1 2 3	TEXTURE OF EXTRA VIRGIN OLIVE OIL-ENRICHED MASHED POTATOES: SENSORY, INSTRUMENTAL AND STRUCTURAL RELATIONSHIPS
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13	Running title
14	OLIVE OIL EFFECT ON MASHED POTATO TEXTURE
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- 32 ABSTRACT
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35 The aim of this work was to study the effect of the addition of extra virgin olive oil (EVOO) on instrumental textural properties, sensory texture profile analysis (TPA) and 36 37 microstructure of fresh and frozen/thawed mashed potatoes formulated without and with 38 added cryoprotectants [kappa-carrageenan (κ -C) and xanthan gum (XG)]. EVOO behaves 39 as soft filler due to droplet aggregates, whereas addition of cryoprotectants led to more 40 structured mashed potatoes (MP) thanks to the gelling properties of κ -C. Both the 41 percentage of added EVOO and processing had a much less significant effect on the texture of the MP containing κ -C and XG, evidencing the ability of this biopolymer blend 42 43 to impart freeze/thaw stability. All samples with added EVOO were perceived as significantly softer and creamier than the samples without EVOO, whereas all MP samples 44 45 with added cryoprotectants were perceived as significantly thicker and creamier than those 46 without hydrocolloids.

47 **KEYWORDS**

48 Extra virgin olive oil, mashed potatoes, texture, microstructure, TPA sensory attributes,49 freezing

51 PRACTICAL APPLICATIONS

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53 Previous studies showed that the quality after freezing and thawing may be improved by 54 the addition of 1.5 g/kg of κ -C and 1.5 g/kg of XG, and/or incorporation of dietary fiber, 55 improvement of mashed potatoes texture by retarding starch retrogradation and increasing 56 water-holding capacity. Growing awareness of the link between diet and health is fast 57 changing consumer habits, so that there has been increasing demand for foods with health 58 enhancing properties. Extra virgin olive oil (EVOO) has important nutritional 59 characteristics linked to its biophenol content and has very important antioxidant properties. The results have shown that although instrumental textural data were able to 60 61 explain differences in consistency perceived, structural information is needed to 62 understand differences in creaminess. Back extrusion test is recommended to industry as 63 practical quality control tool in the commercial production of mashed potatoes with added EVOO. 64

66 INTRODUCTION

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Various health organizations recommend a daily intake of around 600 g of fruit and vegetables, but few people manage to consume this amount. Led by consumer demand, the food industry has shown an increased interest in the manufacture of healthier and more natural fruit and vegetable food products, such as soups, drinks and sauces (Whybrow *et al.* 2006). Mashed potatoes (MP) made from 100 % fresh potato tubers are in addition a natural vegetable semisolid food, which may also be suitable for freezing as a ready-meal component or as a product in itself such as potato gratin (Alvarez *et al.* 2009).

75 Olive oil is an important component of the diet of the countries surrounding the 76 Mediterranean Sea. Due to its composition, olive oil is a good source of biophenols 77 (Boskou and Visioli 2003) as well as lipid-soluble and water-soluble vitamins 78 (tocopherols, β-carotene, ascorbic acid). In addition, thanks to its balanced fatty acid 79 composition virgin olive oil has highly appreciated nutritional characteristics (Mildner-80 Szkudlarz and Jeleń 2010), known for a long time to the people of the Mediterranean 81 region, who use it daily for a variety of culinary purposes. Biophenols with important 82 antioxidant properties and a role in atherogenesis and cancer have been found and 83 quantified in virgin and extra-virgin olive oils (Muniz 2007). However, consumption has 84 also increased in non-Mediterranean areas thanks to growing interest in the Mediterranean 85 diet and a belief that it prevents certain diseases (Boskou and Visioli 2003; 86 Paraskevopoulou et al. 2005). A classic white sauce usually contains flour, milk and butter, 87 but olive oil has been added to a white model-sauce to produce an innovative sauce 88 approximating "Mediterranean cooking" (Mandala et al. 2004).

89 The oil volume fraction exerts profound effects on the physicochemical and 90 viscoelastic properties of emulsions, such as droplet size distribution, creaming, oxidative

91 stability, and rheology (Dickinson and Chen 1999). Fat droplets influence the overall physicochemical and sensory properties of foods in a variety of different ways 92 93 (Chantrapornchai et al. 1999). A great deal of research has been done on the influence of 94 fat droplets on the rheology, stability and flavour of food emulsions, but less is known about their influence on emulsion appearance. Color is one of the major attributes affecting 95 96 consumer perception of the quality of virgin olive oil (Criado et al. 2008), and chloroplast 97 pigments (chlorophyll and carotenoids) are mainly responsible for the color of virgin olive 98 oil, ranging from yellow-green to greenish gold (Ayuso et al. 2004).

99 Texture is by far the most important quality criterion for consumer sensory acceptance 100 of freshly prepared and processed potato products, and particularly of frozen/thawed and 101 dehydrated mashed potatoes. A fluffy and medium-consistency texture is desirable, 102 whereas pastiness, gumminess and stickiness are negative attributes (Lamberti *et al.* 2004). 103 Texture instability remains the most significant challenge for frozen food products, 104 especially with inevitable post-production temperature fluctuations. Loss of moisture and 105 changes in textural attributes often result in significant reduction of product quality.

106 Previous studies showed that the addition of κ -C and XG to MP at a low concentration 107 (each cryoprotectant at 1.5 g/kg) is recommendable on the basis of overall acceptability, 108 especially when the product is going to be frozen (Alvarez *et al.* 2009; Fernández *et al.* 109 2009). κ -C provides the appropriate texture, while XG imparts creaminess and mouthfeel 110 to the product.

111 No research has been done on the addition of olive oil in fresh and frozen/thawed 112 mashed potatoes (designated FMP and F/TMP respectively), particularly with EVOO. The 113 use of EVOO rather than commercial olive oil is preferable because of its high 114 concentrations of both unsaturated fatty acids and antioxidant compounds such as 115 polyphenols and tocopherols (Severini *et al.* 2003). The purpose of the present research

116	was to evaluate the effects of adding EVOO on the textural, physical, structural and
117	sensory characteristics of fresh and frozen/thawed mashed potatoes formulated without and
118	with added cryoprotectants.
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120	MATERIALS AND METHODS
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122	Materials
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124	The potatoes used were fresh tubers (cv Kennebec) from Aguilar de Campoo (Burgos,
125	Spain) grown in 2008. κ -C (GENULACTA carrageenan type LP-60) and XG (Keltrol F
126	[E]) were donated by Premium Ingredients, S.L. (Girona, Spain). EVOO (Carbonell,
127	Spain) was chosen for addition to the MP. Following range-finding experiments, the lower
128	and upper levels of EVOO to be used were set at 10 and 50 g/kg, respectively. A sample
129	without EVOO was also prepared for each type of MP and processing conditions.
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131	Preparation of MP Samples
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133	Tubers were manually washed, peeled and diced. MP were prepared in ~ 2000-g batches
134	from 607.7 g/kg of potatoes, 230.8 g/kg of semi-skimmed in-bottle sterilized milk (fat
135	content, 15.5 g/kg), 153.8 g/kg of water, 7.7 g/kg of salt (NaCl) and the corresponding
136	EVOO concentration (0, 10, 25, and 50 g/kg) using a TM 31 food processor (Vorwerk
137	España, M.S.L., S.C., Madrid, Spain). MP were prepared without and with added κ -C and
138	XG (MPA and MPB samples, respectively). In the latter case, hydrocolloids (each at 1.5
139	g/kg) were also added to the rest of the ingredients in the form of a dry powder. All the
140	ingredients were cooked for 35 min at 90C (blade speed: 40 rpm) (Alvarez et al. 2009;

141 Fernández et al. 2009). The mash was ground for 40 s (1200 rpm) and for 20 s (2600 rpm). 142 The product was at once homogenized through a stainless steel sieve (diameter: 1.5 mm). 143 The highest EVOO concentration was added twice to the MP to evaluate the effect of order 144 of addition and EVOO thermal treatment on MP quality. First, 50 g/kg of EVOO was 145 added along with the rest of the ingredients as indicated above, whereas in the second case 146 the same EVOO concentration (designated "50b" g/kg) was added to the MP before final 147 homogenization. Half of each fresh blend (FMP samples) was analysed immediately and 148 the other half was frozen and thawed (F/TMP samples). Two repetitions of each composition were prepared in different weeks. 149

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151 Freezing, Thawing and Heating Procedures

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153 MP samples were placed on flat freezing and microwave thawing trays, and then frozen by 154 forced convection with liquid nitrogen vapour in an Instron programmable chamber (model 155 3119-05, -70/+250C) at -60C until their thermal centres reached -24C (Fernández et al. 156 2009). After freezing, the samples were packed in polyethylene plastic bags, sealed under 157 light vacuum (-0.05 MPa) on a Multivac packing machine (Sepp Haggenmüller KG, 158 Wolfertschwenden, Germany), and placed in a domestic freezer for storage at -24C. 159 Packed frozen samples were thawed in a Samsung M1712N microwave oven (Samsung 160 Electronics S.A., Madrid, Spain). Samples were heated for 20 min at an output power 161 rating of 600 W. Samples were brought up to 55C by placing them in a Hetofrig CB60VS 162 water bath (Heto Lab Equipment A/S, BirkerØd, Denmark). Sample testing was 55C, 163 where water and product temperatures were monitored by T-type thermocouples as 164 described elsewhere (Alvarez et al. 2005, 2008, 2009; Fernández et al. 2008).

166 Instrumental Texture Measurements

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168 Back extrusion (BE) and cone penetration (CP) mechanical tests were performed in order 169 to study the empirical rheological behavior of "semisolid like" samples. Both experiments 170 were performed using a TA.HDPlus Texture Analyser (Stable Micro Systems Ltd, 171 Godalming, UK) equipped with a 300 N load cell. During tests, MP samples were kept at 172 55C by means of a Temperature Controlled Peltier Cabinet (XT/PC) coupled to a separate 173 heat exchanger and proportional-integral-derivative control unit. For performance of BE 174 tests, a rig (model A/BE, Stable Micro Systems) was used consisting of a flat 45 mm 175 diameter perspex disc plunger that was driven into a larger perspex cylinder sample holder 176 (50 mm diameter) to force down into the MP samples and flow it upward through the 177 concentric annular space between plunger and the container. The measuring cup was filled 178 with 50 ± 1 g of MP. Product was extruded to a distance of 20 mm at 2 mm/s compression 179 rate. At this point (most likely to be the maximum force), the probe returns to its original 180 position. From the recorded force time curves, texture parameters with physical meaning 181 are calculated, which vary from simple consistency indices to a derived flow behavior 182 index, which is obtained according to the mathematical model suggested by Osorio and 183 Steffe (1987). In this study, maximum positive force of extrusion (firmness (N)) and the 184 negative area of extrusion (viscosity index (N s)) have been taken into account in order to 185 describe texture changing in MP samples. For performing the CP tests, a TTC spreadability 186 rig (HDP/SR, Stable Micro Systems) was used, consisting of a 45 degree conical perspex 187 probe (P/45 C) that penetrated a conical sample holder containing 7 ± 0.1 g of MP product. 188 Product was penetrated to a distance of 17.5 mm at 3 mm/s compression rate. CP work per 189 displaced volume (J/m^3) required to accomplish penetration was calculated from the area 190 under the curve up to the "peak" or maximum penetration force, and the average force of the complete curve (N) was also recorded. Texture measurements were performed inquadruplicate and results averaged.

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194 **Other Quality Parameters**

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The color of the MP in the pots was measured with a HunterLab model D25 (Reston, VA, USA) color difference meter fitted with a 5 cm diameter aperture. Results were expressed in accordance with the CIELAB system with reference to illuminant D65 and a visual angle of 10°. The parameters determined were L^* , a^* and b^* . A higher L^* value indicated a brighter or whiter sample and values of a^* and b^* indicated red-green and yellow-blue colors. Yellowness index (YI) was calculated as 142.86 b^*/L^* (Fernández *et al.* 2008).

Expressible water (E_w) was measured by centrifugal force. Centrifuge tubes containing approximately 10 g of MP were centrifuged at 15,000×g for 30 min in a Sorvall®, RC-5B apparatus (Global Medical Instrumentation, Inc, Clearwater, Minnesota, USA). E_w was expressed as the percentage of liquid separated per total weight of sample in the centrifuge tube (Eliasson and Kim 1992). Measurements of color and E_w were performed in quadruplicate and the results averaged.

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209 Sensory Analyses

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MP samples were subjected to texture profile analysis (TPA) modified to evaluate vegetables purees according to UNE 87025 (1996), which was used to select and define the sensory attributes included in the profile. A panel of 4 assessors, previously trained according to the ISO guidelines (ISO 8586-1:1993) and with specific exercise in MP for 8 years (Alvarez *et al.* 2005, 2008; Fernández *et al.* 2008), evaluated the textural attributes of

216 the samples. Profile attributes were classified into four groups (Alvarez et al. 2008). 217 Attributes are listed in the order of the perception according to ISO guidelines (ISO 13299: 218 2003): attributes perceived before putting the sample in mouth (granularity and moisture 219 (1)); attributes perceived at the time of putting the sample in the mouth (stickiness, 220 denseness, homogeneity, moisture (2) and firmness); attributes perceived at the time of 221 preparing the sample in the mouth for swallowing (cohesiveness, adhesiveness and 222 fibrousness (1)); attributes perceived during final and residual phases of mastication (ease 223 of swallowing, palate coating and fibrousness (2)). A description of the sensory attributes 224 evaluated during the TPA can be found elsewhere (Alvarez et al. 2008).

225 Samples were evaluated, in duplicate, in morning sessions (11:00 a.m.-1:00 p.m.). 226 Daily for 40 days assessors were given four samples (about 20 g each), for scoring 227 attributes of each group in the texture profile. All the samples were served at $55 \pm 1C$ on 228 Petri dishes. This sample temperature was reached and kept constant by placing the 229 product in the Hetofrig CB60VS water bath prior to testing. For each sample, panelists 230 evaluated the perceived intensity of the 13 attributes on 8 cm descriptive linear scales 231 labelled at each anchor: (left anchor: 1 = "not detectable; right anchor: 9 = "extremely 232 intense"). To reduce fatigue a rest period of 5 min was taken after scoring each sample.

MP samples were also subjected to an overall acceptability (OA) test based on all sensory attributes (texture, color, taste) on a 9-point hedonic scale (with 8 cm) labelled at each anchor: (left anchor: 1 = "dislike extremely"; right anchor: 9 = "like extremely"). In this case, sensory assessment was conducted by a 14-member untrained panel. Every day, one sample (about 20 g each) was served under the same conditions as indicated above.

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239 Scanning Electron Microscopy (SEM)

MP microstructure was examined by SEM using a Hitachi model S-2.100 microscope (CENIM-CSIC). MP samples were air-dried, then mounted and sputter-coated with Au (200 A aprox.) in a SPI diode sputtering system metallizer. Photomicrographs were taken with a digital system Scanvision 1.2 of RONTEC (800x1.200 pixel).

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246 Statistical Analysis

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248 A three-way ANOVA with interactions was applied to evaluate how the three factors 249 studied-EVOO concentration, presence or absence of hydrocolloids and performance or 250 not of processing -affected the texture, color, sensory attributes and the OA of the MP. 251 $E_{\rm w}$ was always zero for the MPB samples; a two-way ANOVA with interactions was applied to evaluate how EVOO concentration and processing affected the E_w of the 252 products. Minimum significant differences were calculated using Fisher's least significant 253 254 difference test (LSD, 99% for comparison of instrumental parameters and 95% for comparison of sensory attributes and OA). Analyses were performed with Statgraphics® 255 software version 5.0 (STSC Inc., Rockville, MD, USA). 256

258 **RESULTS AND DISCUSSION**

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261 Instrumental Texture Measurements
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265 Table 1 shows the effects of EVOO concentration, cryoprotectant addition and processing 266 on the values of the textural properties derived from the BE and CP tests. Samples with 267 added κ -C and XG, as well as those subjected to freezing/thawing, presented significantly 268 higher and lower textural properties than their respective counterparts. Previous studies 269 showed that when κ -C/XG blends were added to FMP and F/TMP samples, κ -C provided 270 the appropriate texture whereas XG imparted creaminess to the product (Alvarez et al. 271 2009; Fernández et al. 2009; Alvarez et al. in press). Analogously, in starch/XG blends, it 272 was observed that XG does not interfere in potato starch network building (Mandala and 273 Palogou (2003); Mandala et al. 2004). Therefore, addition of both hydrocolloids to MP 274 produces a more structured system which is associated with the gelling properties of κ -C. 275 In natural MP, the product was softer than the fresh control after freezing and thawing 276 (Alvarez et al. 2005). MP is a starchy food, and as such may present quality problems such 277 as syneresis and organoleptic and textural changes. These problems have been ascribed to 278 phase separation caused by retrogradation of the starch (Eliasson and Kim 1992; Kim and 279 Eliasson 1993).

With respect to the effect of EVOO addition, the maximum textural property values were registered in the samples without EVOO, although differences between textural properties of samples with 10 g/kg added EVOO and those without EVOO were nonsignificant (Table 1). However, increasing EVOO concentration produced softer, liquidlike systems, indicating that EVOO behave as soft filler. This result is to be expected as increasing concentrations of liquid oil are added to the product, increasing the oil-phase volume fraction. In oil-in-water emulsions, the extent of the linear region decreased with
increasing oil-phase volume fraction from 20% to 40% v/v (Sun and Gunasekaran 2009).
For their part Dickinson and Chen (1999) suggested that oil/water emulsions may undergo
a behaviour transition from predominantly entropic behaviour to predominantly enthalpic
behaviour with increasing oil-phase volume fraction.

The analysis of variance also showed that the three binary interactions had a significant effect on instrumental firmness, work per displaced volume and average force (Table 1). This means that the effect of EVOO concentration on the texture depended on the presence of κ -C and XG and on the freezing/thawing of the systems. Besides, AB and BC interactions also significantly affected the viscosity index from the BE tests.

296 From the variation in the firmness value based on EVOO concentration for both MPA 297 and MPB samples shown in Fig. 1a, one can observe that firmness was lower in the MPA 298 than in the MPB samples; moreover, the variation in sample firmness was much greater 299 when EVOO content increased from 10 to 50 g/kg in the MPA samples than in the MPB 300 ones. Also, when the concentration of added EVOO was increased, the firmness value 301 behaved similarly in the FMP and F/TMP samples (Fig. 1b); in both cases, the increase in 302 EVOO content led to reduced firmness, without important differences between 50 and 50b 303 g/kg. As droplet concentration increases, the droplets are polydispersed and the samples 304 present a less close packing structure. In mayonnaise, increasing walnut oil content 305 increases the diameter of oil droplets and consequently reduces viscoelastic properties (Cavella et al. 2009). From the variation in the firmness based on processing, the firmness 306 307 value developed differently for the MPA and MPB samples (Fig. 1c). Processing 308 significantly reduced sample firmness in the MPA samples but significantly increased it in 309 MPB samples. This behaviour can be explained taking account that much stronger and 310 more cohesive networks are formed when solutions of XG are frozen and thawed

(Giannouli and Morris 2003). The effect of XG may be explained by amylose/XG
interactions, which compete against amylose/amylose interactions, retarding or even
preventing retrogradation. Also, the addition of small amounts of XG to white sauces made
with starches from different sources significantly improves freeze/thaw stability (Arocas *et al.* 2009).

316 In turn, the variation in average force with EVOO concentration for both MPA and 317 MPB samples (Fig. 1d) was similar to that observed in firmness. In this case, of the MPB 318 samples, the ones with 25 g/kg EVOO added had poorer consistency, whereas in the MPA 319 systems, the ones with 50 g/kg had poorer consistency. When the EVOO concentration was 320 increased the average force decreased in both FMP and F/TMP samples (Fig. 1e), although 321 in the latter case adding 10 g/kg EVOO slightly increased the average force as compared 322 with the control without EVOO. Both the BE firmness and the CP average force values 323 were greater when the EVOO was added after cooking (50b g/kg) in the FMP samples but 324 not in the F/TMP samples. When the processing-dependent variation in average force was 325 plotted (Fig. 1f), the changes in that value were also similar to those observed for firmness 326 (Fig. 1c). Plots for the viscosity index and the work per displaced volume have been 327 omitted for the sake of brevity.

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329 Color Measurements and Expressible Water

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331 All the three factors studied significantly changed the color parameters, although 332 processing did not significantly affect the yellowness index (YI) (Table 2). An increase in 333 EVOO level favours higher L^* value (lightness) due to an increase in the overall light 334 scattering associated with the scattering properties of fat (Chantrapornchai *et al.* 1999). As 335 the EVOO concentration increased there was an increase in redness (decreasingly negative a^* values) and in yellowness (YI), associated with the augmented pigment content of the MP. The pigment profile of the virgin olive oil comprises chlorophyll a, chlorophyll b, and derivative pigments associated with the acidic medium of the oil extraction process (Criado *et al.* 2008).

340 L^* increased when κ -C and XG were added to the MP, which could be partially due 341 to their absolute water-holding capacity (WHC) as discussed below. Also, a^* was higher in 342 the MPB than in the MPA samples, indicating significant raised sample redness. The loss 343 of greenness associated with cryoprotectant addition was probably due to the presence of 344 XG in the system as found previously (Fernández et al. 2008). Increased lightness in the 345 F/TMP samples as compared to their FTM counterparts may have been partly due to the 346 formation of fissures produced by the growth of ice crystals during freezing, which favours 347 the release of water; this would transmit the light more rather than capturing it. For its part 348 the loss of greenness found in the processed samples (a^* values nearer to 0) as compared to 349 the fresh counterparts could be due to slight non-enzymatic browning (Maillard reaction) 350 during microwave thawing.

351 On the other hand, the three interactions had a significant effect on L^* and YI (Table 352 2). Moreover, AB and AC interactions significantly affected the a^* value. The variation in 353 the L^* value based on EVOO concentration in both MPA and MPB samples (Fig. 2a) 354 shows that increased EVOO concentration produced an increase in the L^* value in both 355 samples. The influence of droplet characteristics on the optical properties of colored oil-in-356 water emulsions has been studied (Chantrapornchai et al. 1999). The lightness of the 357 emulsions increased with increasing droplet concentration and decreasing droplet size. As 358 the droplet concentration increases so does the reflectance because the droplets scatter light 359 more effectively and hence the light beam is unable to penetrate further into the product 360 and be absorbed.

The differences between the L^* values of the MPA samples and their MPB 361 362 counterparts increased with increasing the EVOO content (Fig. 2a). In emulsions, XG is 363 added to the aqueous phase to prevent droplets from rapidly creaming and coalescence 364 (Parker et al. 1995; Sun and Gunasekaran 2009). In this study oil droplet diameters were 365 not measured, but it is probable that the droplets in the MPB samples were smaller than in 366 the ones without cryoprotectants as the presence of XG in the system would prevent coalescence. The reason why the L^* values were lower in the MPA samples, then, is that 367 368 reflectance decreases with increasing droplet diameter. Note that in the MPB samples the 369 L^* value was greater when the EVOO was added after cooking (50b g/kg), whereas in the 370 MPA systems it was greater in the samples with 50 g/kg EVOO added before cooking. 371 This discrepancy could also be related to the presence of cryoprotectants in the system. MP 372 with EVOO added before final homogenization would be expected to have larger droplets 373 because the oil was not thoroughly triturated. In the presence of XG, the droplets scatter 374 light more effectively when the oil is not so strongly entrapped in the matrix. In the MPA 375 samples on the other hand, reflectance probably decreased because the scattering efficiency 376 of the droplets decreases above a certain droplet size (Chantrapornchai et al. 1999).

In turn, as the droplet concentration increases, more reflected light travels through the oil phase of the MP being absorbed by the pigments mentioned earlier, intensifying the color of the MP (Figs. 2b, c). However, as regards YI values, there were small differences between FMP and F/TMP samples. Anyway, the color differences found between samples, although significant, should not be of major importance in practical terms.

 $E_{\rm w}$ changed significantly with EVOO concentration and processing (Table 2), and the AC interaction had a significant effect on the WHC of the samples (Fig. 2d). In this study, addition of κ -C and XG reduced the $E_{\rm w}$ of both FMP and F/TMP samples to 0%, corroborating the well-established ability of XG to reduce water separation (Alvarez *et al.* 2008, 2009; Arocas *et al.* 2009), and evidencing the existence of XG-water or XG-water-XG interactions in the systems. XG is an anionic, hygroscopic material of exceptional pseudoplasticity (Baranowska *et al.* 2008); its texturizing effect can be achieved at low gum concentration because of unusual water-holding ability. Also, adding XG (0.3% w/w) to corn starch pastes (10% w/w) minimized amylose retrogradation, syneresis and rheological changes after freezing (Ferrero *et al.* 1994). Certainly, the E_w values confirm that XG effectively stabilizes MP against syneresis when no more than 1.5 g/kg is added.

393 Besides, WHC was greater in the FMP samples than in their F/TMP counterparts at all 394 EVOO concentrations (Fig. 2d). This result is probably related to structural damage caused 395 by freezing. The addition of EVOO at low concentrations significantly increased $E_{\rm w}$, 396 mainly in the processed samples, which is likely due to that the interchain spaces were 397 occupied by oil, displacing the water (Liehr and Kuliche 1996). However, the addition of 398 EVOO at higher concentrations significantly reduced water loss, probably because excess 399 oil hindered the release of water from the starch matrix. EVOO by itself was not effective 400 in enhancing the WHC of MP. In any case E_w percentages were also quite high (> 20) in 401 both FMP and F/TMP samples without added EVOO, evidencing the presence of weak 402 starch-water or starch-water-starch interactions in all the systems. Water separation in the 403 MPA samples is related to starch retrogradation and consequent reduction of WHC 404 (BeMiller and Whistler 1996).

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406 Sensory Analyses

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408 Attributes perceived before putting the sample in mouth. All the three main factors and 409 their interactions significantly (P < 0.05) affected the scores for granularity and moisture 410 (1) (Table 3). One can observe that at all EVOO concentrations granularity scores were 411 greater in the MPA samples (Fig. 3a) and likewise in the fresh products (Fig. 3b). 412 Christianson et al. (1981) indicated that gums like XG affect the gelatinization and 413 retrogradation of starch through strong associations with amylose, resulting in reduced 414 amylose-amylose interactions. In turn, presence of XG reduced granularity in the F/TMP 415 systems by assisting new starch/water interactions and consequent water absorption. In 416 both MPA and MPB samples, panelists judged granularity lowest in the samples with more 417 than 10 g/kg added EVOO. The effects of EVOO on granularity are related to the 418 lubricating and coating properties conferred by the oil as reported for vanilla custard 419 desserts (de Wijk et al. 2003).

In turn, moisture (1) decreased significantly with respect to MPA samples with the addition of cryoprotectants in both FMP and F/TMP (Fig. 3c). Panelists detected greater ability to hold water molecules in MPB samples, confirming the results for E_w values. Similarly, panelists detected less aqueousness in the processed samples than in the fresh ones, probably due to water loss.

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426 Attributes perceived at the time of putting the sample in the mouth. Stickiness scores 427 were significantly higher in the MPB samples, although there were no differences in these 428 scores as a consequence of EVOO concentration or processing (Table 3; Fig. 3d). In turn, 429 the three factors significantly affected scores for denseness, homogeneity, moisture (2) and 430 firmness. Denseness was significantly higher in the processed than in the fresh samples 431 only when EVOO was added at the highest concentrations (Fig. 3e). Also, denseness was 432 lower in the MPA than in the MPB samples (Fig. 3f), and only in this latter case were 433 denseness scores significantly higher in the F/TMP samples than in their FMP 434 counterparts.

435 When EVOO concentration was increased, homogeneity increased in both MPA and 436 MPB samples (Fig. 3g). Note that the presence of EVOO in the systems rendered 437 differences in homogeneity among MPA and MPB samples less appreciable. Also, when 438 EVOO concentration was increased (Fig. 3h), homogeneity increased in the FMP products 439 but was almost constant in the processed samples. This indicates a positive effect of adding 440 EVOO to MP, since the negative effect of freezing on this attribute is masked by the oil. 441 Panelists detected reduced moisture (2) in the MPB samples and in the processed systems, 442 and when the EVOO concentration was increased, moisture (2) significantly increased 443 when cryoprotectants were also added (Fig. 3i).

444 In turn, panelists detected reduced firmness in the samples with added EVOO, without 445 added cryoprotectants and without processing. One can observe that the processed samples 446 with the lower and higher EVOO concentrations were the firmest, whereas in the systems with 25 g kg⁻¹ added EVOO the fresh samples had similar firmness than the control (Fig. 447 448 4a). In the MPA samples there were no differences between firmness scores in fresh and 449 processed samples (Fig. 4b); however, panelists detected increased firmness in processed 450 MP with added κ -C and XG, matching the result for textural properties in MPB samples 451 (Figs. 1c,f).

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453 Attributes perceived at the time of preparing the sample in the mouth for swallowing. 454 EVOO concentration, cryoprotectant addition and processing also had a significant effect 455 on cohesiveness, adhesiveness and fibrousness (1) (Table 3). When EVOO concentration 456 was increased, cohesiveness and adhesiveness scores decreased significantly in the MPB 457 samples (Figs. 4c,d). In the MPA samples there were no significant differences between 458 the adhesiveness scores of fresh and processed samples (Fig. 4e), whereas panelists scored 459 the processed MPB samples higher for adhesiveness than their fresh counterparts. Scores

for fibrousness (1) also decreased with increasing EVOO concentration, with cryoprotectant addition and with processing (Figs. 4f,g). Again, addition of cryprotectants reduced differences in fibrousness (1) between fresh and processed samples. This is probably related to the fact that the hydrocolloids can make systems in the rubbery state more viscous, reducing molecular mobility and preventing retrogradation (Ferrero *et al.* 1994).

466

467 Attributes perceived during final and residual phases of mastication. The three factors 468 studied had a significant effect on ease of swallowing and fibrousness (2) (Table 4), 469 whereas only EVOO concentration had a significant effect on palate coating. In samples 470 both without and with added cryoprotectants (Fig. 4h) and in both FMP and F/TMP 471 samples (Fig. 4i), ease of swallowing scores increased with increasing oil content. 472 However, only when EVOO was added at concentrations of 0 and 10 g/kg, the scores for 473 this attribute were higher in the samples without cryoprotectants and in the processed 474 samples. Panelists also scored the samples with added EVOO significantly higher for 475 palate coating than the ones made without EVOO (Figs. 5a,b). Scores for palate coating 476 were higher in the MPA samples with 25 and 50 g/kg added EVOO than in their MPB 477 counterparts (Fig. 5a), and the EVOO content had a much smaller effect in the F/TMP 478 samples than in the fresh counterparts (Fig. 5b). Palate coating scores for MPA samples 479 decreased after processing whereas scores for MPB samples increased with respect to the 480 fresh products (Fig. 5c). Also, in the MPA samples, the addition of EVOO at all 481 concentrations significantly reduced sample fibrousness (2) (Fig. 5d).

482 A complete dependence study was performed on the instrumental textural properties 483 versus sensory attribute scores. Low correlations between instrumental and sensory ratings 484 were found. Previous publications by other researchers generally agree on good to

excellent correlations for hardness (based on calculated "*r*" values (Szczesniak 2002). Correlations for other parameters are usually less good and product-dependent. In this study, relatively good correlations with sensory denseness and adhesiveness scores were found only in the case of viscosity index ($R^2 = 0.81$ and 0.76, respectively). Differences in consistency observed among samples were explained by viscosity index, but not the variation in granularity or fibrousness, determining the sample creaminess.

491

492 **Overall acceptability.** EVOO concentration, cryoprotectant addition and processing had a 493 significant effect on the OA of the samples (Table 4). Scores for OA increased 494 significantly with increasing EVOO content in both MPA and MPB samples (Fig. 5e), and 495 likewise in both FMP and F/TMP samples (Fig. 5f). Similarly, a positive relationship 496 between oil content and sensory acceptability has been observed in a set of Polish 497 commercial mayonnaises (Juszack et al. 2003) and in salami (Severini et al. 2003). In this 498 study, the main differences between samples without and with added EVOO were ascribed 499 to either an aromatic or a creamy note detected in the oil-added MP. Samples with higher 500 percentages of EVOO produced less sensations of dryness and roughness, more sensations 501 of flavour, creamy and fatty mouth- and after-feel than the samples without added oil. Fat 502 is a well-known enhancer of creaminess sensations (de Wijk et al. 2003). The latter authors 503 suggested that the possible mechanism by which fat affects the sensory attributes include 504 lubrication and flavour release. The effects of fat on odour and flavour attributes may be 505 related to the flavour-releasing properties of fat.

Panelists scored the MPB and F/TMP samples higher for OA (Figs. 5e,f). This is probably related to the presence of XG in the systems. It was found that samples containing blends of κ -C and XG (Alvarez *et al.* 2009; Fernández *et al.* 2009), were preferred organoleptically due to the creamy mouthfeel they produced. The effects of XG

510 on mouth texture may be related to its WHC as perceived by the panelists. Besides, in the 511 processed MPB samples, there were no significant differences between the OA scores 512 given to the MP at any concentration of added EVOO (which were the highest). This has 513 important consequences for the formulation of EVOO-based MP. Results indicate that in 514 the presence of κ -C and XG, if the EVOO content is reduced to below 25 g/kg, the OA 515 score for the product does not decrease, and hence its consumer acceptability is not 516 adversely affected.

517

518 Microstructure Examination

519

520 To achieve a better understanding of the sensory and rheological results and the effect of 521 adding cryoprotectants and of freezing/thawing, the microstructure of the MP samples was 522 studied by SEM (Figs. 6, 7). Fig. 6a shows a microphotograph of the fresh control without 523 either added cryoprotectants or oil. Cooked cells are still distinguishable and firmly bound 524 together by a continuous network of amylose. However, in the fresh control without added 525 EVOO but with added cryoprotectants (Fig. 6b), less complete cells are visible, appearing 526 separated from one another and embedded in a continuous network of amylose and κ -C in 527 which starch granules and XG aggregates are entrapped. Probably, the presence of 528 cryoprotectants occluding a great amount of water probably facilitated loss of the original 529 cell shape.

530 Microphotographs of the corresponding processed counterparts (Figs. 6c,d) show that 531 freezing and thawing of MPA and MPB samples resulted in completely dissolved cells. 532 Part of the intracellular water was drawn out osmotically because of freezing-induced 533 concentration of the cell mass. Cell tearing is probably caused by the formation of ice 534 crystals. Fresh MPA sample contain more complete cells (Fig. 6a), which could give them

535 greater mechanical strength; this would justify that the values of the textural properties 536 were higher in fresh MPA samples than in their processed counterparts. In turn, the 537 processed MPA sample without added EVOO (Fig. 6c) developed a spongy appearance 538 due to amylose and amylopectin retrogradation occurring during freezing and frozen 539 storage (Ferrero *et al.* 1994).

540 The microphotograph of processed MPB sample without added EVOO (Fig. 6d) shows 541 the presence of fibres or strands. According to Giannouli and Morris (2003), during 542 freezing, XG chains are forced to align and associate by conversion of water to ice crystals. 543 The forced associations survive upon thawing to give a cryogel network. It is likely that 544 such strands are related to this XG conformational transition, since they were observed in 545 most of the F/TMP containing cryoprotectants. Formation of strands can be explained by a 546 progressive increase in local concentration of the polymer as liquid water is converted into 547 ice crystals, promoting intermolecular associations.

548 Fig. 7 shows microphotographs of the counterparts of the samples shown in the Fig. 6, 549 but with 50 g/kg added EVOO. When EVOO was added, a dispersed thin phase or layer of 550 oil formed, enveloping all the microstructures constituting the MP. Fig. 7a shows some oil 551 droplets in MPA sample, probably formed by aggregation through steric and/or 552 electrostatic forces (Paraskevopoulou et al. 2005), whereas Fig. 7b shows no oil droplets in 553 presence of κ -C and XG. In MPA samples, freezing also had a negative influence on the 554 formation of oil droplet clusters (Fig. 7c); it is likely that the structural damage caused by 555 freezing enabled the oil droplets to come close enough together to aggregate. 556 Microphotograph of the processed sample with 50 g/kg added EVOO (Fig. 7d) shows that 557 white gel structures are also discernible in the presence of cryoprotectants.

558 Addition of XG to salad dressings induces depletion flocculation of the droplets and 559 formation of a three-dimensional weak gel network structure that retards the process of

560 droplet creaming (Parker et al. 1995). Adding a hydrocolloid causes protein-coated 561 droplets to aggregate and be excluded from the region of continuous phase between them. 562 Therefore, in the MP with added κ -C and XG and oil, the XG may have been adsorbed 563 onto the surface of the droplets, enhancing stability against flocculation and coalescence 564 and forming the white film observed in both microphotographs (Figs. 7b,d). On the other 565 hand, there are no noticeable differences between FMP and F/TMP samples with added κ -566 C and XG and oil, confirming that the addition of κ -C and XG significantly reduced 567 quality differences between FMP and their F/TMP counterparts.

568

569 CONCLUSION

570

571 The addition of either EVOO or cryoprotectants and processing significantly affected the 572 physical, structural and sensory characteristics of MP, although the effect of EVOO 573 concentration depended on the presence of cryoprotectants and on freezing/thawing. 574 Increased EVOO concentration resulted in less structured systems and enhancement of 575 color due to an increase in overall light scattering and pigment content. Addition of κ -C 576 and XG improved thickness, possibly through the exclusion effect of swollen starch 577 granules promoting gelation of the κ -C. Addition of EVOO in increasing concentrations 578 enhanced the sensory quality of MP in terms of reduced granularity, denseness, 579 cohesiveness, adhesiveness and fibrousness, and increased homogeneity, ease of 580 swallowing and palate coating. Instrumental texture measurements were able to distinguish 581 the variations in mechanical textural attributes scored by the panellists. Conversely, 582 geometrical textural attributes (granularity, homogeneity and fibrousness) have to be 583 support by structural traits. Creaminess was the most crucial factor for OA of the products 584 and could be explained by the presence of EVOO aggregates observed by microstructure

- 585 analysis. Samples with 50 g/kg added EVOO were judged the best of all. There is a
- 586 possibility of using EVOO in combination with MP to provide a highly nutritious product
- 587 with improved physicochemical, functional and sensory characteristics.

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699 FIGURE LEGENDS

700 FIG. 1. TEXTURAL PROPERTIES OF MASHED POTATOES WITH ADDED EXTRA

701 VIRGIN OLIVE OIL (EVOO)

- 702 (A-C) Firmness; (D-F) Average force; MPA, MPB: mashed potatoes without and with
- added cryoprotectants respectively; FMP, F/TMP: fresh and frozen/thawed mashedpotatoes respectively.
- FIG. 2. COLOR PARAMETERS AND EXPRESSIBLE WATER OF MASHED
 POTATOES WITH ADDED EXTRA VIRGIN OLIVE OIL (EVOO)
- 707 (A-C) L*, lightness; a*, red-greeness; YI, yellowness index; (D) E_w, expressible water;
- 708 MPA, MPB: mashed potatoes without and with added cryoprotectants respectively; FMP,
- 709 F/TMP: fresh and frozen/thawed mashed potatoes respectively.
- FIG. 3. TPA SENSORY ATTRIBUTES OF MASHED POTATOES WITH ADDED
 EXTRA VIRGIN OLIVE OIL (EVOO)
- (A, B) Granularity; (C) Moisture (1); (D) Stickiness; (E, F) Denseness; (G, H)
 Homogeneity; (I) Moisture (2); MPA, MPB: mashed potatoes without and with added
 cryoprotectants respectively; FMP, F/TMP: fresh and frozen/thawed mashed potatoes
 respectively.
- 716 FIG. 4. TPA SENSORY ATTRIBUTES OF MASHED POTATOES WITH ADDED
- 717 EXTRA VIRGIN OLIVE OIL (EVOO)
- 718 (A, B) Firmness; (C) Cohesiveness; (D, E) Adhesiveness; (F, G) Fibrousness (1); (H, I)
- T19 Ease of swallowing; MPA, MPB: mashed potatoes without and with added cryoprotectants
- respectively; FMP, F/TMP: fresh and frozen/thawed mashed potatoes respectively.
- 721 FIG. 5. TPA SENSORY ATTRIBUTES AND OVERALL ACCEPTABILITY OF
- 722 MASHED POTATOES WITH ADDED EXTRA VIRGIN OLIVE OIL (EVOO)

- 723 (A-C) Palate coating; (D) Fibrousness (2); (E, F) Overall acceptability (OA); MPA, MPB:
- mashed potatoes without and with added cryoprotectants respectively; FMP, F/TMP: fresh
- and frozen/thawed mashed potatoes respectively.
- 726 FIG. 6. MICROPHOTOGRAPHS OF MASHED POTATOES
- 727 (A) Fresh sample without added cryoprotectants; (B) Fresh sample with added 728 cryoprotectants; (C) Processed sample without added cryoprotectants; (D) Processed 729 sample with added cryoprotectants; Magnification was 200 (bar = $100 \mu m$).
- 730 FIG. 7. MICROPHOTOGRAPHS OF MASHED POTATOES WITH ADDED EXTRA

731 VIRGIN OLIVE OIL (EVOO)

- (A) Fresh sample without added cryoprotectants and with 50 g/kg added EVOO; (B) Fresh
- sample with added cryoprotectants and with 50 g/kg added EVOO; (C) Processed sample
- vithout added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with
- added cryoprotectants and with 50 g/kg added EVOO; Magnification was 200 (bar = 100
- 736 μm).
- 737





FIGURE 2









■ MPA □ MPB

FIGURE 4

 $\blacksquare MPA \ \Box MPB$

■ FMP □ F/TMP





Source	Firmness	Viscosity	Work per	Average
	(N)	index (N s)	displaced	force (N)
			volume	
			(J/m^3)	
Main effects:				
A:EVOO concentration (g/kg)				
0	6.21 a	-29.33 a	3518.78 a	1.51 a
10	6.08 a	-28.37 a	3462.25 a	1.49 a
25	5.32 b	-26.12 b	2867.16 b	1.23 b
50	4.69 c	-23.06 c	2681.10 b	1.15 b
50b	4.74 c	-23.69 c	2786.29 b	1.19 b
<i>P</i> values	< 0.001	< 0.001	< 0.001	< 0.001
LSD (99%)	0.26	1.24	227.00	0.097
B:Cryoprotectant addition				
Without κ -C and XG	4.71 a	-20.71 a	2333.51 a	1.00 a
With κ -C and XG	6.10 b	-31.52 b	3792.72 b	1.63 b
<i>P</i> values	< 0.001	< 0.001	< 0.001	< 0.001
LSD (99%)	0.16	0.78	143.57	0.06
C:Processing				
Fresh	5.62 a	-26.73 a	3248.00 a	1.39 a
Frozen/thawed	5.19 b	-25.50 b	2878.23 b	1.23 b
<i>P</i> values	< 0.001	< 0.001	< 0.001	< 0.001
LSD (99%)	0.16	0.78	143.57	0.06
Interactions				
AB	< 0.001	< 0.001	< 0.001	< 0.001
AC	0.001	0.18	< 0.001	< 0.001
BC	< 0.001	< 0.001	< 0.001	< 0.001
ABC	< 0.001	< 0.001	< 0.001	< 0.001

TABLE 1.	EFFECTS	OF EVOO	CONCENTR.	ATION,	CRYOPROT	ECTANT	ADDITION
AND FREE	EZING/THA	AWING ON	TEXTURAL	PROPE	RTIES OF M	Р	

Source	L^*	<i>a</i> *	YI	$E_{\rm w}$ (%)
Main effects:				
A:EVOO concentration (g/kg)			
0	60.79 a	-3.85 a	10.14 a	22.42 b
10	62.95 b	-3.70 b	13.73 b	25.73 a
25	63.68 c	-3.54 c	18.58 c	20.72 d
50	66.04 d	-3.11 d	24.08 d	21.52 c
50b	65.70 e	-3.14 d	23.47 e	21.85 b, c
P values	< 0.001	< 0.001	< 0.001	< 0.001
LSD (99%)	0.07	0.02	0.20	0.71
B:Cryoprotectant addition				
Without κ -C and XG	62.85 a	-3.59 a	16.74 a	-
With κ -C and XG	64.81 b	-3.34 b	19.26 b	-
P values	< 0.001	< 0.001	< 0.001	-
LSD (99%)	0.04	0.01	0.13	-
C:Processing				
Fresh	63.33 a	-3.53 a	18.03 a	21.01 a
Frozen/thawed	64.33 b	-3.40 b	17.97 a	23.89 b
P values	< 0.001	< 0.001	0.17	< 0.001
LSD (99%)	0.04	0.01	0.13	0.45
Interactions				
AB	< 0.001	< 0.001	< 0.001	-
AC	< 0.001	< 0.001	< 0.001	< 0.001
BC	< 0.001	0.34	< 0.001	-
ABC	< 0.001	< 0.001	< 0.001	-

TABLE 2. EFFECTS OF EVOO CONCENTRATION, CRYOPROTECTANT ADDITION AND FREEZING/THAWING ON COLOR MEASUREMENTS AND EXPRESSIBLE WATER OF MP

Sensory attributes Perceived before putting		Perceived at the time of putting the sample in the mouth				Perceived at the time of preparing the sample				
-	the sample	in the mouth						for swallowing		
Source	Granularity	Moisture (1)	Stickiness	Denseness	Homogeneity	Moisture (2)	Firmness	Cohesiveness	Adhesiveness	Fibrousness (1)
Main effects:										
A:EVOO concentration	< 0.001	0.022	0.487	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
B:Cryoprotectant addition	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
C:Processing	< 0.001	< 0.001	0.542	< 0.001	< 0.001	< 0.001	0.002	0.002	< 0.001	< 0.001
Interactions										
AB	< 0.001	0.002	0.002	0.236	< 0.001	< 0.001	0.044	< 0.001	< 0.001	< 0.001
AC	< 0.001	0.003	< 0.001	< 0.001	< 0.001	0.082	< 0.001	0.292	0.818	< 0.001
BC	0.015	0.001	0.611	< 0.001	0.970	0.003	< 0.001	0.083	< 0.001	< 0.001
ABC	< 0.001	< 0.001	< 0.001	0.143	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

TABLE 3. EFFECTS OF EVOO CONCENTRATION, CRYOPROTECTANT ADDITION AND FREEZING/THAWING ON SENSORY ATTRIBUTES PERCEIVED BEFORE AND AT THE TIME OF PUTTING THE SAMPLE IN THE MOUTH, AND AT THE TIME OF PREPARING THE SAMPLE FOR SWALLOWING OF MP

TABLE 4. EFFECTS OF EVOO CONCENTRATION, CRYOPROTECTANT ADDITION AND FREEZING/THAWING ON SENSORY ATTRIBUTES PERCEIVED DURING THE FINAL AND RESIDUAL PHASES OF MASTICATION AND OVERALL ACCEPTABILITY OF MP

Sensory attributes	Perceived du	Overall		
		acceptability		
				(OA)
Source	Ease of	Palate	Fibrousness (2)	
	swallowing	coating		
Main effects:				
A:EVOO concentration	< 0.001	< 0.001	< 0.001	< 0.001
B:Cryoprotectant addition	< 0.001	0.520	< 0.001	< 0.001
C:Processing	< 0.001	0.601	< 0.001	< 0.001
Interactions				
AB	< 0.001	< 0.001	< 0.001	< 0.001
AC	0.003	< 0.001	< 0.001	< 0.001
BC	0.126	< 0.001	< 0.001	0.055
ABC	0.211	< 0.001	< 0.001	0.015







FIG. 7. MICROPHOTOGRAPHS OF MASHED POTATOES WITH ADDED EXTRA VIRGIN OLIVE OIL (EVOO)

(A) Fresh sample without added cryoprotectants and with 50 g/kg added EVOO; (B) Fresh sample with added cryoprotectants and with 50 g/kg added EVOO; (C) Processed sample without added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectants and with 50 g/kg added EVOO; (D) Processed sample with added cryoprotectan