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Original article

Use of enzyme to improve the technological quality of a panettone like baked product

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Summary The aim of this work was to study the influence of amylase, xylanase and lypase on quality parameters of panettone. Two concentrations of each enzyme were utilised. Besides, enzymes were added to dough or to sponge in order to analyse the effect of the time at which the enzymes were added on bread quality. Results showed that enzymes improved the quality of the product. Depending on the enzyme, the effect was more remarkable on bread height, cell distribution or crumb texture. Particularly, lipase and amylase increased bread height and decreased bread hardness. Although xylanase did not modify bread height, it produced better grain crumb structure and changed the amount of water needed for dough development. Results were different when the additive was incorporated in sponge or in dough. Variability of effects and changes in the results depend both on the doses and on the time of incorporation, all of which provide opportunities to optimise the quality of panettone using a combination of enzymes.

Keywords Amylases, breadmaking, colour, flour quality, lipases, texture, xylanases.

Introduction

Panettone is a typical bread from Milan, prepared for Christmas and New Year in Italy and in Latin American countries. It is made of sweet dough and optional ingredients, such as candied fruit, raisins, almonds or chocolate.

In Argentina, during 2007 more than 35 000 000 units of panettone were elaborated; although internal consumption decreased slightly, production increased due to exports (Lezcano, 2007).

During the industrial production of panettone, the main challenges are both to produce dough with a capacity to hold fruits and raisins during proofing and baking, and to obtain products that keep their quality during storage. Generally, sponge and sourdough methods are used in panettone elaboration. The sponge consists of a simple mixture of flour, water and yeast, and is added to bread dough before the mixing and baking process as a substitute for yeast. The use of sponge in panettone elaboration enhances the flavour. Sourdough is a spontaneously fermented mixture of flour and water or more often, a mixture inoculated with a wild microbial starter (yeast and lactic acid bacteria); the latter is a sourdough constantly renewed cyclically, using strict conditions of recipe and ripening (Ottogalli *et al.,* 1996). The use of long methods to improve the aroma due to the large amounts of volatile and aromatic compounds produced by long fermentation also liberates large amounts of amino acids and reducing sugars that act as a substrate for Maillard reactions (Hansen & Hansen, 1996). In the breadmaking industry, several additives are utilised to improve dough properties, tolerance process and bread quality, and in particular to optimise shelf life quality. In general oxidising agents, emulsifiers and enzymes are used.

Among the enzymes added to the bread recipe, amylases are the most common ones. There are different kinds of amylases: α-amylases are typical endo-enzymes, which more or less randomly hydrolyse the α-(1,4)-linkages in amylopectin and amylose, The reaction products of α-amylases are fragments of the starch molecules, known as dextrin′s, which play a role in maintaining bread freshness (León *et al.*, 1997; Durán *et al.*, 2001). Significant research is still performed on the use of starch-converting enzymes as antistaling agents, as evidenced by the big amount of publications and patents. Research works focus on finding new enzymes or modifying the enzyme thermostability (Goesaert *et al.*, 2006). Lipases can be used to retard bread staling, because they catalyse the

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formation of monoglycerides which act as surfactants (van Eijk & Hille, 1996).

Xylanase removes insoluble arabinoxylanes, which hinder the formation of gluten network; besides, it improvessystem stability due to an increase in viscosity. Therefore, the resulting dough is more stable and flexible, and has better oven spring; at the same time, bread pieces have larger volume, better crumb structure and minor hardness (Martínez-Anaya & Jiménez, 1997; Courtin *et al*., 1999). Excessive hydrolysis of arabinoxylanes leads to a weak, wet and sticky dough, yielding bread of poor quality (McCleary, 1986). Today, optimisation of arabinoxylan functionality is obtained by the use of selected endoxylanases (Goesaert *et al*., 2006).

Corsetti *et al*. (2000) reported that pentosans alone or a combination of pentosans and endoxylanases delay bread firming. Physicochemical changes during bread storage lead to crumb firming, which is part of the general process called staling and is the most used parameter to evaluate staling development (Gray & BeMiller, 2003).

Although panettone production is important, few scientific articles discuss this topic (Foschino *et al.,* 1999, 2004; Clarke & Arendt, 2005; Picozzi *et al.,* 2006; Restuccia *et al., 2*007; Rollini *et al.,* 2007); most of them deal with sourdough microbiology and there is no research done on the improvement of panettone quality through the use of enzymes.

The aim of this research was to study the influence of amylase, xylanase, and lypase on panettone quality, when enzymes are added to dough or to sponge.

Materials and methods

Materials

Wheat flour free of additives, was provided by a local milling company (Tiranti SA, Córdoba, Argentina). Wheat flour parameters were the following: Alveograph: deformation energy (W) = 262×10^{-4} J, tenacity (P) = 138mm, extensibility (L) = 43 mm, P/L = 3.21, wet gluten content = 25.9%, falling number = 479 s, protein content = 11.6%, ash content = 0.54% and moisture content = 12.1% on moisture bases (AACC, 2000). Dried yeast (Calsa SA, Lanús, Argentina) and other dough ingredients (food grade) were purchased at a local market. Chemicals used were of reagent grade. Enzymes that are routinely used to improve dough properties and bread quality, were applied to make *panettone*: Multizyme Amylase Conc.: α-amylase, *A. oryzae*, E.C.: 3.2.1.1 (160 000 SKBU g^{-1}) (AMY); Multizyme Xylanase: *A. niger*, E.C.: 3.2.1.8 (12 000 GXU g^{-1}) (XIL); and Multizyme TRD: Lysophospholipase A. *oryzae*, E.C.:3.1.1.5 (2000 PLU g^{-1}) (LPP), were obtained from Nutring SA (Buenos Aires, Argentina).

Breadmaking procedure

The dough base formulation used in this study was sponge, flour, water, shortening, sugar, egg, whole dried milk and salt (Table 1). Enzymes were added according to the experimental design showed in Table 2.

Sponge was prepared by mixing flour, water, sugar and dried yeast during 20 min. The resulting dough was proofed for 70 min in a cabinet at 32 ± 1 °C.

The dough was prepared by mixing ingredients during 30 min (Recco, Shenzhen, China); after 5 min of rest the dough was divided into 780 g pieces, moulded in 185 mm × 125 mm baking pans, proofed for 85 min at 32 ± 1 °C (96% relative moisture, rm), and baked at 185 ± 3 °C for 55 min. Recco, Model FAPA01 (China) bread-making equipment was used to obtain bread.

The bread loaves were wrapped up into polyethylene bags and stored at 20 ± 1 °C and 75 ± 5% rm. After 6-days storage bread loaves were analysed.

Table 1 Sponge and dough ingredients for the production of panettone referred to 100 g flour

Ingredients	Sponge (g)	Dough (g)
Wheat flour	50	50
Water	27.5	
Sugar	2.5	22.5
Dried yeast	0.75	
Shortening		22.5
Egg		17.5
Whole dried milk		2
Salt		0.75
Sponge		80.75

Table 2 Experimental design of enzyme addition for the production of panettone

Sample	Added enzyme	AMY (mg 100 g^{-1} flour) fb	XYL (mg 100 $^{-1}$ flour) fb	LPP (mg 100 $^{-1}$ flour) fb
C	–	–	–	–
SA1	In sponge	0.25	–	–
SA2	In sponge	0.375	–	–
SX1	In sponge	–	5	–
SX2	In sponge	–	10	–
SL1	In sponge	–	–	6
SL2	In sponge	–	–	10
DA1	In dough	0.25	–	
DA2	In dough	0.375	–	
DX1	In dough	–	5	
DX2	In dough	–	10	
DL1	In dough	–		6
DL2	In dough	–		10

SA, Panettone with α-amylase addition in sponge; SX, Panettone with xylanase addition in preferment; SL, Panettone with lysophospholipase addition in sponge; DA, Panettone with α-amylase addition in dough; DX, Panettone with xylanase addition in dough; DL, Panettone with lysophospholipase addition in dough.

Bread properties

Bread loaf height
Height was measured using a millimetric ruler.

Bread crumb texture
Texture profile analysis (TPA) parameters were determined by using a TA-XT2i texturometer (Stable Microsystems Ltd, Goldaming, Surrey, UK) equipped with a 250 N load cell. A cylinder probe of 2.5 cm in diameter was attached to a moving crosshead. The bread loaves were cut into two slices (2.5 cm thick) and the ends were discarded. Each slice was subjected to a double cycle of compression, under the following conditions: deformation rate, 60 mm min^{-1} and maximum deformation, 40%. The texture profile parameters were determined using the Texture Expert version 1.22 (Stable Microsystems Ltd, Goldaming, Surrey, UK). Hardness was the peak force parameter (N) of the first compression cycles of the product and chewiness of the crumb was calculated from a force–distance graph as gumminess * springiness. Two slices per bread sample were analysed, and average values between bread duplicate were reported.

Crumb and crust colour
Bread colour was determined with a Minolta 508d spectrophotometer, 8 mm measurement aperture, D65 illuminant, 10° angle of observer, according to Approved Methods 14-22 (AACC, 2000). Crust colour was measured on the top of each bread loaf. Crumb colour was measured from the centre of the slice. At least five readings were taken from each bread and four bread pieces from each test point: they were recorded as CIE-LAB, L^* (lightness), a^* (redness-greenness), and b^* (yellowness-blueness) values.

Characterisation of crumb structure
For each bread loaf, two slices were obtained from the central region and photographed with a digital camera (Canon, Mississauga, Canada) (2048×1536 image size). JPEG image file formats were analysed with an image analyser (Image J 1.38n; National Institute of Health, Bethesda, USA). Colour images were converted to eight-bits 256 grey level images. A single field of view (FOV) was evaluated for each image. This FOV captured the majority of the crumb area of each slice. Images were taken from the centre of the slice. The segmentation method (conversion to a binary image) of the 256 grey level digital images was used to extract coherent information from raw image data. A threshold method was used for image segmentation. Crumb images were considered to contain grey level information from pixels of which the darkest individuals belong to cell and the brightest belong to cell wall. A grey level histogram of each digital grey scale image was obtained by means of the Image J software. A grey level image composed of a dark, distinct and uniform object on a brighter background contains homogenous regions with well defined boundaries that generally lead to a bimodal histogram with sharply defined intensity peaks. The two peaks correspond to the relatively large number of points inside and outside the object. The dip between the peaks corresponds to the relatively few points around the edge of the object. The threshold is placed in the valley between both peaks, then pixels with a grey level higher than the threshold value will be associated to the background while the remaining pixels will be associated to the object. Nevertheless, in bread crumb images the non-uniform transition between cell and non-cell uniform produces results in a histogram with bimodal characteristics. In order to obtain a representative grey level threshold, the grey level histogram obtained from FOV was deconvolutioned in two Gaussian peaks and finally fitted (coefficient of determination, $R^2 > 0.996$) by means of PeakFit v4 for win32 (Jandel Scientific, San Rafael, CA, USA). The intersection of the two Gaussian curve, peak 1 and 2, was selected as the grey level threshold. A threshold level was obtained for each FOV and it was used to produce a binary image, where pixels with a grey level higher than the threshold value will be associated to the cell wall, while pixels with a grey level lower than the threshold value will be associated to the cells. The crumb cell features chosen were the total number of cells (N), the total cell area, the mean cell area (Am), and the ratio between cell areas to total area (Ar). Grain uniformity was determined as the ratio of small to large cell counts (i.e. the ratio of the number of small cells to large ones, 4 mm^2); higher values indicate greater uniformity of crumb grain (Zghal *et al.,* 2002).

Statistical analysis

Two bread loaves were obtained. The experiments were achieved in duplicate. Results were expressed as mean values ± SD. The data were statistically treated by multivariate analysis of variance (MANOVA) (Cox, 1992); where significant differences were compared using Bonferroni test and confidence level of 95%, using the INFOSTAT statistical software, 2008 version (Facultad de Ciencias Agropecuarias, UNC, Argentina).

Results and discussion

Bread height

Height was considered indicative of bread development because the moulds were identical and rigid, Due to the surfactant nature of the hydrolysis products of lipases, which improved dough machinability and

increased oven spring (Si, 1997), the largest bread loaves were obtained using LPP. The effect was more notable when enzyme was added in the dough (DL1 and DL2) (Fig. 1); the low influence of lipase in preferment could be due to the acid environment that reduces enzyme activity (Di Cagno *et al.,* 2003). However, according MANOVA ($\alpha \leq 0.05$), the values of bread height were significantly larger when LPP was used.

The lowest level of XYL in dough (DX1) developed larger breads than controls, but this effect was not observed in sponge samples. The effect of XYL on bread height was less than expected because xylanase showed a positive influence on bread quality (Martínez-Anaya & Jiménez, 1997); since every sample was produced with the same water content, probably, water was not enough for dough development in panettones with XYL. Besides, XYL added to sponge produced smaller bread loaves, probably due to the long action time, which allowed an extensive degradation of arabinoxylan (Courtin *et al.,* 1999). Amylase hydrolyses starch into dextrins and maltose, thus allowing yeast to work continuously during dough fermentation, proofing and the early stage of baking. Besides, starch hydrolysis produces a rapid decrease of the viscosity, which gives rise to larger alveolus. Consequently, bread volume is improved (Gujral *et al.*, 2003). However, panettone height did not differ from controls with the addition of AMY to the dough. Increase of height was observed when AMY was added to sponge, in agreement with Corsetti *et al.* (2000), who stated that the addition of fungal amylase markedly increased volume compared to standard sourdough bread. Height differences between SA1, SA2 and DA1, DA2 were attributed to difference in enzyme action time: the use of AMY in sponge led to an increase in the level of fermentable sugars in dough.

Crumb bread texture

Bread hardness was significantly changed with enzyme addition (Fig. 2); the effect was more remarkable when the enzyme was added to sponge ($\alpha \leq 0.05$). The use of XYL produces panettones with less hardness than control. Jiménez & Martínez-Anaya (2001) established that water-insoluble pentosans were positively correlated with hardness during storage. XYL would lead to cleavage of the backbone of arabinoxylans with the consequent release of water and water-insoluble pentosan diminution (Rouau *et al.,* 1994), explaining therefore the positive effects of XYL on bread freshness.

LPP showed an significant improving effect on crumb hardness ($\alpha \leq 0.05$), probably due to lyso-phospholipids generated, which were able to interact with starch and to diminish the amylopectin retrogradation (Kweon *et al.,* 1994). Besides, softer crumbs correspond with higher specific volume bread loaves (Armero & Collar, 1998).

Sponge samples with amylases showed lower crumb hardness than controls, but when the enzyme was added to the dough this effect was not observed. Amylases hydrolyse starch producing dextrins. The softening effect of amylase on dough is due to starch retrogradation inhibition produced by dextrins (León *et al*., 1997; Durán *et al.,* 2001). The highest panettone obtained with amylases showed lesser crumb hardness. Coincidentally, other authors using sourdough informed that amylases increased specific volume and decreased crumb hardness (Corsetti *et al.,* 2000).

Staled and dried bread samples need more salivation and more mastication before swallowing (Bramesco & Setser, 1990). In breads with complex formulation as panettone, chewiness is affected by fat and sugar content.

When enzymes were used to obtain bread, chewiness was lower (AMY: 15.0%; XYL: 21.10%; LPP: 14.6%)

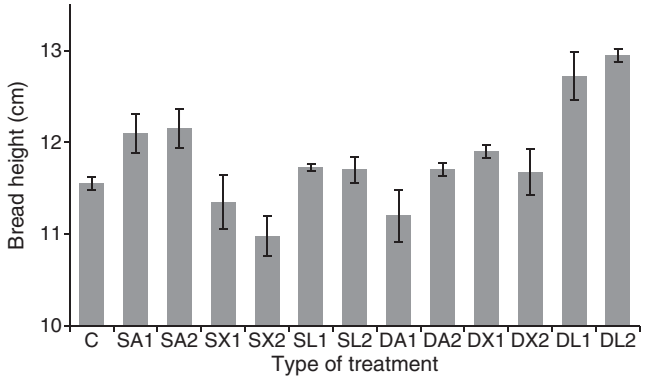

Figure 1 Bread height (cm) of panettone samples as affected by the dough fermentation method and the addition of enzymes. S: sponge, D: dough, A: amylase, X. xylanase, L: lipase, 1: the lowest dose, and 2: the highest dose.

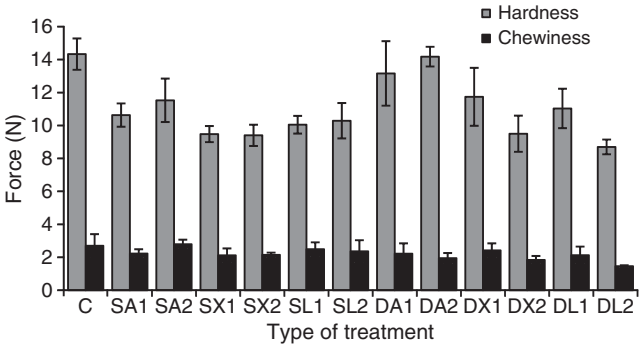

Figure 2 Effect of enzymes on the hardness and chewiness of the panettone crumb after 6 days of storage at room temperature. S: sponge, D: dough, A: amylase, X: xylanase, L: lipase, 1: the lowest dose, and 2: the highest dose.

(Fig. 2); however, there were no significant differences among the samples, except for DL2, due to high SD of measurements.

Crust and crumb colour

No significant differences in a and b colour parameters ($P \leq 0.05$) were observed among panettones, while both crumb and crust lightness (L) were affected by enzyme addition.

The 100 – L^* parameter was considered by Resmini *et al.* (1993) and Fernández-Artigas *et al.* (1999) as browning index in the control of pasta-drying and flour toasting. Table 3 shows browning index from bread samples.

The addition of AMY did not significantly modify crumb or crust colour of panettone.

Enzyme XYL produced an increase in crust 100 – L^* values, especially when it was added in sponge. This darkness increase could be related to the enzyme activity, which produced a dryer crust and therefore there were more Maillard reactions.

The use of LPP, mainly in high doses, produced a clearer crumb due to the emulsifying activity of enzyme products which facilitate major oxygen retention (Néron *et al.,* 2005).

Crumb structure

The effect of enzymes on crumb grain characteristics was assessed by digital image analysis. Results showed that main changes were produced by XYL and LPP (Table 4). Pictures were converted to binary image, where clear regions correspond to crumb wall and dark areas to cells (Fig. 3a).

Ratio between cell area to total area (Ar) was higher than controls in all samples with enzymes; therefore the incorporation of air in dough and sponge where the enzyme had been added was higher; however, the differences were not significant ($\alpha \leq 0.05$). XYL added to sponge produced homogeneous crumb, with greater cell mean area (Am) and less amount of cell (Fig. 3b). Panettones obtained with LPP and with AMY added in dough had smaller amounts of bigger cells, and hence more aired crumb (Fig. 3c); however, cell mean area is greater than controls and the amount of cell is lower than controls.

Table 3 Browning index (100 – L^*) of panettone crumb and crust as affected by the dough fermentation method and the addition of enzymes

	100 – L^*	
Sample	**Crumb**	**Crust**
C	26.22 ± 0.66	26.42 ± 3.07
SA1	25.28 ± 0.07	26.79 ± 1.89
SA2	24.03 ± 0.06	25.73 ± 3.22
SL1	23.67 ± 0.04	26.67 ± 3.73
SL2	24.39 ± 1.18	27.44 ± 3.22
SX1	27.03 ± 0.78	32.19 ± 2.42
SX2	24.85 ± 0.40	28.93 ± 2.81
DA1	25.24 ± 0.13	26.05 ± 2.09
DA2	25.48 ± 0.01	26.35 ± 3.45
DL1	26.29 ± 0.06	29.43 ± 3.41
DL2	22.62 ± 0.23	27.39 ± 2.46
DX1	25.04 ± 1.48	28.06 ± 1.88
DX2	25.42 ± 1.53	26.95 ± 3.26

SA, Panettone with α-amylase addition in sponge; SX, Panettone with xylanase addition in preferment; SL, Panettone with lysophospholipase addition in sponge; DA, Panettone with α-amylase addition in dough; DX, Panettone with xylanase addition in dough; DL, Panettone with lysophospholipase addition in dough.

Table 4 Effect of enzymes on crumb structure

Samples	Am (mm2)	N	Ar
C	4.04 ± 0.00	2644 ± 182	0.295 ± 0.003
SA1	4.39 ± 0.13	2720 ± 72	0.317 ± 0.001
SA2	4.87 ± 0.19	2635 ± 110	0.355 ± 0.014
SL1	4.37 ± 0.36	2729 ± 75	0.320 ± 0.014
SL2	3.96 ± 0.14	2745 ± 298	0.298 ± 0.042
SX1	5.58 ± 0.43	2267 ± 202	0.374 ± 0.003
SX2	5.32 ± 0.30	2468 ± 144	0.362 ± 0.028
DA1	4.88 ± 0.08	2445 ± 112	0.331 ± 0.018
DA2	4.66 ± 0.15	2526 ± 170	0.325 ± 0.002
DL1	5.22 ± 0.57	2501 ± 141	0.362 ± 0.001
DL2	5.42 ± 0.16	2413 ± 134	0.362 ± 0.016
DX1	4.65 ± 0.40	2480 ± 35	0.318 ± 0.048
DX2	4.28 ± 0.73	2704 ± 72	0.318 ± 0.001

Am, Cell mean area; N, Total number of cells; Ar, Ratio between cell area to total area; SA, Panettone with α-amylase addition in sponge; SX, Panettone with xylanase addition in preferment; SL, Panettone with lysophospholipase addition in sponge; DA, Panettone with α-amylase addition in dough; DX, Panettone with xylanase addition in dough; DL, Panettone with lysophospholipase addition in dough.

Conclusion

Results showed that enzymes improve the quality of the product. Depending on the enzyme, the effect was more remarkable on bread height, cell distribution or crumb texture.

LPP led to improved bread quality because it increased bread height and decreased bread hardness. AMY also increased height, but to a lesser extent than LPP. XYL produced a better grain crumb structure, with higher ratio between cell areas to total area. Results were different when additive was incorporated into sponge or into dough; action of AMY was more notable in long method, while LPP was more effective when it was added to the dough.

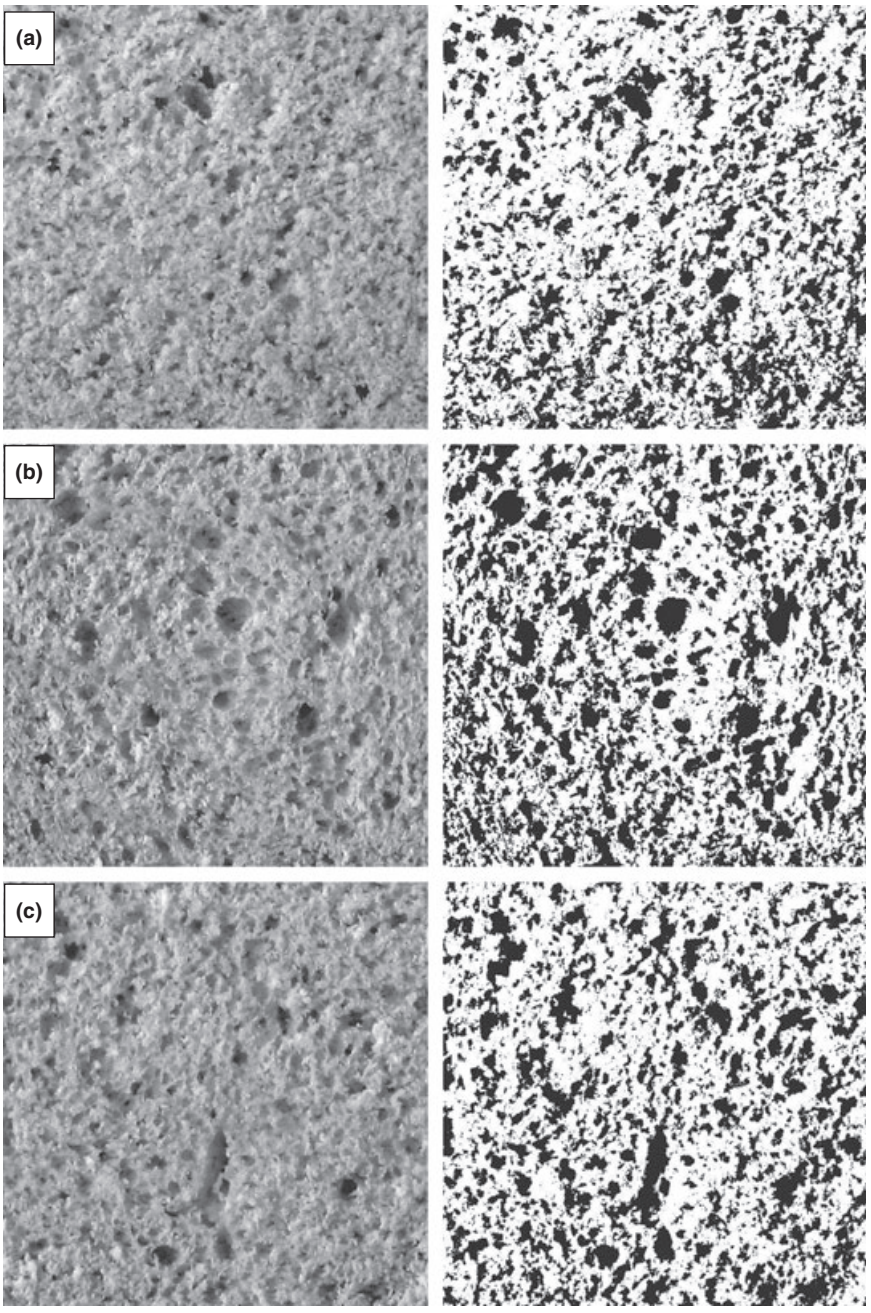

Figure 3 Greyscale (left) and binary images (right) of samples C (a), SX1 (b), and DL2 (c).

Variability of effects and changes in the results depending on the doses and time of incorporation provide opportunities to optimise panettone quality, using a combination of enzymes.

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