UNDERSTANDING EMULSIFIERS EFFECT ON BREAD AERATION DURING BREADMAKING

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Running tittle: Emulsifiers role in bread aeration

Abstract

BACKGROUND: Much research has been done to explain emulsifiers action during breadmaking, but there is still plenty unknown to elucidate their functionality despite their diverse chemical structure. The aim of the present study was to provide some light about the role of emulsifiers on air incorporation into the dough and gas bubbles progress during baking and their relationship with bread features. Emulsifiers like diacetyl tartaric acid ester of monoglycerides (DATEM), sodium stearoyl lactylate (SSL), distilled monoglyceride (DMG-45 and DMG-75), lecithin and polyglycerol esters of fatty acids (PGEF) were tested in very hydrated doughs. RESULTS: Emulsifiers increased the maximum dough volume during proofing. Emulsifiers increased the number of bubbles incorporated during mixing, observing higher number of bubbles, particularly with PGEF. Major changes in dough occurred at 70 K when bubble size augmented, becoming more heterogeneous. DMG-75 produced the biggest bubbles. As a consequence, emulsifiers tend to increase the number of gas cells with lower size in the bread crumb, but led to greater crumb firmness, which suggested different interactions between emulsifiers and gluten, affecting protein polymerization during baking. CONCLUSION: Bubbles progress during baking allowed discriminate among emulsifiers, which could explain their performance in breadmaking.

Keywords: emulsifier, image analysis, bubble, dough aeration, bread, crumb

Introduction

Bakery products are extensively consumed worldwide due to their nutritional and physical characteristics. [1] Among the diversity of bakery products obtained from either different raw ingredients or making processes, the most appreciated products are the sponge baked wheat bread, with low density and soft crumb. In the course of flour and water mixing, gluten formation and aeration brought about during kneading will be responsible of the subsequent cellular structure of the baked bread. [2] Air incorporated into the dough during mixing must be kept through the breadmaking process to attain low density breads. Bread contains about 70% of gas that comes from the initial aeration and the fermentation, both are important stages to take into account during the making process. [3] Because of that air bubbles incorporation during mixing have been the focus of many studies that stated the influence of mixer type and mixing time, [4, 5] besides the important role of ingredients. [6, 7] Certainly, the progress of those initial nuclei bubbles throughout fermentation when carbon dioxide is generated [8] and final expansion of the gases occluded into the bubbles during baking determines the diversity of cellular structures encountered on bread crumbs. [3] Bubbles are very fragile and whatever changes in their number and size will have a direct impact on the internal crumb structure. [9]

Nowadays, large-scale production and consumers demand for higher quality, homogeneity and longer shelf life that have been achieved with the use of processing aids such as enzymes, hydrocolloids, emulsifiers, etc. to adjust doughs properties. These additives are essentials for improving dough properties and final quality of fresh product. [10] Specifically, emulsifiers are active surfactant composites used in breadmaking for their ability to stabilize dough, a thermodynamically unstable system, through their interactions with gluten proteins. [11] During mixing, the use of emulsifiers

increases the strength and the extensibility of the dough; in the fermentation stage they improve gas retention and avoid dough collapse, [11, 12] leading to softer bread crumbs, [13] although their effect is greatly dependent on the wheat flour protein content [14] and proofing duration. [15] Those studies confirmed the effect of different emulsifiers in breadmaking processes, specifically in improving the internal structure of bread. [16] In spite of the knowledge acquired on emulsifiers action during breadmaking, they are still attracting research due to there is still much unknown to explain their functionality despite their chemistry diversity. For instance, despite the impact of dough aeration into bread crumb features, there is no information about the role of emulsifiers on dough aeration and the bubbles number and size along the process. To understand the role of emulsifiers on determining the cellular structure of bread crumb, the main objective of this study was to assess the amount of gas occluded into the dough and bread along bread making and how several emulsifiers with diverse chemical structure affected the bubble size distribution.

Materials and methods

Breadmaking wheat flour was supplied by Harinera La Meta (Lleida, Spain) and compressed yeast by (DHW Europe, Germany). The selected emulsifiers included: diacetyl tartaric acid ester of monoglycerides (DATEM), sodium stearoyl lactylate (SSL) and distilled monoglyceride (with potassium citrate added) with two different particle sizes 45 microns (DMG-45) and 75 microns (DMG-75), which were provided by Danisco (Grindsted, Denmark), defatted hydrolyzed sunflower lecithin (Tricalcium phosphate) from Lasenor (Barcelona, Spain), and Polyglycerol esters of fatty acids containing polysorbate 80 (PGEF) from Palsgaard (Juelsminde, Denmark).

85

86 *Gas bubbles during fermentation and baking*

87 A very hydrated dough recipe containing wheat flour, water (900 ml kg^{-1} based on
88 wheat flour weight) and 10 g kg^{-1} compressed yeast was used. Emulsifiers were added
89 at 5 g kg^{-1} (f.b.) whenever tested. Ingredients were mixed during 3 minutes at 328 rpm
90 in a mixer (RZR-1 Heidolph, Schwabach, Germany).

91 The gas released and dough development characteristics during fermentation were
92 recorded using the Rheofermentometer F3 (Chopin, France), slightly modifying the
93 instructions given by supplier. Briefly, hydrated dough (315 g) were confined in a glass
94 recipient. The tests were performed on dough at 30 K for 3 hours, with a slight
95 cylindrical weight. Registered parameters included: Hm (mm), maximum dough
96 fermentation height; T1, the time (min) at which Hm is attained; H′m (mm) maximum
97 height of gaseous release; T′1, the time (min) at which H′m is reached; Tx, the time
98 (min) at which gas starts to escape from the dough, thus when porosity of dough
99 develops. All determinations were made at least in duplicate, and the average values
100 were adopted.

101 A microscope was used to follow bubble changes of dough during baking as previously
102 described Rodríguez-García, Salvador and Hernando [17] For that purpose, doughs were
103 prepared as described before but without the addition of yeast to follow behavior of
104 bubbles from air incorporation. Microbaking was performed using a system controller
105 unit for heating and freezing stages (Analysa-LTS350, Linkam, Surrey, UK) mounted
106 under the lens of a light microscope (Nikon ECLIPSE 80i, Nikon Co., Ltd., Tokyo,
107 Japan). The temperature profile settings were from 30 K to 105 K increasing at 1.5 K
108 min^{-1}. Samples were captured at ×4 magnification (objective lens ×4/0.13∞/– WD 17.1,

109 Nikon). During microbaking, a video film was recorded with an attached camera
110 (ExWaveHAD, model no. DXC-190) and images were acquired every 10 K. The
111 analysis software (Linksys 32, Linkam) was directly interfaced with the microscope,
112 enabling temperature control and image recording control. Duplicates were recorded.
113 The number, size and distribution of the bubbles in the dough were analyzed using the
114 ImageJ software (National Institutes of Health, Bethesda, MD, USA).

115

116 *Bread making and characterization*

117 A scale-down breadmaking method was carried out [18] to identify the emulsifiers effect.
118 Recipes were prepared as described before, and then four grams of dough were placed
119 in previously oiled cylindrical glass molds (17 mm x 300 mm, diameter x height). They
120 were fermented for 100 min at 30 K and finally baked at 130 K for 11 min. Two batches
121 were run for each sample.

122 Texture profile analysis of crumbs was carried out in a TA-XTPlus (Stable Micro
123 Systems, Surrey, UK). A 10 mm thick slices were compressed twice with a 0.6 mm
124 diameter probe up to 50% at 1 mm s^{-1} speed. The registered parameters were crumb
125 hardness (g), springiness, cohesiveness, chewiness (g) and resilience. In order to study
126 cell crumb distribution and morphogeometric characteristics of the loaves, both cross
127 and longitudinal sections of breads were captured using a scanner (HP Scanjet G3110,
128 Hewlett-Packard, USA) with 600 dpi resolution. The 2D area and perimeter of
129 longitudinal section was assessed using ImageJ software. The same software was used
130 to analyze the cell crumb distribution in 10x10 mm crumb cross-sections. Image section
131 was improved by splitting RGB channels and selecting the channel with greater contrast
132 between background and object. Finally, Otsu algorithm (predefined by the software)

was applied to convert image into a binary image and particle analysis of the image was carried out. The parameters assessed were cell/cm^2, mean area (mm^2) and circularity (from 0, rectangle, up to 1, perfect circle). Six slices were used for each determination.

Statistical analysis

Experimental data were statistically analyzed by analysis of variance (ANOVA) using Statgraphics Centurion XVI.I 16.1 software (Statistical Graphics Corporation, UK), to identify significant differences among them. Cluster analysis and principal components (PCA) were also performed to discriminate among emulsifiers with the tested variables.

Results and Discussion

Dough development and gaseous release characteristics

To evaluate the action of diverse emulsifiers on dough performance during breadmaking, very hydrated doughs were used. The effect of emulsifiers on gas retention during dough fermentation was recorded in the rheofermentometer plots (Figure 1). After an initial elapsed time, a steady increase of dough volume was displayed, but certain variation was observed in the presence of emulsifiers (Figure 1a). Lecithin and PGEF delayed the onset of volume increase compared to the control and the other emulsifiers. All emulsifiers increased the proofing rate, calculated as the initial slope of dough volume increase (Table 1). The maximum dough development (Hm) reached in the presence of the emulsifiers was higher than the one observed in the control dough, being greater in the case of PGEF (34.6 mm), followed by SSL and DMG-75 (33.0 mm and 32.4 mm, respectively). The presence of polysorbate blended

156 with the PGEF might contribute to the high volume obtained due to its action as
157 dispersing agent. This result agrees with those obtained by Gómez et al., Gómez, del
158 Real, Rosell, Ronda, Blanco and Caballero. [19] where polysorbate addition to low
159 hydrated doughs led to higher dough volumes than other emulsifiers as DATEM and
160 SSL. Nevertheless, the time (T1) required to reach the maximum dough development
161 was higher in the presence of emulsifiers than in the control, confirming that emulsifiers
162 are much more effective when longer dough fermentations are applied. [19] This
163 improvement has been ascribed to the emulsifiers ability for strengthening the gluten
164 network, increasing dough extensibility [19] and dough volume, [16] which in turn was
165 attributed to the formation of aggregates with gluten proteins. [20] However, that effect
166 cannot be explained only by the emulsifier chemical structure, given that distilled
167 monoglycerides with different particle size (DMG-45 and DMG-75) produced different
168 responses. Dough stability during fermentation was greatly dependent on the emulsifier
169 tested, and only lecithin and DMG-45 extended the stability of the dough longer than
170 the control.

171 Regarding the gas production during fermentation (Figure 1b), the most evident effect
172 was the decrease in the initial CO_2 production when emulsifiers were present. It seems
173 that emulsifiers, independently of their chemical structure, affected the initial release of
174 carbon dioxide. Taking into account that no sugar was added in the recipe, possible
175 explanations could be related to either some interactions between emulsifiers and the
176 free sugars, available in the flour for proofing, that decrease their readiness for the yeast
177 or due to physical constraints derived of the more ordered and stronger protein structure
178 in the presence of emulsifiers. [21] As the proofing progresses, main difference was
179 observed during the last hour of fermentation when a decrease on the CO_2 production
180 was observed, due to dough permeability to gas in some of the doughs. Doughs with

181 DMG-45, DMG-75 and lecithin showed greater permeability than the control, which
182 resulted in a decrease of the ability to retain CO_2 at the end of the fermentation. The
183 highest CO_2 production was with DATEM addition.

184

185 **Microscopy and analysis image of simulated microbaking**

186 The ability of the emulsifiers to stabilize the gas bubbles, incorporated into the doughs
187 during mixing, was continuously monitored under a microscope. Very hydrated doughs
188 were subjected to a steady temperature increase to simulate the baking process and
189 consequently the capacity of the dough to hold the gas. Turbin-Orger, Boller, Chaunier,
190 Chiron, Della Valle and Réguerre [22] suggested that the liquid fraction present in the
191 dough influence the cellular structure by affecting the connectivity of bubbles and their
192 possible coalescence. In this study, very hydrated doughs were used to discard the
193 possible interference of liquid effect. The captured images of doughs along temperature
194 increase are shown in Figure 2. Initially, differences in the structure of the doughs were
195 barely visible. Junge, Hoseney and Varriano-Marston [23] reported that emulsifiers
196 increase the incorporation of gas bubbles during mixing, but they did not find
197 modifications during the baking stage. However, dough images (Figure 2) showed
198 progressive changes with the temperature increase and major changes were observed
199 when reaching 70 K. Babin, Della Valle, Chiron, Cloetens, Hoszowska, Pernot,
200 Réguerre, Salvo and Dendievel [24] reported that the cell structure stabilization occurs
201 with the temperature range 50–70 K when main changes associated to starch granule
202 swelling and gluten cross-linking are produced. In all cases, the bubble size augmented
203 as the temperature increased and their number and size were dependent on the type of
204 emulsifier. The most important differences were observed when using DMG-75: bigger
205 bubbles were observed at low temperature (40 K) if compared to the doughs prepared

206 with the other emulsifiers, and these bubbles were really big at 70 K, giving place to the
207 biggest bubbles at 100 K.

208 Quantitative analysis of the bubbles distribution and size is shown in Figure 3, where
209 distributions were ordered from smaller to larger bubble width when temperature
210 increased. In all the samples, the addition of emulsifiers increased the number of
211 bubbles incorporated during mixing if compared to control, which may be due to the
212 lower surface tension induced by the addition of emulsifiers. Kokelaar, Garritsen and
213 Prins [25] showed that addition of some emulsifiers as SSL and DATEM originated more
214 and smaller bubbles during mixing, because of the lower surface tension of dough
215 inducing the subdivision of the entrapped air bubbles. When comparing the doughs
216 prepared with the different emulsifiers (Figure 3), the dough formulated with PGEF
217 presented greater incorporation of air bubbles during mixing, as the diagram
218 corresponding to this emulsifier shows greater frequency of bubbles at the beginning of
219 the micro baking process. Through temperature rise, all the samples, including control,
220 showed an increase in the amount of detected bubbles, and bubbles size distribution
221 became more heterogeneous due to expansion and interaction of the bubbles. The
222 doughs prepared with DATEM, Lecithin and DMG- 45 presented a frequency
223 distribution similar to that obtained for the control dough; in fact, the size of the bubbles
224 increased in a uniform, controlled way (Figure 2). All these doughs showed small
225 bubbles at low temperatures and a tendency to regular distribution of bubbles during
226 heating; moreover, bubbles exceeding 120.000 μm^2 were not generally detected
227 regardless of the heating temperature. Nevertheless, the samples prepared with SSL,
228 PGEF and DMG-75 exhibited bigger bubbles, over 120.000 μm^2. Specifically, DMG-75
229 dough diagram presented very big bubbles, which continued interacting and coalescing
230 even at 100 K. When temperature reached 100 K the samples containing SSL, PGEF

231 and DMG-75 presented coarser distribution of bubbles, while DATEM, DMG-45 and
232 lecithin had more bubbles but smaller ones. When baking temperature rises, the bubbles
233 expand increasing the coalescence due to Ostwald maturation, [26] where big bubbles
234 grow up at the expense of small ones, consequently there is an increase in its size. With
235 the addition of the emulsifiers, this phenomenon often decreased, due to the
236 stabilization of the interface. [27] However, in the present work, it can be observed that
237 depending on the emulsifier used in the dough formulation, the expansion of bubbles is
238 controlled in a different way, being DATEM, DMG-45 and lecithin more effective for
239 controlling this mechanism. It must be stressed that besides the different chemical
240 structure of the emulsifiers, their physical structure must be considered, since DMG-45
241 and DMG-75 induced different bubble stabilization.

242

243 **Image digital analysis and texture profile of breads**

244 The effect of emulsifier addition on technological characteristics is summarized in
245 Table 2. Compared to the control, significantly smaller longitudinal area was produced
246 by PGEF addition, which meant a reduction in the size of the loaves. The rest of the
247 emulsifiers did not significantly modify this parameter. Previous studies reported that
248 adding SSL and DATEM resulted in higher area and volume of breads, due to the
249 increase in dough aeration and volume. [28, 29] Probably the use of high hydrated doughs
250 is responsible for the differences with previous studies. In addition, a negative
251 correlation (r=-0.8754) was observed between the longitudinal area of the small scale
252 breads and the maximum height of the proofed dough (Hm). This correlation indicated
253 that dough volume increased during fermentation with emulsifiers addition but likely
254 they did not confer enough resistance to improve final volume. Likewise, no significant

differences were found in the longitudinal perimeter, except with DATEM and PGEF that gave smaller values.

The analysis of the bread cross section revealed significant differences in the number of gas cells (cell cm^{-2}) and mean cells area (mm^2) on account of emulsifiers addition (Table 2, Figure 4). The more number of cells, the less mean cell area and vice versa. DMG-45, DMG-75, DATEM and Lecithin showed greater cell number with smaller area than the control. In the case of distilled monoglycerides emulsifier (DM) with different particle size (DMG-45 and DMG-75), no significant difference was observed in these parameters, showing that particle size did not affect the cell number and area. Emulsifiers did not induce a significant effect on the circularity compared to the control. However, significant differences between DMG-75 (0.60) and PGEF (0.74) were found. Perfect circularity is difficult to obtain in bread, due to the pressure differences in the gas bubbles and changes occurred during process. [25]

All samples showed significant differences in all texture parameters compared to control (Table 2). With the hydrated recipe used very soft crumbs were obtained, and emulsifiers increased the crumb hardness although variation ranged from 79 to 100 g. The highest hardness was obtained with the PGEF, followed by SSL and DATEM. DMG-45, DMG-75 and lecithin were the emulsifiers that less rise the crumb hardness. Hardness showed a strong positive correlation (r=0.9373) with the maximum height of dough (Hm) during fermentation, but that contrasts with results obtained when optimum hydration of wheat flour (500-600 ml kg^{-1}) was used. [12] Dough hydration affects the size of the bubbles diameter, [30] leading to higher bubbles, but since all recipes were prepared with the same hydration it should be expected no additional effect due to the liquid phase. Considering the high number of smaller cells mostly found in the breads

containing emulsifiers, the hardness increase must respond to the thickness of the cell walls. Therefore, in this study the interaction of emulsifiers with proteins and starch leading to the cell walls had greater impact on texture than the bubbles feature. It has been previously reported that a higher degree of gluten polymerization during baking results in higher firmness of the baked products. [11] At the same time, emulsifiers, like SSL or DATEM interact with gluten, changing the solubilization of polymeric aggregates and that interaction was dependent on the type of emulsifier, [20] particularly SSL reduces the incorporation of gliadins into the gluten network having a direct effect on the subsequent polymerization during baking, [11] and in turn affecting crumb firmness. Therefore, at the level of hydration used in the present study, emulsifiers contributed to increase dough aeration and in consequence the number of gas cells in the crumb, but simultaneously their interaction with gluten changed the proteins polymerization during baking affecting cell walls thickness and in turn crumb firmness.

Considering the other texture parameters, chewiness was significantly higher in the samples with emulsifiers, except with DMG-75, than in the control, and resilience decreased especially in samples with distilled monoglycerides (DMG-45 and DMG-75), which again differed than the previously reported with optimum hydrated doughs, [12] confirming the important role of water on the dough aeration and crumb texture.

Statistical analysis

In order to understand the effect produced by the emulsifiers and the differences or correlations between them, a cluster analysis (Figure 5) and an analysis of principal components (Figure 6) were carry out. Cluster analysis showed the discrimination between breads containing emulsifiers and the control by combining the two

observations that were closest to form the groups. Three well differentiated groups were drawn, with the control and DMG-75 being more separated from the rest of the samples. The other emulsifiers were closer in relation to the analyzed variables, evidencing more similar effects in the doughs and final product. According to their performance with dough and bread, the closest emulsifiers were DATEM and lecithin, followed by DMG-45, SSL and finally PGEF. In this study, the closeness observed between lecithin and DATEM was attributed to the production treatment of lecithin that included a hydrolysis stage, thus it behaves as a monoglyceride despite of being from a diglyceride.

Principal component analysis (PCA) of the experimental data obtained containing emulsifiers resulted in two principal components explaining 48.3 and 22.0 % of the data variation (Figure 6). Thus, the model explained 70.3% of the total variation in data. The first principal component weight (PC1) was defined by the longitudinal 2D area, the longitudinal 2D perimeter and cell cm^{-2} in the positive axis, and on the negative axis were located resilience, cohesiveness, springiness, Tx and mean cell area. Component 2 (PC2) was defined by T1, bubbles cm^{-2}, longitudinal 2D area, H′m and the mean bubble area. DATEM, Lecithin, SSL, and PGEF were found in the negative PC1 component where the majority of dough and bread responses were located. As shown in cluster analysis (Figure 5), DMG-75 was the furthest emulsifier attending to its experimental responses, particularly longitudinal 2D area and perimeter, and H′m. Results obtained from DATEM and Lecithin were explained due to responses to chewiness, cohesiveness, resilience, cell circularity and Tx. However, PGEF and SSL, adjacent in cluster analysis, were related with the mean cell area, hardness, T′1 and Hm. Eventually, DMG-45 position was related to T1 and the number of gas cells cm^{-2}. Overall, emulsifiers could be grouped into four categories attending to the responses

obtained with dough and bread performance. In the first group, DATEM and Lecithin due to their effect on crumb texture and dough permeability; second group would include SSL and PGEF that showed bigger bubbles, with less and bigger gas cells and higher crumb hardness; third group with an intermediate behavior respect to the previous ones DMG-45; and finally DMG-75, with a more distant behavior than the control, which led to big bubbles.

CONCLUSIONS

Emulsifiers role on the progress of bubbles during proofing and baking was evaluated. Emulsifiers showed different functionality that was attributed to their diverse chemical structure and also physical characteristics (particle size). Furthermore, results shown that emulsifiers functionality was dependent on the dough hydration. All emulsifiers studied, increased the maximum dough volume during proofing, but showing different effect on dough permeability or ability to retain CO_2. Digital image analysis of the recorded baking under microscope, allowed quantifying both bubbles number and size and understand emulsifiers role on aeration. Emulsifiers allowed greater air incorporation into the dough observing higher number of bubbles, particularly with PGEF. Major changes in dough occurred at 70 ºC when bubble size augmented and became more heterogeneous, and emulsifiers affected the size and number of bubbles, with DMG-75 producing the biggest bubbles. In bread, emulsifiers tend to increase the number of gas cells with lower size, but that gave greater crumb firmness, which suggested different interactions between emulsifiers and gluten, affecting protein polymerization during baking. Despite the diverse chemical structure of the emulsifiers, experimental data following dough proofing and bread features allowed to discriminate among them.

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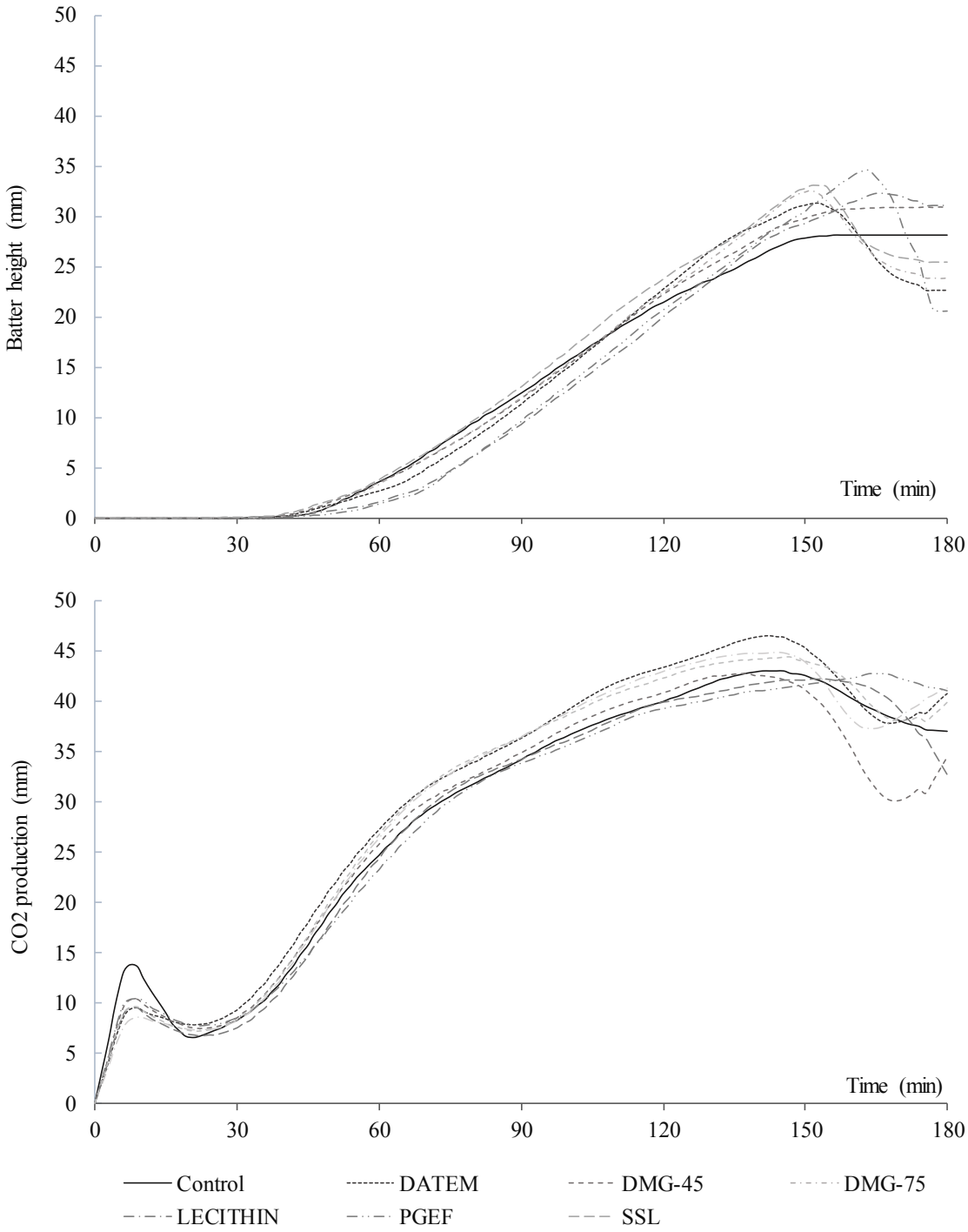

445

446 **Figure 1.** Rheofermentometer curves consisted of dough development time curves (a)

447 and gas release curves (b).

448

449

450 **Table 1.** Analysis of fermentation stage of batters containing emulsifiers by
451 reofermentometer.

	Dough development			Gas behaviour		
	Hm (mm)	T1 (min)	Proofing rate (%)	H'm (mm)	T′1 (min)	Tx (min)
Control	28.5±0.5^{a}	145±0^{a}	30.79	43.7±1.0^{a}	146±3^{a}	140±1^{b}
DATEM	31.4±1.0^{b}	167±9^{c}	33.44	47.0±0.8^{c}	161±7^{c}	145±2^{c}
DMG-45	31.0±1.0^{b}	169±4^{c}	31.13	43.6±0.1^{a}	140±6^{a}	136±5^{a}
DMG-75	32.4±0. 8^{b}	150±2^{b}	31.62	45.8±1.3^{b}	161±8^{c}	134±0^{a}
Lecithin	32.3±0.6^{b}	165±3^{c}	33.77	43.4±0.8^{a}	165±4^{c}	136±3^{a}
PGEF	34.6±0.9^{d}	164±6^{c}	34.77	44.0±0.5^{ab}	176±7^{d}	175±5^{d}
SSL	33.0±0.8^{c}	153±4^{b}	31.46	44.5±0.9^{b}	150±3^{b}	148±4^{c}

452 Mean ± standard deviation. Different letters within the same parameter differ
453 significantly (P<0.05)
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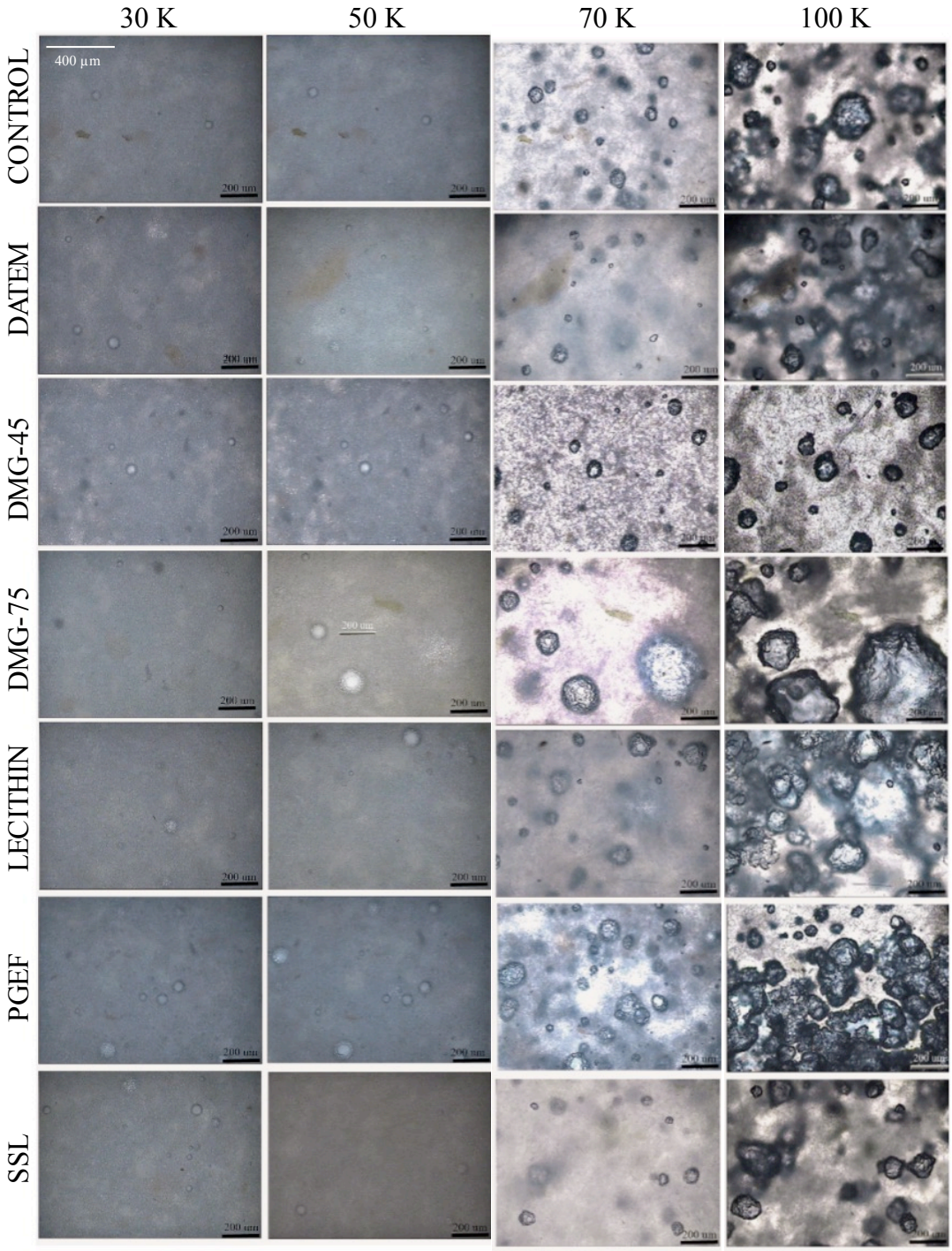

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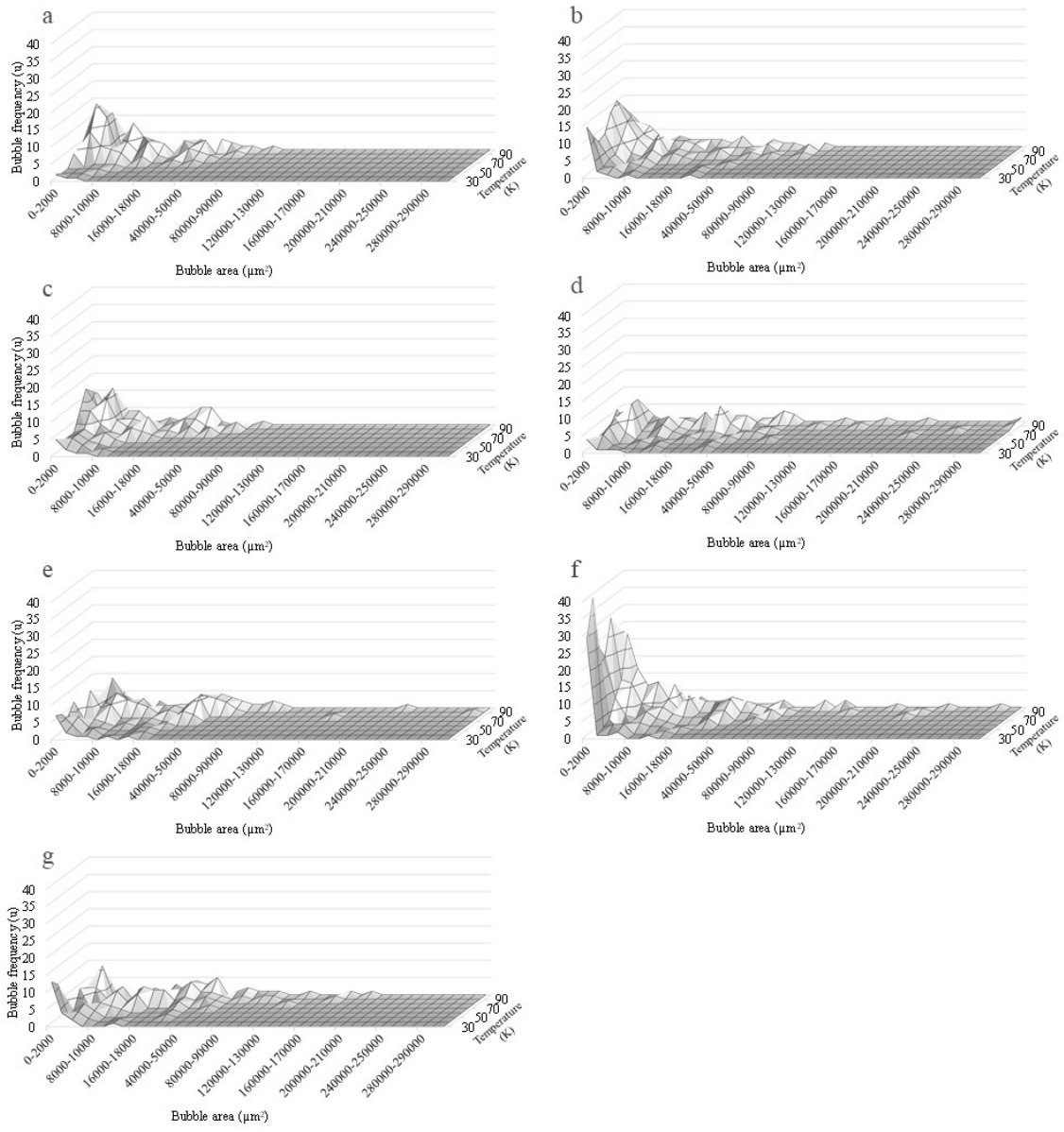
458 **Figure 2.** Captured images of gas bubbles during simulated microbaking at microscope.



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460 **Figure 3.** Bubble size distribution during baking for each emulsifier: Control (a),

461 DATEM (b), DMG-45 (c), DMG-75 (d), Lecithin (e), PGEF (f) and SSL (g).

462 **Table 2.** Emulsifiers effect on loaves morphogeometrics characteristics, cell crumb distribution and texture profile of small scale breads.

Sample	Control	DATEM	DMG-45	DMG-75	Lecithin	PGEF	SSL
Longitudinal section							
Area (cm^2)	5.37 ± 0.48^{bc}	5.31 ± 0.24^{bc}	5.09 ± 0.19^{ab}	5.12 ± 0.32^{ab}	5.00 ± 0.24^{ab}	4.95 ± 0.20^{a}	5.50 ± 0.30^{c}
Perimeter (cm)	1.25 ± 0.04^{c}	1.17 ± 0.07^{ab}	1.23 ± 0.05^{bc}	1.25 ± 0.07^{bc}	1.23 ± 0.1^{bc}	1.11 ± 0.04^{a}	1.20 ± 0.08^{bc}
Cross section							
Number of cells cm^{-2}	10 ± 2^{a}	13 ± 2^{bc}	15 ± 1^{cd}	16 ± 2^{d}	15 ± 2^{cd}	9 ± 2^{a}	12 ± 3^{ab}
Mean cell area (mm^2)	3.31 ± 0.81^{b}	1.92 ± 0.89^{a}	1.57 ± 0.47^{a}	1.61 ± 0.40^{a}	1.88 ± 0.34^{a}	3.23 ± 1.70^{b}	2.48 ± 0.48^{ab}
Minimum cell area (mm^2)	0.15 ± 0.02^{c}	0.14 ± 0.04^{c}	0.10 ± 0.03^{ab}	0.06 ± 0.02^{a}	0.12 ± 0.03^{bc}	0.13 ± 0.02^{bc}	0.12 ± 0.03^{bc}
Maximum cell area (mm^2)	11.80 ± 3.61^{bc}	7.88 ± 1.79^{ab}	7.22 ± 1.94^{a}	9.12 ± 2.60^{abc}	10.13 ± 2.37^{cd}	17.73 ± 4.77^{d}	13.67 ± 3.65^{abc}
Circularity	0.68 ± 0.16^{ab}	0.68 ± 0.12^{ab}	0.62 ± 0.11^{ab}	0.60 ± 0.11^{a}	0.70 ± 0.06^{ab}	0.74 ± 0.07^{b}	0.72 ± 0.05^{ab}
Crumb texture							
Hardness (g)	55 ± 4^{a}	85 ± 6^{c}	79 ± 3^{b}	79 ± 3^{b}	80 ± 4^{bc}	100 ± 6^{e}	93 ± 5^{c}
Springiness	0.95 ± 0.01^{c}	0.93 ± 0.01^{c}	0.94 ± 0.02^{a}	0.86 ± 0.06^{ab}	0.94 ± 0.04^{c}	0.91 ± 0.02^{bc}	0.85 ± 0.08^{c}
Cohesiveness	0.83 ± 0.03^{c}	0.73 ± 0.02^{b}	0.72 ± 0.03^{a}	0.66 ± 0.05^{a}	0.73 ± 0.04^{b}	0.74 ± 0.03^{b}	0.64 ± 0.04^{b}
Chewiness (g)	35 ± 5^{a}	56 ± 6^{bcd}	53 ± 5^{bc}	47 ± 5^{ab}	56 ± 3^{bc}	58 ± 6^{d}	51 ± 7^{b}
Resilience	0.49 ± 0.03^{c}	0.38 ± 0.01^{b}	0.37 ± 0.04^{a}	0.29 ± 0.03^{a}	0.39 ± 0.04^{b}	0.40 ± 0.03^{b}	0.26 ± 0.01^{b}

463 Mean ± standard deviation. Different letters within the same parameter differ significantly ($P<0.05$)
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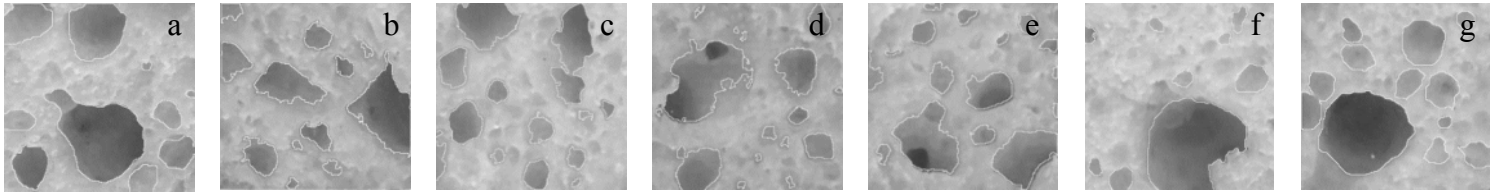


Figure 4. Captured images and bubbles count of small scale breads. a: Control, b: DATEM, c:DMG-45, d:DMG-75, e: Lecithin, f: PGEF, g: SSL.

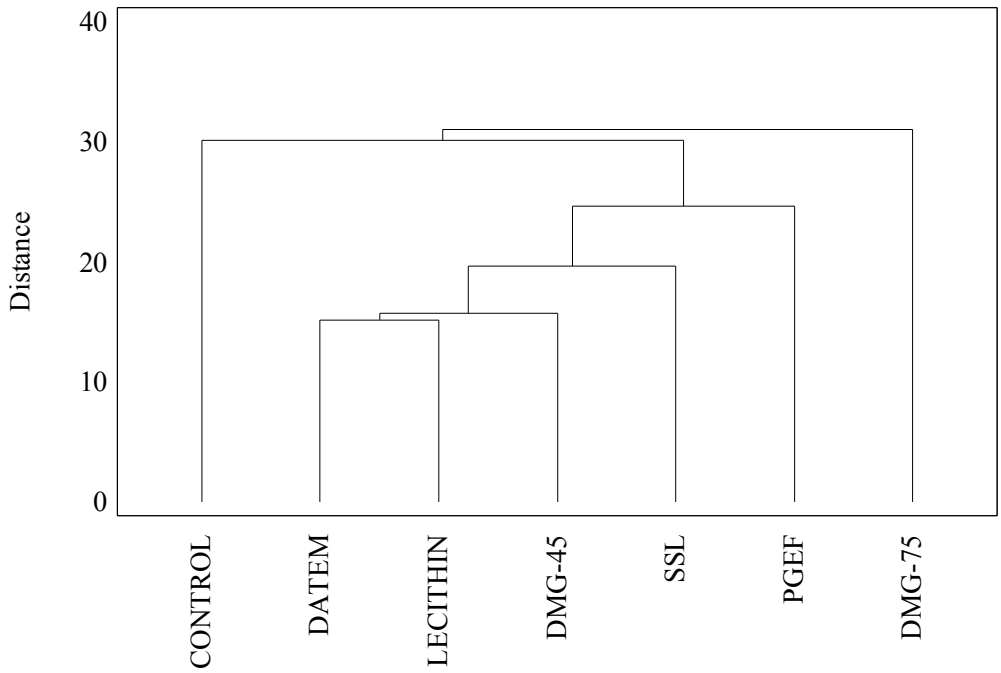
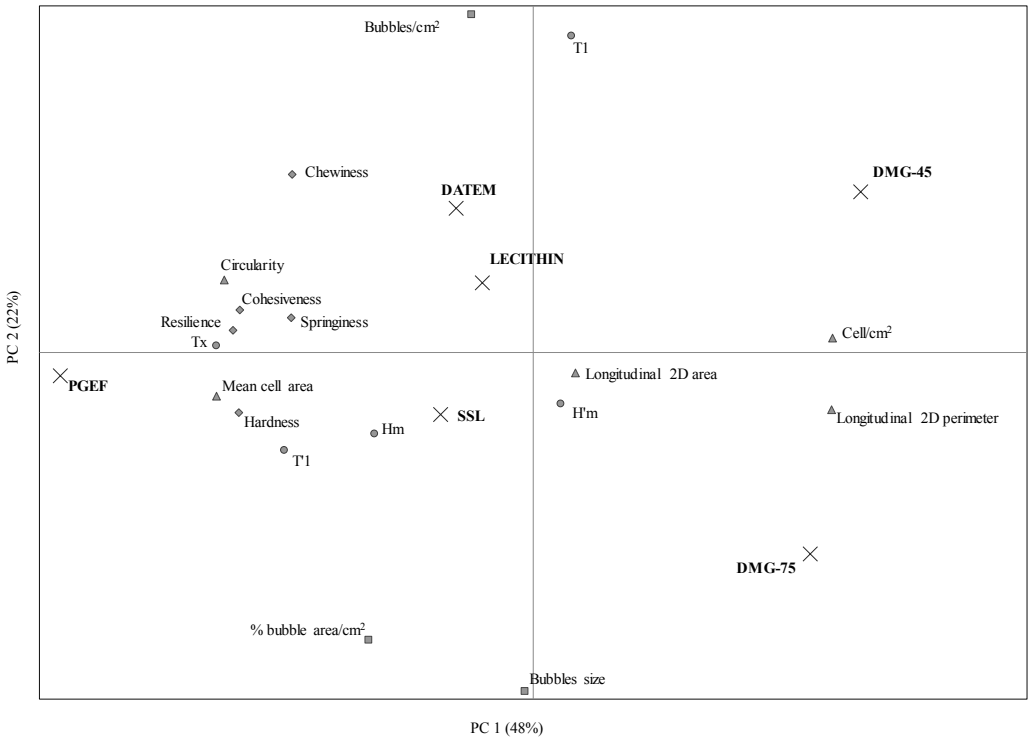


Figure 5. Cluster statistical analysis by using closest neighbor method.



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476 **Figure 6.** Score plot from a principal component analysis of the combination of

477 components weight (■ simulated microbaking, ◆ texture properties, ●

478 rheofermentometer variables and ▲ digital image analysis of breads) and principal

479 components (× emulsifiers).

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