

## Accepted Manuscript

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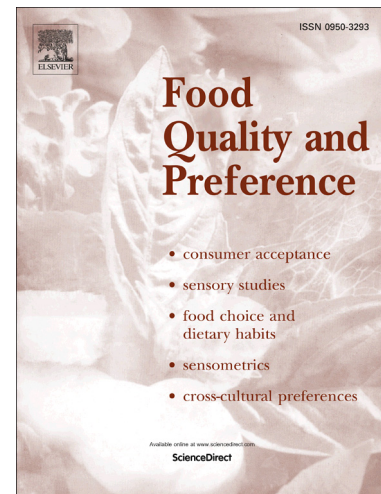
PII: S0950-3293(17)30095-2  
DOI: <http://dx.doi.org/10.1016/j.foodqual.2017.04.011>  
Reference: FQAP 3312

To appear in: *Food Quality and Preference*

Received Date: 20 December 2016  
Revised Date: 23 March 2017  
Accepted Date: 26 April 2017

Please cite this article as: Pentikäinen, S., Sozer, N., Närväinen, J., Sipilä, K., Ariful Alam, S., Heiniö, R-L., Paananen, J., Poutanen, K., Kolehmainen, M., Do rye product structure, product perceptions and oral processing modulate satiety?, *Food Quality and Preference* (2017), doi: <http://dx.doi.org/10.1016/j.foodqual.2017.04.011>

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*Do rye product structure, product perceptions and oral processing modulate satiety?*

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**Abstract**

Food structure and cephalic phase factors are hypothesized to contribute to postprandial satiety in addition to established food properties such as energy content, energy density, and macronutrient and fibre composition of a preload. This study aimed to evaluate if the structure of rye products has an impact on subjective feelings of satiety, and whether cephalic phase factors including oral processing, satiety expectations and perceived pleasantness modulate the interaction. Four wholegrain rye based samples (extruded flakes and puffs, bread and smoothie) were studied in terms of texture characteristics, *in vivo* oral processing, and expected satiety (n=26) and satiety as well as perceived pleasantness (n=16) (ClinicalTrials.gov number: NCT02554162). The vast textural differences between products were reflected in mastication process, perceived pleasantness and satiety expectations. Extruded products required the most intensive mastication. Rye puffs and rye bread which were characterized by a solid and porous structure, and showed better satiety effect in the early postprandial phase compared to other products. Mastication effort interacted with satiety response. However, the products requiring the highest mastication effort were not the most satiating ones. It seems that there are some food structure related mechanisms that influence both mastication process and postprandial satiety, the mastication process itself not being the mediating factor. Higher palatability seems to weaken postprandial satiety response.

**Keywords:**

satiety; cross-over; postprandial; food structure; texture; oral processing

## 1 1 Introduction

2 The feeling of satiety has been proposed to support weight management through various routes such  
3 as greater food reward, reduced hunger and better control of energy intake (Hetherington et al.,  
4 2013). For instance, the amount and type of dietary fibre in food, macronutrient composition and  
5 energy density of food contribute to the modulation of satiety. In addition, cognitive and sensory  
6 signals generated before and during eating (cephalic phase) are proposed to influence satiation  
7 (intra-meal satiety) and satiety (inter-meal satiety) (Blundell et al., 2010). Cephalic phase responses  
8 such as stimulation of hormone and enzyme secretion are hypothesized to enhance nutrient  
9 processing and thus to enhance also satiety response (Smeets, Erkner, & De Graaf, 2010).

10 Signals that are generated already during oral processing are needed for optimal appetite regulation,  
11 in addition to signals originating from later phases of digestion (Smeets et al., 2010). The  
12 importance of oral phase for appetite regulation has been well established in studies where appetite  
13 suppression has been incomplete after infusing food directly to stomach. Hogenkamp and Schiöth  
14 recently reviewed studies on oral processing of food, satiation and satiety, and concluded that  
15 viscosity of food had consistent impact on *ad libitum* food intake (satiation) and that orosensory  
16 exposure was the mediating factor between viscosity and satiation (Hogenkamp & Schiöth, 2013).  
17 Later, Bolhuis et al. showed that hard foods which were eaten in smaller bites than soft foods and  
18 processed longer in mouth, reduced the energy intake during the meal, and that the effect was  
19 sustained over the following meal (Bolhuis et al., 2014). They concluded that the differences in oral  
20 processing might mediate this effect. Mastication process has also shown to suppress gastric  
21 emptying rate (Ohmure et al., 2012).

22 The effects of preload texture and resulting oral processing on postprandial satiety have been  
23 investigated in several studies. Energy intake at next meal context is adjusted only partly after a

24 liquid preload while it is fully adjusted after semi-solid or solid preload (Almiron-Roig et al., 2013).  
25 This leads to lower overall caloric intake (preload and *ad libitum* meal) after semi-solid or solid  
26 preloads compared to liquid preload. This indicates that food texture, at least when liquids are  
27 compared to solids or semi-solids, plays a role not only in satiation but also in satiety response.  
28 However, the results concerning food textures other than liquids, resulting in varying orosensory  
29 exposure, are somewhat inconsistent (Hogenkamp & Schiöth, 2013). Satiety effect of foods with  
30 either solid or heterogeneous texture, assumed to induce high orosensory exposure, or  
31 corresponding comminuted texture, assumed to induce low orosensory exposure, have been  
32 compared by various groups: Mattes et al. found that there were no differences in satiety responses  
33 between solid and semi-solid foods (apple vs. apple soup, peanut vs. peanut soup or chicken vs.  
34 chicken soup) (Mattes, 2005) whereas later (Flood-Obbagy & Rolls, 2009) a whole apple was  
35 concluded to induce satiety more than apple sauce and the whole apple also reduced energy intake  
36 in the following meal. Martens et al. showed that solid food (steamed chicken breast) resulted in  
37 enhanced satiety response compared to liquefied food (blended steamed chicken breast) (Martens,  
38 Lemmens, Born, & Westerterp-Plantenga, 2011) whereas Flood and Rolls showed that there was no  
39 difference in satiety response whether soup was offered as separate broth and vegetables versus  
40 pureed soup (Flood & Rolls, 2007). Also heterogeneous and homogeneous yoghurts resulted in  
41 similar satiety response (Tsuchiya, Almiron-Roig, Lluch, Guyonnet, & Drewnowski, 2006). To  
42 summarize, the evidence regarding the importance of food texture and oral processing on satiety is  
43 inconsistent. Most of the studies do not report oral processing precisely. The influence of oral  
44 processing on appetite has been studied also in experimental settings where the same foods have  
45 been eaten varying the number of chews or mastication time as instructed by the researchers. The  
46 results of such studies have been inconsistent: some reports indicate that increasing number of  
47 chews or mastication time improves satiety but others show no connection (Hogenkamp & Schiöth,  
48 2013).

49 Sensory characteristics of foods such as chewiness and saltiness (Forde, van Kuijk, Thaler, de  
50 Graaf, & Martin, 2013), anticipated creaminess (McCrickerd, Lensing, & Yeomans, 2015) and  
51 thickness and creaminess (Yeomans & Chambers, 2011) have been found to influence on expected  
52 satiety. Even expectations about the satiating capacity of foods evoked by visual and other sensory  
53 perceptible cues have shown to influence the actual satiety response: In the study of Brunstrom et al  
54 participants were shown either a large or a small portion of fruits prior to consuming an equal size  
55 fruit smoothie (Brunstrom, Brown, Hinton, Rogers, & Fay, 2011). The participants who saw the  
56 larger fruit portion reported higher expectations of satiety and in fact also experienced enhanced  
57 satiety for three hours. Liking of food has also been repeatedly shown to influence appetite reflected  
58 as an increased intake as palatability increases (Sørensen, Møller, Flint, Martens, & Raben, 2003).  
59 However, results concerning the importance of palatability on postprandial satiety remain  
60 inconclusive. To summarize, cephalic phase factors including oral processing, perception about  
61 pleasantness of food as well as expectations about its satiating capacity may all work together to  
62 modulate the satiety response.

63 The current study aimed to evaluate if the structure of rye products influences subjective feelings of  
64 satiety, and if cephalic phase factors including oral processing, satiety expectations and evaluated  
65 pleasantness are mediating the interaction. The use of rye products as model foods allowed the  
66 comparison of extreme food structures with only minor differences in chemical composition.

67

## 68 2 Materials and Methods

### 69 2.1 Products and their nutrient contents

70 The test foods were wholegrain rye products representing varying structures; wholegrain sourdough  
71 rye bread, extruded wholegrain rye flakes, extruded wholegrain rye puffs and wholegrain rye  
72 smoothie (Table 1 and Figure 1). Wheat bread was included as a control product. Wholegrain  
73 sourdough rye bread (wholegrain rye flour, water, salt) and refined wheat bread (wheat flour, water,  
74 yeast, sugar, rapeseed oil, salt) were commercially available products by local bakery (Emil  
75 Halme). Wholegrain rye puffs and flakes were prepared at VTT using whole grain rye flour (Oy  
76 Karl Fazer AB/Fazer Mills and Mixes, Lahti, Finland) and salt (0.8%) as ingredients. A twin screw  
77 extruder (APV MPF 19/25, Baker Perkins Group Ltd, Peterborough, UK) was used to produce the  
78 extrudates with a constant feed rate of 60 g/min and temperature profile of 80-95-110-120 °C  
79 (section 1 to die exit) with the screw speed of 350 and 250 rpm for puffs and flakes, respectively.  
80 Water was pumped into the extruder barrel in order to obtain desired moisture contents in the  
81 extrudates. Extruded products were collected continuously from the exit die (diameter 3 mm) and  
82 dried immediately in an oven at 100 °C, 30 min for puffs and 90 min for flakes. Wholegrain rye  
83 smoothie was prepared mixing grinded wholegrain rye flakes with blackcurrant juice and letting the  
84 mixture stand for 15 minutes resulting in a thick smoothie-like heterogeneous texture. Blackcurrant  
85 juice was a commercial product (Marli).

### 86 2.1.1 Instrumental texture

87 Texture profile analysis was used to extract the primary and secondary mechanical characteristics of  
88 breads by using a texture analyser (TA-XT plus Texture Analyser, Stable Micro System,  
89 Godalming, Surrey, UK) with a 25-mm diameter cylinder probe (P/25L Lap Perspex), 30-kg load  
90 cell, 60% strain on 25-mm thick cylindrical pieces of breads which were cut by the help of a mould.  
91 Upper crust was included in the pieces. The acquisition rate was 200 points/s and the test speed was  
92 1.7 mm/s. TPA software (Exponent v.6, Stable Micro System, Godalming, Surrey, UK) was used to  
93 extract force-deformation curve. Hardness, cohesiveness, chewiness, and adhesiveness were  
94 calculated based on force-deformation curve.

95 Textural properties of extruded puffs and flakes were analyzed by the uniaxial compression test  
96 using a texture analyser (Texture Analyser TA-HDi, HD3071, Stable Micro Systems, United  
97 Kingdom) equipped with a 250 kg load cell and a cylindrical 36 mm aluminium probe using a  
98 protocol used by Alam et al. (Alam et al., 2014). Snack samples were prepared by cutting the  
99 extruded ribbons to 10 mm height and flakes were analysed as is. The samples (50 replicates for  
100 each samples) were deformed at 70% strain with a test speed of 1 mm/s and the acquisition rate 200  
101 points/s. Texture Exponent software v.5.1.2.0 (Stable Micro Systems, UK) was used to obtain  
102 values of hardness ( $F_{max}$ ), crispiness work ( $C_w$ ) and crispiness index ( $C_i$ ). High crispiness is  
103 accompanied by a high  $C_i$  and low  $C_w$  value, whereas low crispiness corresponds to a low  $C_i$  and  
104 high  $C_w$  value. The analysis was performed using the algorithms described by Alam et al. (Alam et  
105 al., 2014).

### 106 2.1.2 Perceived characteristics

107 All assessors of VTT's internal trained sensory panel (n=12) have passed the basic taste test, the  
108 odour test and the colour vision test and trained for sensory profiling. The trained sensory panel was



109 first familiarized with the sensory assessment of diverse cereal samples. The method in sensory  
110 profiling was descriptive analysis (Lawless & Heymann, 2010). The vocabulary of the sensory  
111 attributes was developed by describing the differences between the samples. The assessors  
112 familiarized themselves with the products, discussed and defined the key attributes differentiating  
113 the products in a training session aiming to produce the descriptors for the sensory profile. The  
114 selected attributes included *colour darkness, rye flavour intensity, flavour intensity, visual porosity,*  
115 *hardness, crispiness, crunchiness, crumbliness, moisture, adhesion to teeth and work needed for*  
116 *mastication*. In sensory profiling the latter was evaluated according to the instructions: “Masticate  
117 the sample using your back teeth until the sample is ready to be swallowed. After that, please  
118 evaluate how much work was needed for mastication”. Actual reference samples were used to  
119 define the extremes for most of the attributes, and all descriptors were also verbally anchored. All  
120 sensory intensities were evaluated using 10 cm scale anchored from “not at all” to “extremely”. All  
121 samples were evaluated by sensory profiling in duplicate sessions in two consecutive days by all the  
122 panellists. The samples were blind-coded by 3-digit numbers, and the presentation order of the  
123 samples was randomized within each test day. Water was served to the assessors for cleaning the  
124 palate between the different samples. The scores were recorded and collected using computerized  
125 software (Compusense Five, Ver 5.4.15, CSA, Computerized Sensory Analysis System,  
126 Compusense Inc., Guelph, ON, Canada).

## 127 2.2 Participants

128 Participants (n=26) were recruited through public advertisements and email advertisements in  
129 Otaniemi campus area nearby the study location. The eligibility of the volunteers was checked  
130 beforehand through screening questionnaire. The criteria were: female gender, age 20-40 years,  
131 BMI between 18.5 and 27 kg/m<sup>2</sup>, stable body weight ( $\pm$  4 kg during the previous year) and a habit  
132 of eating breakfast. Smokers, pregnant or lactating women, persons with missing teeth (except 3<sup>rd</sup>

133 molars) or with diagnosed acute temporomandibular disorders (TMD) (self-reported) and persons  
134 with dietary restrictions possibly affecting the study participation (celiac disease, allergies or  
135 aversions to cereal foods or high carbohydrate foods) or abnormal eating behaviour according  
136 Eating Disorder Diagnostic Scale (EDDS) were excluded. Young healthy females were recruited to  
137 diminish the variation in mastication pattern. The interested volunteers fulfilling the inclusion  
138 criteria were invited to an info visit. Volunteers deciding to participate signed an informed consent  
139 form. The whole study population (n=26) participated in mastication trial and a subgroup of 20  
140 participants started the satiety trial. The both trials were conducted during October-December 2015.  
141 Sixteen of these participants completed all the study visits and four discontinued due to personal  
142 reasons. Characteristics of the participants are described in Table 2. Two participants were older  
143 than 40 years (48 and 50 years). However, since they fulfilled all the other inclusion criteria they  
144 were included in the study, as the number of recruited participants was not as high as desired. The  
145 participants were given one movie ticket per study visit to compensate their time and effort. The  
146 study protocol was approved by the Coordinating Research Ethics Committee of the Helsinki and  
147 Uusimaa Hospital District. The study was conducted according to the ethical principles of good  
148 research and clinical practice described in the declaration of Helsinki. The trial was registered in  
149 ClinicalTrials.gov (NCT02554162).

## 150 2.3 Mastication trial

### 151 2.3.1 Procedure

152 The mastication trial followed a cross-over, single-blind design, in which all participants masticated  
153 the five samples in random order. The participants were instructed to eat a breakfast 1 - 1.5 hours  
154 before the visit scheduled between 8 - 11 a.m. The study procedure was first practiced with a test  
155 sample and the coded food samples were served to the participant in random order, each sample in  
156 three portions. Portion sizes represented a mouthful of food: 2 x 2 x 2 cm-size cube of bread

157 (including crust in one side) (approx. 7.7 g), one table spoon of flakes (3.5 g), two 2 cm pieces of  
158 puffs (1 g) and one table spoon of rye smoothie (16.8 g). The participants were instructed to  
159 masticate each portion of sample until subjective swallowing point and then expectorate the bolus.  
160 The three portions of each sample were masticated in a row and there was break between different  
161 samples during which mouth was rinsed with water and the expected satiety rating for each sample  
162 was evaluated. As a final sample, the participant was served three portions (=piece) of chewing gum  
163 and she was asked to chew each piece for 20 seconds. Electromyography measures electrical  
164 activity of the facial muscles and even if the measured voltage is linearly relative to the force  
165 generated by the muscle, the calibration varies between different subjects and even the four muscles  
166 monitored. Thus, to get an indication of the relative force needed to masticate each of the samples  
167 individual data on oral processing of chewing gum was used as a reference for force parameters.  
168 The mastication trial visits were video recorded to support data analysis.

### 169 2.3.2 Electromyography (EMG) measurements

170 The mastication process was characterised by measuring the electrical activity of masticatory  
171 muscles by EMG equipment (Mega Electronics, Kuopio, Finland) using disposable dermal  
172 Ag/AgCl electrodes. The skin was cleaned with 70 % ethanol alcohol, masseter and temporal  
173 muscles were identified by touch when the participant gritted her teeth and bipolar electrodes were  
174 placed on them on both sides of the face. A reference electrode was placed on cervical vertebra.  
175 EMG activity was measured continuously throughout the whole mastication trial. The data block  
176 starts and ends for each chewing period were both marked in the EMG acquisition system (Figure  
177 2A) and recorded manually. From the EMG time series, the onset, duration and amplitude of each  
178 chew were extracted by applying chemometric techniques for the elimination of high frequencies  
179 and background fluctuations as in the study of Pentikäinen et al. (Pentikäinen, Sozer, et al., 2014)  
180 (Figure 2B). Chewing force and work parameters were normalized to chewing process of chewing

181 gum. As a result of data processing and analyses, the duration of oral processing, duration of EMG  
182 activity, duty cycle (duration of EMG activity/duration of chewing), number of chews, relative  
183 chewing force (highest EMG amplitude for the product normalized to highest EMG amplitude for  
184 chewing gum) and relative work (time of EMG activity x relative chewing force) were calculated  
185 for each test food. All analysis of EMG data was done using Matlab® (The MathWorks Inc.,  
186 Natick, MA, USA). The values for duration of EMG activity, duration of oral processing, number of  
187 chews and relative work were extrapolated to represent the amount served later in the satiety trial.  
188 The coefficients were determined by dividing the weight of the whole portion served in the satiety  
189 trial by the weight of one mouthful of food used in mastication trial. Coefficients for rye bread, rye  
190 smoothie, rye puffs, rye flakes and wheat bread were 12.4; 32.8; 58; 16.9 and 19.2, respectively.

### 191 2.3.3 Expected satiety

192 The participant was asked to evaluate the satiating capacity of the samples before and after  
193 mastication of each study product. This part was included in order to find out whether food  
194 structure evaluated based on visual cue (picture) or with both visual and sensory cues (mastication)  
195 influences anticipated satiety effect. The evaluation was based on a photograph showing a portion  
196 including a fixed amount of sample and a glass of juice. The portions in photographs were the same  
197 size as the portions that were later used in the satiety trial. The questions, as translated from Finnish  
198 were: (before mastication) “Imagine that you would eat the whole portion of food shown in the  
199 photograph. Evaluate how satiated you would feel after one hour.” and (after mastication) “You  
200 have just masticated the product shown in the photograph. Imagine that you would eat the whole  
201 portion of food shown in the photograph. Evaluate how satiated you would feel after one hour”. The  
202 evaluation was done on 10 cm visual analogue scale (VAS) anchored with 0=not at all satiated,  
203 10=extremely satiated.

## 204 2.4 Satiety trial

205 The satiety trial followed a cross-over, single-blind design, in which all participants tested the five  
206 study portions in random order, each portion on a separate day. There were at least two washout  
207 days between two consecutive study visits. The participants were instructed to follow their usual  
208 eating and exercise habits during the day preceding each study visit and to fast at least 10 hours  
209 before arriving to the study visit.

210 The study visits started in the morning between 7 and 9 a.m. The test portion sizes were matched by  
211 energy content each portion providing 380 kcal of energy (Table 1). The portions consisted of  
212 blackcurrant juice (5 dl) and of either 95 g of wholegrain (WG) sourdough rye bread, 59 g of WG  
213 rye flakes, 58 g of WG rye puffs or 75 g refined wheat bread. WG rye smoothie was prepared by  
214 mixing 59 g of grinded rye flakes in 5 dl blackcurrant juice. The participants were instructed to eat  
215 and drink the test products at their own pace but not to spend more than 20 min on eating. Satiety  
216 related sensations were evaluated before and right after consuming the test portion and repetitively  
217 every 30 min until 210 min after starting point of the consumption using 10 cm visual analogue  
218 scales (VAS) anchored with extremes (0=not at all, 10=extremely). The evaluated sensations were  
219 *hunger, fullness, satiety, desire to eat* and *prospective food consumption* (“How much would you be  
220 able to eat right now?”). In addition, *pleasantness* of the test portion was evaluated after consuming  
221 the portion. Average appetite score was afterwards calculated as [desire to eat + hunger + (10-  
222 fullness) + prospective food consumption]/4. Computerised data-collecting system (CSA,  
223 Computerised Sensory Analysis System, Compusense, Guelph, Canada, Compusense five 5.2) was  
224 used to collect the evaluations.

225 2.5 Statistical analyses

226 IBM SPSS Statistics 22 was used to analyse the data.

227 Oneway ANOVA was used to study the sensory differences of study products. Pair-wise  
228 comparison was conducted by using Tukey's test. Repeated measures ANOVA was used to study  
229 the differences in satiety expectations and pleasantness evaluations. Friedman's non-parametric test  
230 for related samples was used to compare the parameters describing mastication process. P-value  
231  $<0.05$  was considered as statistically significant.

232 Regarding the satiety evaluations, baseline value of each visual analogue scale parameter was  
233 subtracted from the values of subsequent time points to take into account the possible effect of  
234 baseline differences on the analysis. Linear mixed-effects models were used to compare the effects  
235 of the test portions on the profiles of postprandial satiety responses. The used models included  
236 participant as a random factor, and product, time, and product \* time interaction as fixed factors.  
237 When a significant main effect of a product or product \* time interaction was observed, post hoc  
238 analyses were performed using the Sidak correction for multiple comparisons in order to identify  
239 the statistically significant differences between the test portions. The contribution of cephalic phase  
240 factors was evaluated by adding parameters of oral processing, evaluated pleasantness and satiety  
241 expectations to the model as fixed factors one at a time and Schwarz's Bayesian Criterion (BIC)  
242 was then used to compare goodness of fit between the models. The smaller the BIC value is the  
243 better the model fit is.

## 244 3 Results

### 245 3.1 Characteristics of study products

#### 246 3.1.1 Instrumental texture

247 Instrumental texture of the solid products was measured using a texture analyser. The extrudates  
248 were dry products with hard and fragile texture whereas breads were springy and moist (Table 3).  
249 Rye flakes had the hardest texture and wheat bread the least hard. Hardness of rye puffs and rye  
250 bread was similar whereas they had otherwise different textural properties rye puffs being crispy  
251 and rye bread being springy. Rye bread was less cohesive, more chewy and adhesive than wheat  
252 bread. Puffs were crispier than flakes, indicated by higher crispiness index and lower crispiness  
253 work.

#### 254 3.1.2 Perceived characteristics

255 The sensory characteristics of the samples were evaluated by a trained sensory panel. The products  
256 varied significantly in all the evaluated sensory attributes ( $p < 0.001$  for all) (Figure 3) as was  
257 intended. Rye flakes and rye bread were evaluated to require more work for mastication than the  
258 other products (rye flakes vs. rye puffs, smoothie and wheat bread  $p < 0.001$ ; rye bread vs. rye puffs  
259 and smoothie  $p < 0.001$ , rye bread vs. wheat bread  $p = 0.004$ ). Rye puffs adhered to teeth more than  
260 the flakes, breads or smoothie ( $p < 0.001$  for all). Rye flakes and puffs were crumblier, crunchier and  
261 crispier compared to the other products ( $p < 0.001$  for all). Rye flakes were crunchier than rye puffs  
262 ( $p = 0.15$ ) and rye puffs were crispier than rye flakes ( $p < 0.001$ ). Rye flakes were harder than the  
263 other products ( $p < 0.001$  for all) and rye bread was harder than wheat bread ( $p = 0.009$ ). Rye puffs  
264 and both breads were more porous than rye flakes or smoothie ( $p < 0.001$ ). Both overall flavour and  
265 rye flavour were more intense in rye bread than in other products ( $p < 0.001$  for all).

### 266 3.1.3 Expected satiety and evaluated pleasantness

267 The participants of the mastication trial (n=26) evaluated the expected satiating capacity of the  
268 products before and after masticating them. The evaluation was based on picture representing  
269 isocaloric portions of the products. The satiety expectations differed significantly between the  
270 products ( $p<0.001$  for both before and after mastication) (Figure 4A). The portion containing  
271 wholegrain sourdough rye bread was evaluated to be more satiating than the other portions both  
272 before mastication (rye bread vs. rye flakes, smoothie and wheat bread  $p<0.001$ ; rye bread vs. rye  
273 puffs  $p=0.031$ ) and after mastication ( $p<0.001$  for all) whereas wholegrain rye smoothie portion was  
274 evaluated as less satiating than the other portions before mastication ( $p<0.001$  for all) and less  
275 satiating than rye bread and rye flakes ( $p<0.001$  for both) and wheat bread ( $p=0.005$ ) after  
276 mastication. Expected satiety effects of rye bread, rye flakes and rye smoothie were evaluated  
277 higher after than before mastication ( $p=0.001$ ,  $p<0.001$ , and  $p<0.001$ , respectively). There were no  
278 differences in the evaluations before and after mastication of rye puffs or wheat bread. The  
279 participants of the satiety trial (n=16) evaluated the pleasantness of the consumed portions. There  
280 were significant differences in the ratings of pleasantness between the portions ( $p<0.001$ ) (Figure  
281 4B). The rye bread portion was evaluated as more pleasant than the other portions (rye bread vs.  
282 smoothie  $p=0.002$ ; vs. rye puffs  $p<0.001$ ; vs. wheat bread  $p=0.011$ ; vs. rye flakes  $p=0.005$ ) and  
283 extruded rye puff portion was evaluated less pleasant than rye bread ( $p<0.001$ ), wheat bread  
284 ( $p=0.001$ ) and rye flake portion ( $p=0.006$ ).

### 285 3.2 Mastication properties

286 Mastication was characterized by monitoring the electrical activity of facial muscles during  
287 masticating mouthful of sample. There were significant differences between products in all the  
288 measured oral processing attributes: number of chews, total oral processing time, total EMG  
289 activity time, duty cycle, relative force and relative work ( $p<0.001$  for all). Table 4 shows the  
290 values for the parameters and the results of pairwise comparisons. Total oral processing time, total



291 EMG activity time and relative work for mouthful of sample were the highest for rye bread and rye  
292 flakes and the lowest for puffs and smoothie. The number of chews was the highest for mouthful of  
293 rye flakes and the lowest for puffs and smoothie. It should be noted, however, that for smoothie the  
294 events detected as chews are mostly other muscle motions than actual chewing.

295 When the measured oral processing attributes were extrapolated to represent the process of chewing  
296 the whole portion of the product (as amount served in the satiety trial) there were also statistically  
297 significant differences between products in all the attributes ( $p < 0.001$ ). Total oral processing time,  
298 EMG activity time and relative work per portion were the highest for flakes and puffs and the  
299 lowest for smoothie. Number of chews per portion was higher for flakes, puffs and wheat bread  
300 than for rye bread or rye smoothie.

### 301 3.3 Postprandial satiety responses to food portions

302 Portions of the test products were served to subgroup of 16 participants in the satiety trial. Each  
303 portion was served in separate day. The mean VAS ratings for hunger, fullness, desire to eat,  
304 prospective food consumption, satiety and average appetite score for the 210 min period are  
305 presented in Figure 5. Hunger (Figure 5A) was significantly lower and fullness (Figure 5B) higher  
306 at 30 min after consumption of puff portion compared to flake portion ( $p < 0.012$  and  $p < 0.028$ ,  
307 respectively) whereas there were no statistically significant differences between other portions.  
308 Desire to eat (Figure 5C) was significantly higher at 60 min after consumption of flake portion than  
309 rye bread portion ( $p < 0.038$ ) but there were no differences between other portions. Prospective food  
310 consumption (Figure 5D) was significantly higher after consuming flakes compared to puffs at 30  
311 min and 60 min ( $p < 0.002$  and  $0.028$ , respectively) and compared to rye bread at 30 min ( $p < 0.018$ ).  
312 However, there were no other differences between products or in other time points. There were no  
313 statistically significant differences in satiety ratings (Figure 5E). Average appetite (a parameter

314 derived from fullness, prospective food consumption, hunger and desire to eat) (Figure 5F) was  
315 significantly higher after consuming flakes compared to puffs at 30 min and 60 min ( $p < 0.011$ ,  
316  $p < 0.045$ , respectively) and compared to rye bread at 30 min ( $p = 0.034$ ). Between other products no  
317 differences were seen.

318 3.4 Postprandial average appetite in relation to oral processing, evaluated pleasantness and satiety  
319 expectations

320 Mixed model including product and time as fixed factors, subject as a random factor and average  
321 appetite as dependent factor was taken as starting point to study the contribution of cephalic phase  
322 factors on average appetite (a parameter derived from fullness, prospective food consumption,  
323 hunger and desire to eat). BIC value describing the goodness of fit for this model was 2195.  
324 Parameters of oral processing (number of chews per portion and relative work); evaluated  
325 pleasantness and satiety expectations were then added to the model as fixed factors one at a time to  
326 see whether they influenced the goodness of model fit. Adding the number of chews in the model  
327 did not improve the fit (BIC value 2165, p-value for product 0.051) but adding a parameter for  
328 relative work did improve it (BIC value 1911, p-value for product 0.001). Including evaluated  
329 pleasantness improved the fit as well (BIC 1965, p-value for product 0.001). The differences  
330 between products were abolished when the evaluations about expected satiety before mastication  
331 (BIC 1966, p-value 0.109) and after mastication (BIC 1968, p value for product 0.304) were added  
332 in the model.

333 4 Discussion

334 The results showed that rye product portions matched by energy content but varying in structure  
335 required different type of mastication process and influenced on postprandial satiety measures  
336 differently in the early postprandial period. Mastication effort, measured as relative mastication

337 work, and perceived pleasantness seem to interact with satiety response. The portion with rye flakes  
338 showed the weakest satiety impact, puffs and rye bread showing the strongest impact and rye  
339 smoothie intermediate. Rye puffs and rye bread, having the most beneficial influence on satiety,  
340 were both characterized by a solid and porous structure with comparable instrumental and sensory  
341 hardness. However, there were many characteristics that differentiate these products: rye bread was  
342 soft and springy product and rye puffs crispy, with strong adhesion to teeth, probably attributable of  
343 the combination of high content of arabinoxylan and big particle surface area in mastication. Rye  
344 flakes, resulting in the weakest satiety response, were characterised as hard and crunchy, and having  
345 a non-porous structure requiring intensive mastication effort. The differences in satiety responses in  
346 this study occurred already in the early postprandial phase (30 min and 60 min) indicating that  
347 cephalic and gastric phase factors were behind the differences.

348 The mastication process was analysed in a mastication trial measuring the process with EMG. The  
349 method makes it possible to evaluate not only mastication time or number of chews but also relative  
350 chewing force and mastication effort that is needed to disintegrate the sample in the mouth. The  
351 results show that the mouthfuls of samples required different mastication patterns, rye bread and  
352 flakes needing the highest number of chews and the longest processing time. Since the number of  
353 mouthfuls needed to consume a portion of food (with fixed energy amount) varies, the mastication  
354 parameters were extrapolated to represent the values for portions served in the satiety trial. The  
355 results show that the number of chews, oral processing time and mastication effort were the highest  
356 for portions of rye flakes and rye puffs. Thus, the driest products required the most mastication  
357 effort among the studied products.

358 Number of chews and mastication effort (derived as a product of chewing time and force), were  
359 used to represent the mastication process in the statistical models to reveal possible contributions to  
360 the satiety. These two parameters were chosen because they are reasonably uncorrelated, while e.g.

361 number of chews and chewing time are strongly dependent. Mastication effort was found to  
362 improve the model while the number of chews did not influence the goodness of the fit. This  
363 indicates that mastication effort would be more relevant oral processing factor than the mere  
364 number of chews with respect to the appetite response. However, the obtained result does not  
365 support the hypothesis that higher mastication effort would be beneficial for satiety response since  
366 the flakes requiring the most intense effort actually resulted in the weakest satiety response. We  
367 assume that there are structural properties that are reflected in mastication parameters but actually  
368 are relevant for other satiety inducing mechanisms in the body. Differences in stomach distention  
369 could offer one plausible explanation: rye bread and rye puffs were porous products which most  
370 probably were disintegrated into fairly small particles with good hydration capacities compared to  
371 the flakes that have hard and dense structure resulting assumedly bigger particles in mastication.  
372 The beverage consumed alongside the flakes is probably emptied rapidly from stomach causing less  
373 stomach distention which is among factors influencing satiety. The period of the observed  
374 differences supports this hypothesis: the differences in the satiety responses were seen during the  
375 first hour after consumption. The rheology of the boluses would be interesting to study *in vitro* to  
376 better understand the impact of food structure for stomach digestion phase.

377 Rye smoothie portion and portion with rye flakes and juice is an interesting pair to compare since  
378 these portions include exactly the same ingredients and similarly produced cereal product (extruded  
379 flakes), energy content and volume but in different forms. The smoothie was designed to represent  
380 the flakes portion without the need for extensive mastication. Despite being structurally very  
381 different, both the products possess properties potentially beneficial for satiety: the flakes required  
382 more mastication effort which might be a beneficial property for satiety whereas rye smoothie was a  
383 soup-like product which is a food type generally considered having good satiating capacity. Some  
384 researchers believe that for maximum satiating power, the water should to be incorporated in the  
385 food, as opposed to being consumed alongside the food as a beverage (Almiron-Roig et al., 2013).

386 Indeed, rye smoothie tended to induce better satiety compared to rye flake portion although the  
387 difference was not statistically significant. One possible explanation may be again in hydration: the  
388 rye smoothie was let stand for 15 min before the satiety trial thus resulting in thick texture with  
389 hydrated rye flake particles. Dry rye flakes, which are characterised with low porosity and which  
390 have been shown to remain in bigger particles than extruded puffs in mastication (Alam et al.,  
391 2016), assumedly do not absorb water promptly and the beverage consumed alongside the flakes is  
392 probably emptied rapidly from stomach causing less stomach distention than the juice that is  
393 incorporated in the food product. Dhingra et al. concluded in their review about dietary fibre in  
394 foods that hydration properties are relevant in explaining the physiological effects of fibres and that  
395 for example substrate pore volume impacts the hydration capacity (Dhingra, Michael, Rajput, &  
396 Patil, 2012). Also our earlier study showed that beta-glucan which was added in juice resulted in  
397 better satiety response than the same ingredient added in biscuits in study setting having the same  
398 basic products (Pentikäinen, Karhunen, et al., 2014).

399 In addition to mastication process other cephalic phase related factors, such as perceived  
400 expectations about the satiating capacity of the food as well as perceived pleasantness may  
401 influence the actual satiety response. In the current study the study portions, even though matched  
402 with energy, were evaluated differently regarding their satiating capacity: rye bread was evaluated  
403 as the most powerful satiety-maintaining product whereas the rye smoothie was evaluated to be  
404 poorest to suppress appetite. In addition, the evaluations of the satiating capacities were enhanced  
405 after oral processing of the food, especially for rye flakes and rye smoothie which apparently were  
406 also unfamiliar foods for the participants. It has been shown that expectations about the satiating  
407 capacity of food can influence the actual satiety response and that the effect can last up to three  
408 hours (Brunstrom et al., 2011). Adding the evaluated satiety expectations into the mixed model  
409 abolished the differences between products. Thus, we assume that the expectations about the  
410 satiating capacity of the portions influenced the results.

411 Rye puff portion was evaluated as the least pleasant, rye bread portion as the most pleasant and  
412 other portions intermediate. Regarding the previous studies about the possible influence of  
413 pleasantness on satiety these clear differences could not be neglected. Addition of pleasantness  
414 ratings into statistical model enhanced the model as well as made the between-product differences  
415 more statistically significant ( $p=0.001$  vs. original  $p$ -value of 0.044). Thus the evaluated  
416 pleasantness of the products indeed was influencing the result. Lower pleasantness ratings for rye  
417 puffs may have resulted from considerably big volume of the portion resulting from airy structure.  
418 Also strong adhesion to teeth might have influenced the poorer pleasantness ratings.

419 Differences in oral processing can be achieved either by instructing participants to masticate food  
420 during a fixed time or by applying fixed number of chews or by providing textures that lead to more  
421 longer oral processing patterns. The latter approach is preferable when trying to develop products  
422 that would naturally help to control food intake and enhance satiety response. The current study was  
423 successful in producing varying food structures resulting in different oral processing pattern. They  
424 were not only foods as such and with comminuted structure but realistic products with structural  
425 differences including ductile and chewy texture (bread), hard and crunchy texture (flakes) and hard,  
426 airy, crispy texture (puffs) and a soup-like texture (smoothie).

427 As a drawback the current study's setting is that the familiarity of the products (even though it was  
428 not specifically asked) assumedly was different. Rye bread is a staple food in Finland whereas both  
429 extruded rye products and rye smoothie are uncommon food items. It has been seen in earlier  
430 studies that earlier experiences about foods help to evaluate their satiety effect (Brunstrom,  
431 Shakeshaft, & Scott-Samuel, 2008). Thus, in further study settings it would be good to familiarize  
432 the study participants to each study product beforehand to exclude the possible mixing impact of  
433 familiarity. Postprandial satiety responses were measured during 210 minutes following the  
434 established practices (3-5 hours) (Blundell et al., 2010). However, in the current study or similar

435 studies where differences in satiety responses are hypothesized to occur mainly due to cephalic  
436 phase or stomach phase factors it might be more informative to measure the responses more  
437 frequently during a shorter period.

438 To conclude, the vast textural differences between products were reflected in mastication process  
439 and also in the satiety response to food portions with similar energy contents. The results did not  
440 support the hypothesis that mastication process itself would mediate the interaction between food  
441 structure and postprandial satiety but there appears to be other mechanisms possibly related to  
442 stomach phase digestion modulating the interaction. Palatability seems to weaken postprandial  
443 satiety response.

444 Acknowledgments: We Riitta Pasanen, Leila Kostamo, Tarja Wikström, Eero Mattila, Anna-Liisa  
445 Ruskeepää (VTT Technical Research Centre of Finland) for skilful assistance in preparing the  
446 samples, analysing nutrient contents and structural properties of the samples as well as assisting in  
447 data collection.

448 Funding: This research did not receive any specific grant from funding agencies in the public,  
449 commercial, or not-for-profit sectors.

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528

Table 1 Nutrient content of the samples and nutrient content and portion sizes of portions offered in the satiety test.

	<i>Samples (/100 g)</i>					<i>Satiety test portions (/portion)</i>				
	WG sourdough h rye bread	Extruded WG rye flakes	Extruded WG rye puffs	Refined wheat bread	Black- currant juice	WG sourdough rye bread + juice	Extruded WG rye flakes + juice	Extruded WG rye puffs + juice	WG rye smoothie	Refined wheat bread + juice
Nutrient content										
Energy (kcal)	200	322	330	253	38	382	382	382	382	382
Starch (g)	35.4	57.7	59.8	46.4	ns	33.7	34.1	34.5	34.1	34.8
Protein (g)	6.5	9.7	9.8	9.1	ns	6.2	5.7	5.6	5.7	6.8
Fat (g)	0.6	1.2	1.3	2.4	ns	0.6	0.7	0.7	0.7	1.8
Total dietary fibre (g)	13.3	20.7	19.8	4.7	ns	12.6	12.2	11.4	12.2	3.6
Soluble dietary fibre (g)	7.5	9.5	10.7	2.3	ns	7.2	5.6	6.2	5.6	1.7
Insoluble dietary fibre (g)	3.6	3.7	4.0	1.5	ns	3.4	2.2	2.3	2.2	1.1
Oligosaccharides (g)	2.2	7.6	5.2	1.0	ns	2.0	4.5	3.0	4.5	0.7
Sugar (g)	-	-	-	-	9.6	48	48	48	48	48
Portion sizes (g)										
Cereal product						95	59	58	58	75
Juice						500	500	500	500	500
Total						595	559	558	559	575

Table 2 Characteristics of the study participants. Values are means  $\pm$  SD, n=26 in the mastication trial and n=16 (subset) in satiety trial.

	Mastication trial n=26		Mastication trial and satiety trial n=16 (subset)	
	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
Age	31.7 $\pm$ 7.5	19-50	32.9 $\pm$ 8.2	22-50
BMI	22.2 $\pm$ 1.9	19.1-27.3	22.4 $\pm$ 2.2	19.8-27.3
Eating behaviour <sup>1</sup>				
<i>Cognitive restraint</i>	45.7 $\pm$ 16.6	11-72	51.7 $\pm$ 12.1	17-72
<i>Uncontrolled eating</i>	27.6 $\pm$ 10.3	11-48	27.6 $\pm$ 11.2	11-48
<i>Emotional eating</i>	33.3 $\pm$ 24.7	0-89	41.4 $\pm$ 26.8	0-72

<sup>1</sup> Eating behaviour was measured with 18-item Three-Factor Eating Questionnaire (TFEQ) (Karlsson, Persson, Sjöström, & Sullivan, 2000)

Table 3 Moisture contents of the samples and textural properties measured with TPA (breads) and TA (extrudates).

	WG sourdough rye bread	Refined wheat bread	Extruded WG rye flakes	Extruded WG rye puffs
Moisture (%)	39.3 ± 0.1	32.3 ± 0.4	7.0 ± 0.0	5.5 ± 0.0
Hardness (N)	24 ± 8	4 ± 1	1530 ± 390	27 ± 3
Cohesiveness	0.4 ± 0.1	0.7 ± 0.0	-	-
Chewiness	5.1 ± 1.8	2.0 ± 0.5	-	-
Adhesiveness	-0.010 ± 0.014	-0.133 ± 0.332	-	-
Crispiness work			98.3 ± 37.3	0.6 ± 0.1
Crispiness index (x 10 <sup>-3</sup> )			0.004 ± 0.002	21 ± 5

Table 4 Oral processing parameters. Values are means  $\pm$  SD, n=26. Different superscript letters in a row indicate statistically significant difference ( $p < 0.05$ ) between products. Extrapolated parameters represent oral processing parameters for the portion size served in the satiety trial.

	WG sourdough rye bread	Extruded WG rye flakes	Extruded WG rye puffs	WG rye smoothie	Refined wheat bread	$\chi^2$	Sig.
Parameters for mouthful of food							
Number of chews	27 $\pm$ 10 <sup>b</sup>	28 $\pm$ 7 <sup>b</sup>	11 $\pm$ 5 <sup>a</sup>	7 $\pm$ 4 <sup>a</sup>	20 $\pm$ 8 <sup>b</sup>	85.8	<0.001
Total oral processing time (s)	20 $\pm$ 9 <sup>c</sup>	21 $\pm$ 8 <sup>c</sup>	8 $\pm$ 4 <sup>a</sup>	4 $\pm$ 3 <sup>a</sup>	14 $\pm$ 6 <sup>b</sup>	84.9	<0.001
Time of EMG activity (s)	9 $\pm$ 3 <sup>bc</sup>	10 $\pm$ 3 <sup>c</sup>	4 $\pm$ 2 <sup>a</sup>	2 $\pm$ 1 <sup>a</sup>	7 $\pm$ 3 <sup>b</sup>	85.6	<0.001
Duty cycle (%) <sup>1</sup>	46 $\pm$ 3 <sup>a</sup>	48 $\pm$ 4 <sup>a</sup>	53 $\pm$ 6 <sup>b</sup>	61 $\pm$ 13 <sup>b</sup>	48 $\pm$ 3 <sup>a</sup>	46.6	<0.001
Relative force (%) <sup>2</sup>	90 $\pm$ 15 <sup>b</sup>	101 $\pm$ 25 <sup>b</sup>	75 $\pm$ 23 <sup>ab</sup>	45 $\pm$ 23 <sup>a</sup>	80 $\pm$ 17 <sup>b</sup>	60.0	<0.001
Relative work <sup>3</sup>	8 $\pm$ 3 <sup>bc</sup>	11 $\pm$ 3 <sup>c</sup>	3 $\pm$ 1 <sup>a</sup>	1 $\pm$ 1 <sup>a</sup>	5 $\pm$ 2 <sup>b</sup>	80.7	<0.001
Extrapolated parameters for food portion							
Number of chews	340 $\pm$ 130 <sup>a</sup>	480 $\pm$ 120 <sup>b</sup>	640 $\pm$ 260 <sup>b</sup>	210 $\pm$ 130 <sup>a</sup>	380 $\pm$ 160 <sup>b</sup>	80.3	<0.001
Total oral processing time (s)	250 $\pm$ 110 <sup>ab</sup>	360 $\pm$ 130 <sup>c</sup>	440 $\pm$ 210 <sup>c</sup>	140 $\pm$ 100 <sup>a</sup>	280 $\pm$ 110 <sup>b</sup>	73.7	<0.001
Time of EMG activity (s)	110 $\pm$ 40 <sup>ab</sup>	170 $\pm$ 50 <sup>c</sup>	220 $\pm$ 90 <sup>c</sup>	70 $\pm$ 40 <sup>a</sup>	130 $\pm$ 50 <sup>b</sup>	82.2	<0.001
Relative work <sup>3</sup>	100 $\pm$ 30 <sup>b</sup>	190 $\pm$ 50 <sup>c</sup>	160 $\pm$ 70 <sup>c</sup>	40 $\pm$ 40 <sup>a</sup>	100 $\pm$ 40 <sup>b</sup>	70.2	<0.001

<sup>1</sup> Time of EMG activity/Total oral processing time

<sup>2</sup> Chewing force of the product related to chewing force of chewing gum

<sup>3</sup> Time of EMG activity x relative force

## FIGURE CAPTIONS

Figure 1 Photographs of the food samples. Rye smoothie was prepared mixing grinded wholegrain rye flakes with blackcurrant juice and letting the mixture stand for 15 minutes

Figure 2 A: EMG data after 50 Hz notch filtering for a single participant, chewing gum sample. The three mastication sequences are each labeled with 'start' and 'stop'. B: Further analysis of the second mastication sequence of the data above. EMG power was computed, highpass-filtered, squared (blue curve) and smoothed (red curve), after which chews were detected (black block curve). The event data were used for number of chews, total oral processing time, time of EMG activity and duty cycle. The smoothed EMG power was used for relative force and, when multiplied by time of EMG activity, the relative work.

Figure 3 Perceived characteristics of the samples evaluated by the trained sensory panel (n=2x12). Sensory intensities were evaluated on an intensity scale 0-10. Values are means. There were statistically significant differences ( $p<0.001$ ) between the samples in each attribute.

Figure 4 A) Expected satiety before and after mastication (n=26) and B) pleasantness of the portions after eating the portion (n=16). Expected satiety was evaluated based on photograph representing study portions together with mastication trial. Pleasantness of each study portion was evaluated together with satiety trial right after consuming the portion. The evaluations were done on a VAS scale 0-10. Values are means  $\pm$  SD. Different letters above bars indicate statistically significant difference between evaluations (in 2A uppercase letters for values before mastication and lowercase letters for values after mastication). Asterixes in 2A indicate significant difference within product before and after mastication trial \*\* $p<0.01$ , \*\*\* $p<0.001$ .

Figure 5 Changes VAS ratings for A) hunger, B) fullness, C) desire to eat, D) prospective food consumption, E) satiety and F) average appetite score during 210 min postprandial period in healthy women for wholegrain rye bread (--■--), wholegrain rye smoothie (··· ◆···), wholegrain rye puffs (--x--), wholegrain rye flakes (--▲--) and refined wheat bread (--□--). Values are means with their standard errors represented by vertical bars, n=16. Significant product effect was found for hunger, fullness, desire to eat, prospective food consumption and average appetite score. The time points with statistically significant differences ( $p<0.05$ ) between products are marked with asterix (\*).



Rye bread



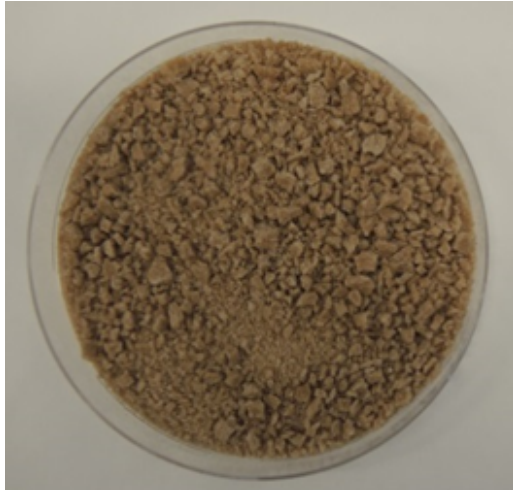
Rye puffs

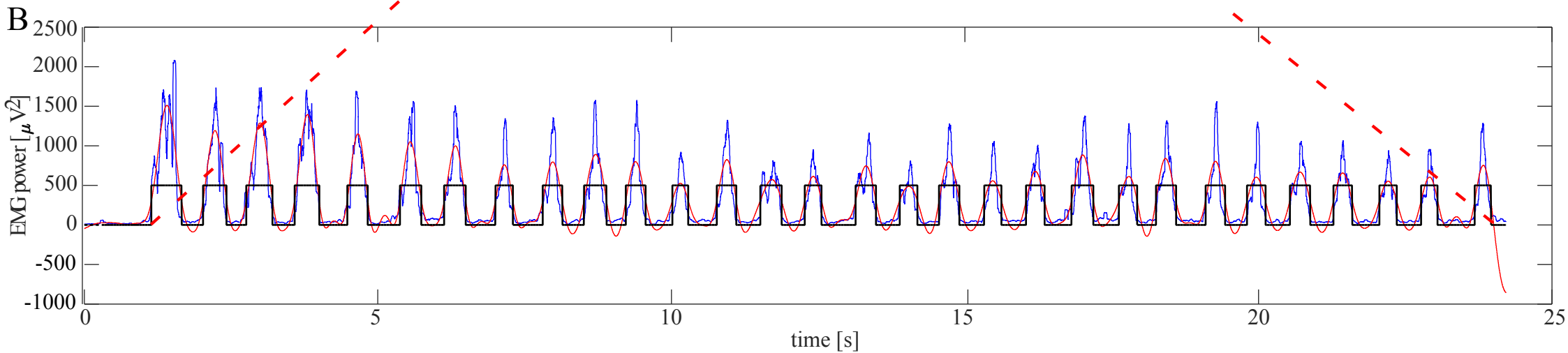
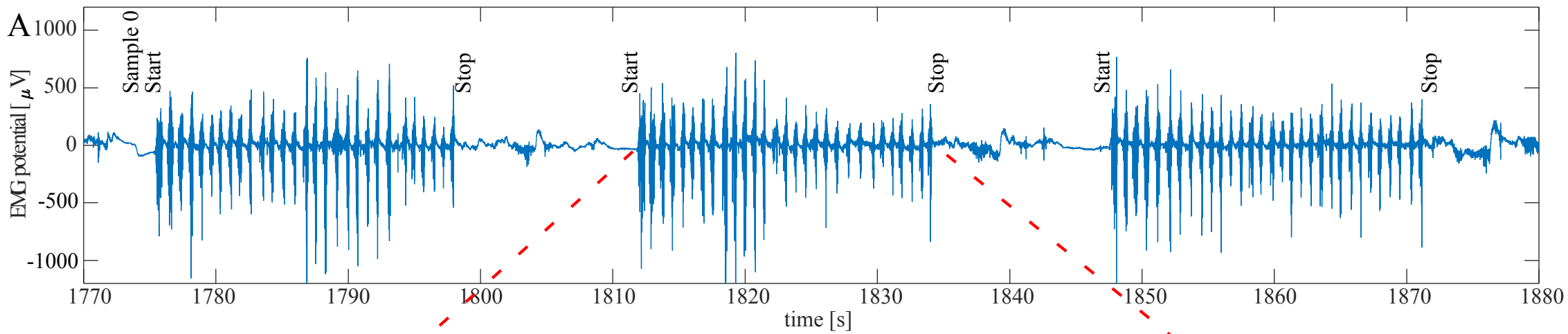


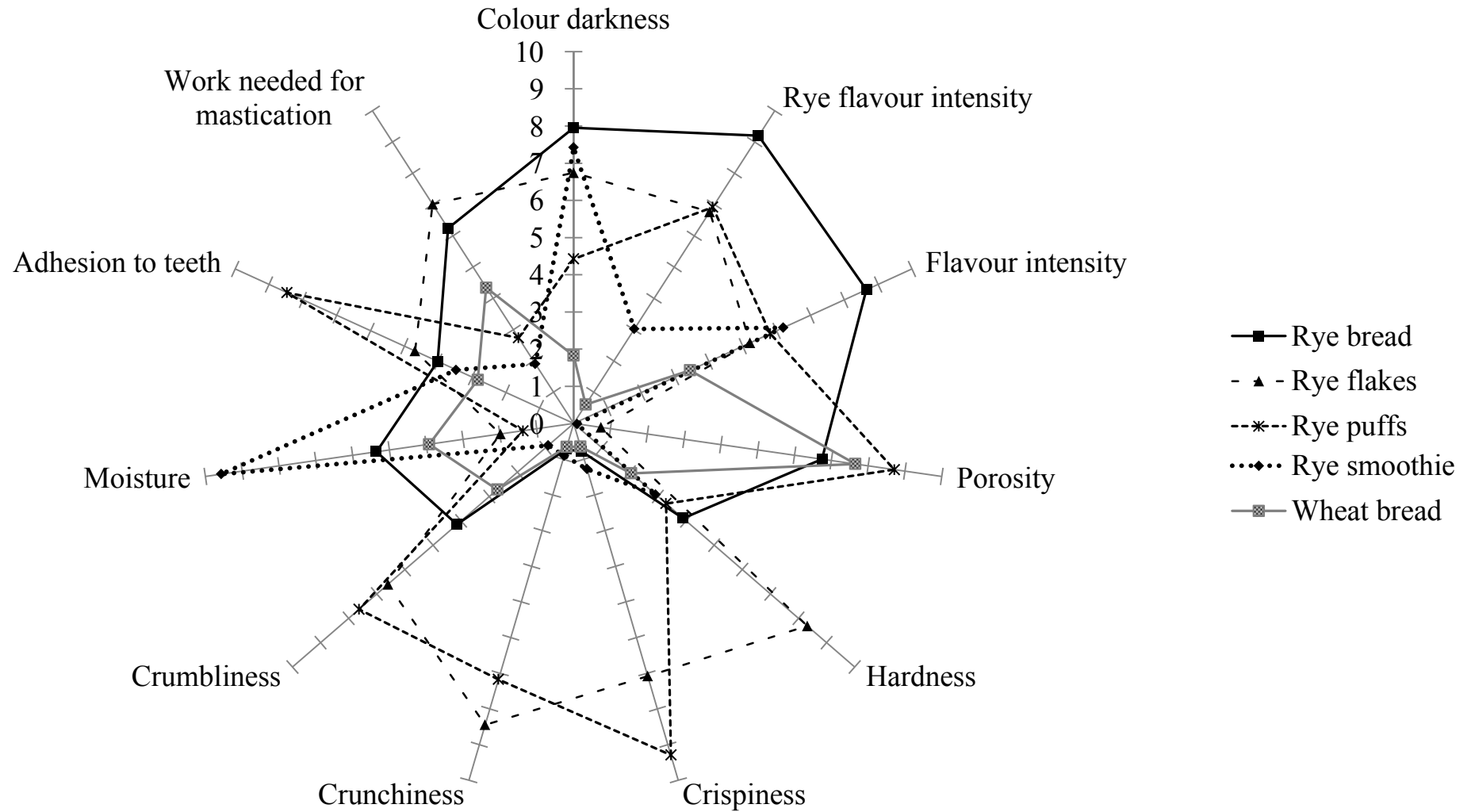
Rye flakes



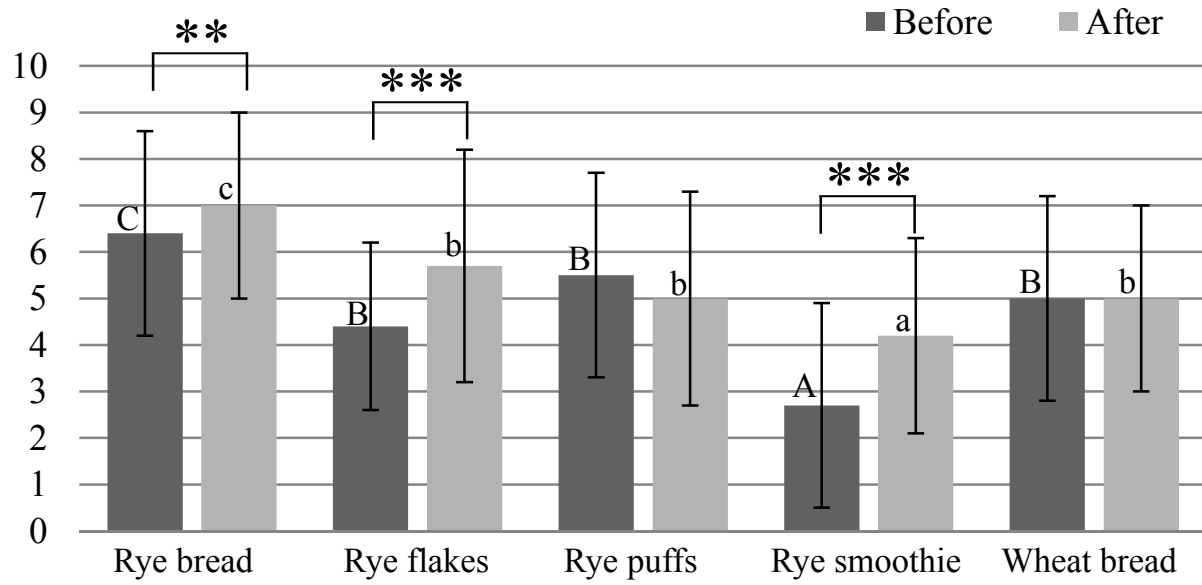
Grinded rye flakes    Blackcurrant juice



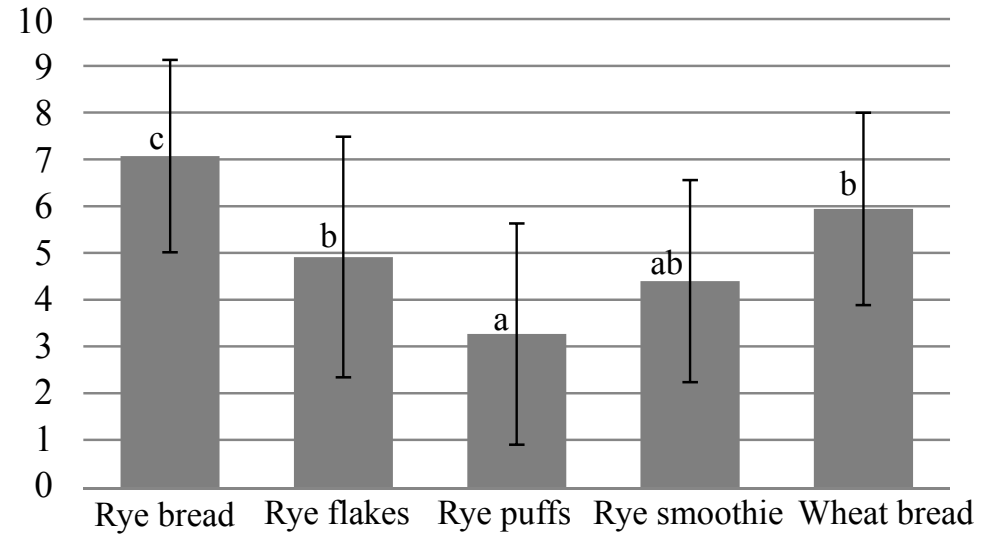




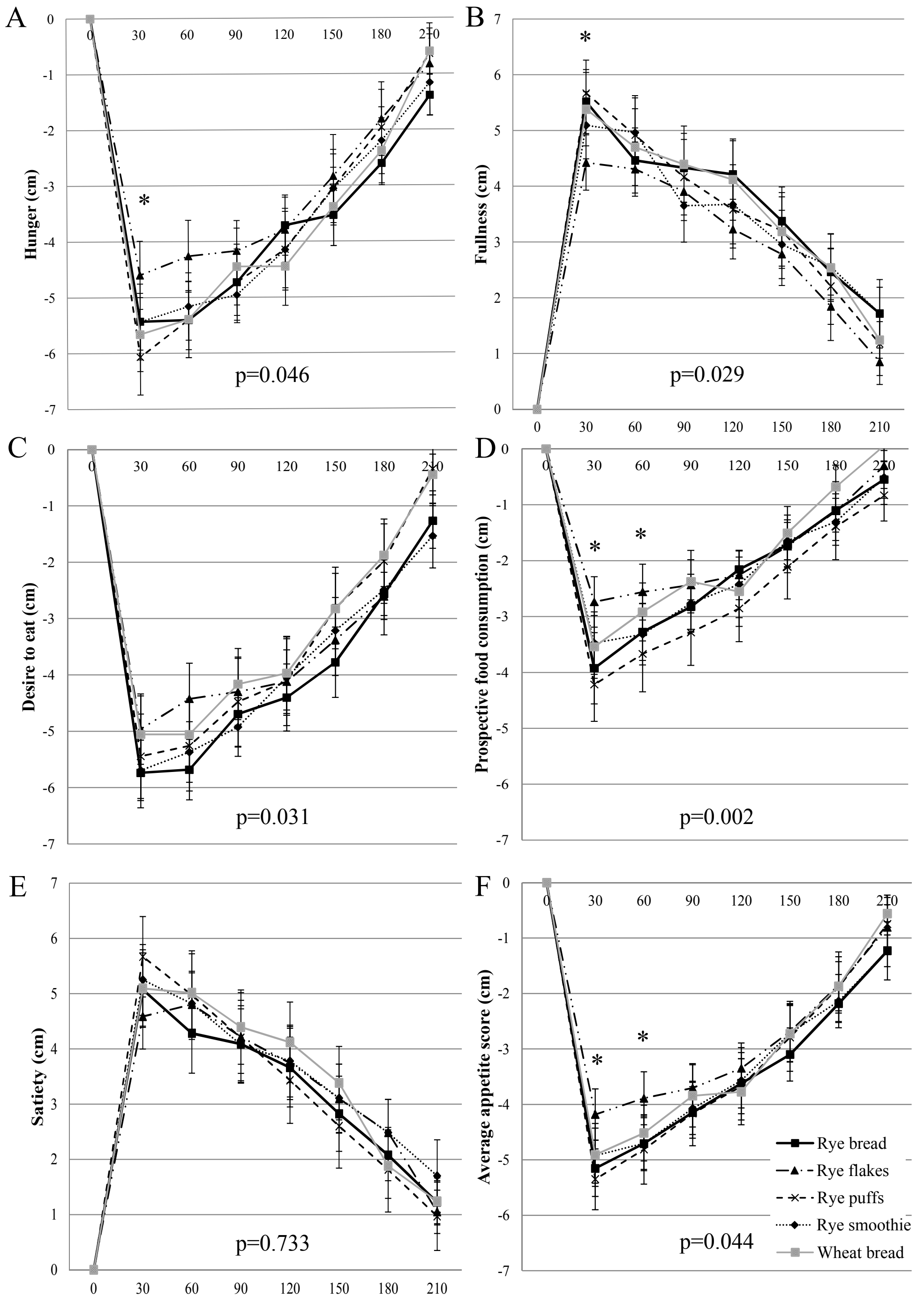
A) Expected satiety before and after mastication



B) Pleasantness



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**Highlights:**

- Food structure influences satiety in the early post-prandial period
- There is a link between mastication effort and satiety
- Evaluated pleasantness modulate satiety response

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