Aguayo-Mendoza, E. C. Ketel, E. van der Linden, C. G. Forde, B.
716 Piqueras-Fiszman and M. Stieger, *Food Qual. Prefer.*, 2019, **71**, 87–95.
- 717 75 D. Braxton, C. Dauchel and W. E. Brown, *Food Qual. Prefer.*, 1996, **7**, 217–223.
- 718 76 W. E. Brown and D. Braxton, *Food Qual. Prefer.*, 2000, **11**, 259–267.
- 719 77 M. Devezeaux de Lavergne, J. A. M. Derks, E. C. Ketel, R. A. de Wijk and M.
720 Stieger, *Food Qual. Prefer.*, 2015, **41**, 189–200.
- 721 78 E. Neyraud, M. A. Peyron, C. Vieira and E. Dransfield, *J. Dent. Res.*, 2005, **84**,
722 250–254.
- 723
- 724

725 Table 1. Composition per 100 g before fermentation of each sample.

726

Sample*	Composition per 100 g before fermentation			Total protein content (g)
	SMP** (g)	Lactic culture (mL)	Extra-ingredient (g)	
C	9.1	0.4	-	3.0
MP	16.6	0.3	-	5.5
MPMD	16.3	0.3	2.0 g maltodextrin	5.4
MPF	15	0.3	9.9 g apple cubes	5.0
WPM	8.8	0.4	3.3 g whey protein microgel	6.1
WPI	8.8	0.4	3.4 g whey protein isolate	6.1

727 *All samples were completed to 100g with water. C: control; MP: double skimmed milk powder;
 728 MPF: double skimmed milk powder and apple cubes; MPMD: double skimmed milk powder and
 729 2% of maltodextrin; WPI: whey protein isolate added; WPM: whey protein microgels added.

730 **SMP: skimmed milk powder.

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732

733 Table 2. Mastication parameters extracted from oral processing analysis and their
734 definitions.
735

Technique and parameter	Definition
EMG	The values were determined by the sum of EMG activities from first bite to swallow. Mean values were calculated across all subjects and replicates for each yogurt sample.
<i>Eating time (s)</i>	Total duration of the chewing sequence
<i>Peak activity</i>	Muscle peak activity values were calculated separately for right (R) and left (L) AT, MM and DA muscles as well as their total (R + L) across chewing sequence
<i>Total activity</i>	Muscle total activity values were calculated separately for right (R) and left (L) AT, MM and DA muscles as well as their total (R + L) across chewing sequence divided by number of cycles
JT-3D	Jaw movements measurements were reported starting from placement of the sample in the mouth until the final swallow and are means across all subjects and replicates for each sample treatment
<i>Number of chews</i>	Number of chewing cycles from ingestion until terminal swallow clearly identifiable
<i>Range of movements (mm)</i>	The total range of vertical, anteroposterior and lateral movements of the jaw during a chewing cycle
<i>Total movement (mm)</i>	The total of vertical, anteroposterior and lateral movements of the jaw during a chewing cycle
<i>Maximum velocity (mm/s)</i>	Maximum jaw velocities in vertical, anterior posterior and lateral dimensions
<i>Average velocity (mm/s)</i>	Average jaw velocities in vertical, anterior posterior and lateral dimensions

736
737

Table 3. Yield stress, hysteresis loop area and flow behavior parameter values for samples of yogurt.

Sample	σ_0^* (Pa)	Flow behavior parameter			Hysteresis loop area* (W/m ³)
		K (Pa·s)	n	η_{50} (Pa·s)	
C	166 ^c (7)	35.1 ^b	0.17 ^c	1.35 ^b	4798 ^a
MP	494 ^a (7)	55.7 ^a	0.26 ^b	3.06 ^a	4535 ^a
MPMD	448 ^b (5)	17.1 ^c	0.39 ^a	1.52 ^b	882 ^b
WPI	77 ^d (5)	9.2 ^c	0.30 ^b	0.59 ^c	584 ^b
<i>Standard error</i>		2.0	0.02	0.16	109

Different letters in the same column indicate statistically significant differences ($p < 0.05$).

*Values between parentheses are the standard error.

C: control; MP: double skimmed milk powder; MPMD: double skimmed milk powder and 2% of maltodextrin; WPI: whey protein isolate added.

745 Table 4. Facial jaw-muscle activity parameters during consumption of test foods.
746

Eating parameter	Sample					
	C	MP	MPF	MPMD	WPI	WPM
Eating time (s)	3.5 ^c	6.9 ^b	11 ^a	5.9 ^b	4.1 ^c	3.9 ^c
Peak muscle activity*						
TA	0.22 ^d	0.32 ^{bc}	0.67 ^a	0.34 ^b	0.22 ^{cd}	0.26 ^{bcd}
MM	0.20 ^d	0.31 ^b	0.70 ^a	0.30 ^{bc}	0.22 ^{cd}	0.27 ^{bcd}
DA	0.51 ^d	0.56 ^c	0.74 ^a	0.61 ^{bc}	0.48 ^d	0.63 ^b
Total muscle activity*						
TA	0.16 ^d	0.32 ^b	0.85 ^a	0.29 ^{bc}	0.18 ^d	0.21 ^{cd}
MM	0.14 ^d	0.31 ^b	0.89 ^a	0.31 ^b	0.17 ^{cd}	0.23 ^c
DA	0.28 ^d	0.53 ^b	0.90 ^a	0.50 ^b	0.31 ^d	0.42 ^c

747 Different letters in the same row indicate statistically significant differences (p < 0.05).
748 *MM: masseter muscles; AT: anterior temporalis; AD: anterior digastric.
749 C: control; MP: double skimmed milk powder; MPF: double skimmed milk powder and apple
750 cubes; MPMD: double skimmed milk powder and 2% of maltodextrin; WPI: whey protein isolate
751 added; WPM: whey protein microgels added.
752
753

Table 5. Average jaw movements during consumption of test foods.

Eating parameter	Sample					
	C	MP	MPF	MPMD	WPI	WPM
Number of chews	3.7 ^c	7.0 ^b	12 ^a	6.5 ^b	3.9 ^c	4.2 ^c
Range of movement (mm)						
<i>Vertical</i>	5.6 ^d	6.9 ^{bc}	8.4 ^a	7.4 ^b	6.0 ^{cd}	6.7 ^{bc}
<i>Anterior-posterior</i>	5.0 ^c	6.5 ^{ab}	7.3 ^a	6.8 ^{ab}	5.1 ^c	5.8 ^{bc}
<i>Lateral</i>	3.1 ^c	5.8 ^b	11 ^a	6.7 ^b	3.3 ^c	4.3 ^c
Total movement (mm)						
<i>Vertical</i>	32 ^c	72 ^b	147 ^a	68 ^b	39 ^c	45 ^c
<i>Anterior-posterior</i>	26 ^c	55 ^b	97 ^a	48 ^b	31 ^c	33 ^c
<i>Lateral</i>	17 ^c	41 ^b	118 ^a	41 ^b	19 ^c	24 ^c
Maximum velocity (mm/s)						
<i>Vertical</i>	3.8 ^b	5.1 ^b	8.5 ^a	5.1 ^b	4.1 ^b	4.6 ^b
<i>Anterior-posterior</i>	3.5 ^d	4.4 ^{bc}	6.5 ^a	4.9 ^b	3.7 ^{cd}	3.9 ^{cd}
<i>Lateral</i>	2.6 ^c	4.4 ^b	7.7 ^a	4.8 ^b	2.8 ^c	3.2 ^c
Average velocity (mm/s)						
<i>Vertical</i>	0.79 ^c	1.00 ^{bc}	1.53 ^a	1.06 ^b	0.82 ^{bc}	0.90 ^{bc}
<i>Anterior-posterior</i>	0.62 ^d	0.78 ^{bc}	1.06 ^a	0.82 ^b	0.65 ^{cd}	0.67 ^{bcd}
<i>Lateral</i>	0.41 ^c	0.60 ^b	1.19 ^a	0.66 ^b	0.41 ^c	0.47 ^c

Different letters in the same row indicate statistically significant differences ($p < 0.05$).

C: control; MP: double skimmed milk powder; MPF: double skimmed milk powder and apple cubes; MPMD: double skimmed milk powder and 2% of maltodextrin; WPI: whey protein isolate added; WPM: whey protein microgels added.

761 **Figure captions**

762

763 Figure 1. Flow curves of the different yogurts. *C: control; MP: double skimmed milk
764 powder; WPI: whey protein isolate added; MPMD: double skimmed milk powder and
765 2% of maltodextrin. Blue diamonds (first upward curve), orange squares (downward
766 curve) and gray triangles (second upward curve).

767

768 Figure 2. Viscosity profiles of the different samples along the pseudoplastic zone
769 values.

770

771 Figure 3. Average sensory space generated by MFA from Flash Profile data of yogurt
772 samples (C: control; MP: double skimmed milk powder; MPF: double skimmed milk
773 powder and apple cubes; MPMD: double skimmed milk powder and 2% of
774 maltodextrin; WPI: whey protein isolate added; WPM: whey protein microgels added).
775 (A) First and second dimensions and (B) the first and third dimensions. Descriptors
776 correlating ($r > 0.6$ or $r < 0.6$) with each of the dimensions are listed in the text boxes; the
777 number of times the descriptor appeared is shown in brackets.

778

779 Figure 4. PCAs for normalized (A) electromyography and (B) jaw tracking parameters
780 obtaining during consumption of yogurt samples. *C: control; MP: double skimmed milk
781 powder; MPF: double skimmed milk powder and apple cubes; MPMD: double skimmed
782 milk powder and 2% of maltodextrin; WPI: whey protein isolate added; WPM: whey
783 protein microgels added. Identification of parameters on tables 5 and 6 respectively.

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171x106mm (300 x 300 DPI)

Figure 2. Viscosity profiles of the different samples along the pseudoplastic zone values.

82x55mm (300 x 300 DPI)

171x68mm (300 x 300 DPI)

Figure 4. PCAs for normalized (A) electromyography and (B) jaw tracking parameters obtaining during consumption of yogurt samples. *C: control; MP: double skimmed milk powder; MPF: double skimmed milk powder and apple pieces; MPMD: double skimmed milk powder and 2% of maltodextrin; WPI: whey protein isolate added; WPM: whey protein microgels added. Identification of parameters on tables 5 and 6 respectively.

171x73mm (300 x 300 DPI)