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Effect of ultrasonic assisted blanching on size variation, heat transfer and quality parameters of mushrooms

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1. Introduction

The button mushrooms (*Agaricus bisporus*) are one of the most popular and valuable edible fungi. The shelf life of these mushrooms is limited to a few days, mainly because they have no cuticle to protect them from physical or microbial attacks and water loss. In the same way, the important content in nutrients, the high respiration rate and their high tyrosinase and phenolic content make them very susceptible to enzymatic reactions (Aguirre et al. 2009). All these factors induce a fast deterioration after harvest (Kotwaliwale et al. 2007) including the softening, due to the loss of moisture through respiration, and the browning due to enzymatic breakdown of cells (Mohapatra et al. 2010). These phenomena result in reduced product acceptability since consumer´s preference is for white, unblemished and hard texture mushrooms. In view of their highly perishable nature, mushrooms must be processed to extend their commercial shelf life for off-season use (Devece et al. 1999). In this sense, most mushroom crops are preserved by canning and only a small portion treated by other methods such as freezing or drying (Coskuner and Ozdemir, 1996). The production of heat-sterilized preserves represents most usual long-term preservation and accounts for more than 60 % of industrial processed mushrooms (Biekman et al., 1996). In the preparation process of sterilized mushrooms, blanching is an important pre-treatment, which main objectives are: i) to inactivate enzymatic browning by thermal inactivation of the enzyme polyphenoloxidase (PPO) and ii) to induce shrinkage in such a way that it will not occur during sterilization (Wu et al., 1981) and ensure control of post-process yield (ratio of drained weight to fill weight) (Sensoy and Sastry, 2003). Although both objectives are important, it has been found (Lespinard, et al., 2009) that shrinkage is the limiting factor to determine the processing time at blanching temperatures above 60 ºC.

However, depending on the processing conditions applied, the quality and bioactivity of the final product can be negatively affected due to the destruction of nutrients relatively unstable to heat, the loss of water-soluble components by leaching and the induced changes in texture and colour (Gamboa-Santos et al. 2012).

In this regard, blanching at low temperature, in the range of 55–75 °C, can be used in order to improve the firmness of cooked vegetables and fruits, reducing physical breakdown during further processing and providing an excellent and safe way of preserving texture (Verlinden et al. 2000).

The increased consumer's awareness by the relationship between diet and health has increased the interest of the food industry for mild processing technologies that provide final products with improved

characteristics as compared to those obtained by conventional thermal treatments (Soria and Villamiel 2010). In this sense, the introduction of new technologies could lead to a reduction of the processing time, the improvement in operating conditions or the reduction of the processes energy needs, thereby decreasing both environmental and economic costs. Ultrasound is an example of these new technologies to intensify food processes (Cárcel et al. 2011). The use of high intensity ultrasound has been considered to enhance heat and mass transfer for different products and processes such as drying (De la Fuente et al. 2006; García-Pérez et al. 2006; Gallego-Juárez et al. 2007), atmospheric freeze drying (García-Pérez et al. 2012), osmotic dehydration (Cárcel et al. 2007a; Fernandes and Rodrigues, 2007; Jambrak et al. 2007b), brining (Cárcel et al. 2007b; Gabaldón-Leyva et al. 2007; Siró et al. 2009), freezing (Delgado et al. 2009) and in many other food applications such as sterilizing, blanching, extracting, degassing, filtrating or enhancing oxidation (Leadley and Williams 2002; Mason 1998; Ortuño et al. 2013; Peralta-Jimenez and Cañizares-Macías 2012; Horžić et al. 2012). In blanching process, the combination of ultrasound with classical heat treatments is an interesting alternative since it allows using milder conditions reducing processing time and increasing efficiency of enzyme inactivation processes (López et al. 1994; López and Burgos 1995; De Gennaro et al. 1999; Cruz et al. 2009; Cheng et al. 2013).

Lespinard et al. (2009) found that the volume change, the rate of heat transfer and the deteriorative reactions are the main features to be taken into account during the design and optimization of mushrooms blanching processes. In this respect, in the available literature only a few references can be found on modelling of volume contraction and heat transfer during the thermal processing of mushrooms (McArdle and Curwen 1962; Konanayakam and Sastry 1988; Sheen and Hayakawa 1991; Biekman et al. 1997; Sensoy and Sastry 2004). With regard to the application of ultrasound during blanching of mushrooms, only some studies had been found (Sastry et al. 1989; Lima and Sastry 1990; Jambrak et al. 2007a; Cheng et al. 2013), being the effect of ultrasound treatment on the shrinkage not reported elsewhere. On the other hand, mushroom quality is defined by a combination of parameters, including whiteness and texture (Gormley 1975). In this regard, none of the aforementioned references have studied the influence of ultrasound on texture and colour variations during the blanching of mushrooms.

The overall goal of this work was to evaluate the effect of the simultaneous application of heat and ultrasound on the volume contraction, heat transfer and the changes in quality factors of mushrooms, and develop a mathematical model that allows finding the optimal processing conditions.

2. Materials and Methods

2.1. Samples

Freshly harvested mushrooms (*Agaricus bisporus*) were purchased in a local market in Valencia (Spain) and maintained in refrigeration (4 ºC) until experiments were carried out (less than 24 h). Mushrooms were selected taking into account visual similarity of size (with an average diameter and height of 0.036 and 0.040 m, respectively) and colour and gently washed with tap water at room temperature to remove foreign materials that could be adhered to their surface.

2.2. Blanching processing

In order to evaluate the effects of ultrasound on the mushrooms, conventional (CB) and ultrasonic assisted blanching (UB) experiments were carried out. For that purpose, mushrooms were placed in a sample holder and submerged into distilled water in a thermostatically controlled bath. Blanching processes were carried out at different water-bath temperatures (50, 60, 70, 80 and 90 ºC) until a sample size contraction of 18 % (80 % of the highest size reduction) was reached in all cases as suggested by Lespinard et al. (2009). These authors studying the influence of blanching on shrinkage and PPO activity found that this size contraction value was the limiting factor to estimate the processing time for blanching temperatures higher than 60 °C. Then, samples were removed from the bath and immediately sunk in a water-ice mixture for 2 min. Finally, they were conveniently drained and dried with absorbent paper to remove the excess of water.

In the case of UB experiments, ultrasound (25 kHz, 400W) was applied through a probe system (UP400S, Hielscher Ultrasonics GmbH, Teltow, Germany) provided with a 40 mm diameter titanium alloy sound probe. For that purpose, the probe was immersed (1 cm) in the thermostatically controlled bath and placed above the samples at a fixed distance (3 cm; Fig. 1). The treatments were carried out at the maximal power capacity of the equipment and ultrasound was continuously applied.

Fig.1 Scheme of experimental set-up for ultrasonic assisted blanching treatments

2.3. Size variation determination

The size variation of mushrooms during the blanching process was determined by measuring, for each of the five temperatures tested, the diameter (*d*) and height (*l*) of the samples with a Vernier calliper, before processing and at different times of processing. Two independents runs were performed for each

condition and three different pieces were measured in each run. The experimental variation of *d* and *l* with time was studied from a dimensionless shape factor (*D**) and it was fitted to a first-order kinetics model (Eq. 1),

$$D^* = \frac{D_t - D_{eq}}{D_0 - D_{eq}} = exp(-K_1 * t) \quad (1)$$

where D_t is the instantaneous value of the characteristic dimension (*d* or *l*) at a time *t* (min); D_0 is its initial value; D_{eq} the equilibrium dimension (after blanching for over an hour) and K_1 is the temperature-dependent rate constant.

Considering that the radial and longitudinal shrinkages were similar (Lespinard et al. 2009), the values of the rate constants (K_1) for the variation of *d* and *l* were averaged obtaining an overall rate constant (K_m) that represented the global shrinkage for each process condition tested.

The K_m temperature dependence was modelled through an Arrhenius-type relationship (Eq. 2), where K_0 is the pre-exponential factor, *Ea* is the activation energy, *Rg* is the universal gas constant (0.00831 kJ mol^{-1} K^{-1}), and *T* is the water bath temperature.

$$K_m = K_0 e^{-\frac{Ea}{RgT}} \quad (2)$$

Shrinkage experiments were performed in three replicates and in duplicate runs.

2.4. Modelling of heat transfer

To model the heat transfer in the mushroom, a set of experiments were independently carried out at the same test conditions described in Section 2.2. In these experiments, the temperature in the water bath and in the thermal centre of three mushrooms (geometric centre of mushroom head) were monitored every 15 s using rigid type K thermocouples and recorded using a multi-channel data acquisition system (HP Data Logger 34970 A, Hewlett-Packard Española, S.A., Madrid, Spain).

A mathematical model was developed to describe conduction heat transfer through the mushroom (Eq. 3), for conventional and ultrasonically assisted blanching, considering as uniform the initial temperature (Eq. 4) and convective boundary conditions (Eq. 5).

$$\rho C_P \frac{\partial T}{\partial t} = \nabla \left(k \nabla T \right) \quad (3)$$

$$T(x,y,z,t=0)=T_0 \quad (4)$$

$$k\nabla T = h(T_\infty - T) \quad (5)$$

where ρ, C_p, k are the density, specific heat capacity and thermal conductivity of mushroom, respectively (C_p = 3883 J kg^{-1} K^{-1} and k = 0.4324 W m^{-1} K^{-1} obtained from Sastry et al. 1985; ρ = 699.4 ± 20.5 kg m^{-3} obtained by liquid displacement method); h is the convective heat transfer coefficient; T_∞ is the heating medium temperature and T_0 is the initial temperature of mushroom.

The mathematical model was solved using the finite element method. Shrinkage was coupled to the heat transfer model through Eq. 1 using an arbitrary Lagrangian-Eulerian method (COMSOL AB, 2005). The boundary condition for shrinkage was computed through the velocity of the geometric boundaries changes-variation obtained from time derivative of the characteristic dimension (Eq. 6),

$$v_D = \frac{dD_t}{dt} = -K_1(D_0 - D_{eq})\exp(-K_1 t) \quad (6)$$

where v_D is the velocity of size change in the characteristic dimension direction D. The moving boundary displacement was then propagated throughout the domain, obtaining a smooth mesh deformation over all the sample volume. To construct simulation domain, mushrooms were assumed to be bodies with rotational symmetry. To make valid this assumption the mushrooms employed in experiments were selected taking into account its symmetry grade. Therefore, geometries of the mushrooms, employed as simulation domain, were built from images of transversal cuts of samples. These images were digitally processed to obtain the mushroom contour according to the procedure described by Santos and Lespinard (2011). Finally, to obtain the two-dimensional axial-symmetric domain, the contour was transformed into a solid object and it was scaled considering the measured dimensions (d and l).

To run the finite element model the two-dimensional axial-symmetric domain was imported into a mesh generator and discretized using triangles. An unstructured mesh with 685 nodes and 1264 triangular elements was developed. To achieve this meshing, a maximum element size of 1 mm and an element growth rate of 1.3 were specified. This will give the adequate number of elements. The use of finer mesh showed no significant effect on the accuracy of the solution.

The heat transfer coefficient between the mushroom and the liquid medium was estimated from the evolution of temperature measured in the centre of a bronze mushroom shaped object (Lespinard et al. 2009).

Finally, the heat transfer model developed was validated by comparing experimental and simulated mushroom temperatures. These comparisons were performed calculating the correlation coefficient (R^2) and the average relative differences ($E_{r\,ave}$) (Eq. 7),

$$E_{r\,ave} = \frac{100}{m}\sum_{i=1}^{m}\left\|\frac{T_s - T_e}{T_e}\right\| \quad (7)$$

where m is the number of experimental values, T_s are simulated temperatures and T_e are experimental temperatures.

2.5. Evaluation of quality indexes

All the quality parameters considered were measured in three different samples for each of the two run carried of each experimental condition tested (time, temperature of treatment and ultrasound application). That means a minimum of 120 samples were used for the determination of each quality parameter. The results were presented as percentage of relative variation with regard the unprocessed sample.

2.5.1. Determination of texture

Hardness of mushrooms was estimated from compression tests carried out with a texturometer TA-XT2i (Stable Micro Systems Ltd, Godalming, Surrey, UK). The experimental data were recorded and processed with the Texture Expert Exceed software. After cutting the tail, the heads of mushrooms were compressed on their round face with a cylindrical probe (10 mm in diameter) at a test speed of 5 mm s^{-1} and 30 % compression of the sample height. From the force-deformation curves, the maximum force (N), as an indicator of hardness, was obtained.

2.5.2. Determination of colour

The influence of different treatments on colour of samples was studied from the measurement of the lightness, L^* parameter of the CIELab scale (CIE, 1978). This parameter was considered since whiteness is the most important parameters used to evaluate mushroom quality (Gonzales-Fandos et al. 2000). In fact mushroom colour has been commonly measured using only the L value (Ananthewaram et al. 1986; Jolivet et al. 1998; Brennan et al., 2000; Cliffe-Byrnes and O'Beirne 2007; Gonzales-Fandos et al. 2000). The determinations were carried out using a Minolta colorimeter CR 300 Series (Osaka, Japan) with a measuring area of 8 mm diameter and provided with a 10^o standard observer and a D65 standard illuminant. The instrument was calibrated with a standard white plate (sY = 93.2, sx = 0.3133, sy =

0.3192). Measurements on each sample were performed at three points on the mushrooms surface and averaged.

2.5.3. Cooking value

The final quality of the blanched product depends on the received average intensity of the thermal treatment. The effect of the heat treatment on quality factors can be evaluated by *C*-value (Cooking value) equations, which are similar to the *F*-value equations that represent the effect on the microorganisms. The cooking value concept was introduced by Mansfield (1962) and nowadays is included in the standard nomenclature used for heat treatments (Holdsworth, 1997). In the present work, to evaluate an average deterioration of quality parameters in mushrooms, the average cooking value (C_{ave}) was determined by numerical integration (Eq. 8), using the simulated temperatures for the mushroom domain (Ω) obtained through the simulation model. A reference temperature (T_{ref}) of 100 ºC and a thermal reference factor (z_c) value of 23 ºC were considered for estimations. The value of z_c was chosen as the average of those values corresponding to the deterioration kinetics of mushroom quality parameters (Ohlsson 1980).

$$C_{ave} = \int_0^{t_p} \left(\frac{\int_\Omega 10^{\frac{T(t,\Omega)-T_{ref}}{z_c}} \partial\Omega}{\int_\Omega \partial\Omega} \right) \partial t \qquad (8)$$

2.5.4. Enzymatic activity of polyphenoloxidase

Considering that the enzymatic activity of polyphenoloxidase (PPO) is a limiting factor of the processing time, the influence of ultrasound application on this parameter was also studied. For that purpose, an enzymatic kinetic model developed by Cheng et al. (2013) was linked to the predictive heat transfer model to determine surface enzyme activity retention (AR_{sur}) of PPO (Eq. 9). The residual enzyme activity was calculated as the percentage of remaining activity,

$$AR_{sur}(\%) = \left(\frac{A_t}{A_0} \right) \cdot 100 = \frac{1}{\Gamma} \int_0^\Gamma 10^{\left[-\frac{1}{D_{ref}} \int_0^t 10^{\left(T-T_{ref}\right)/z_c} dt \right]} d\Gamma \cdot 100 \qquad (9)$$

where A_0 is the initial activity, A_t is the residual activity at time t and Γ is the mushroom surface. The D_{ref} -value is the time (min) needed to reduce the initial activity by 90 % and it was calculated in terms of *K*-value as given by Eq. 10,

$$D_{ref} = \frac{\ln(10)}{K} \quad (10)$$

K is the inactivation rate constant and can be estimated by an Arrhenius-type relationship (Eq.11),

$$K = K_0 e^{-\frac{Ea}{RgT}} \quad (11)$$

where Ea is the activation energy, K_0 is the pre-exponential factor, Rg is the universal gas constant (0.00831 kJ mol^{-1} K^{-1}) and T is the mushroom temperature. The kinetic parameters values employed were 214 kJ mol^{-1}, 2.43 10^{32} min^{-1} and 10.3 °C for Ea, K_0 and zc, respectively. These values were based on those obtained for inactivation kinetics of PPO in mushrooms during blanching by Cheng et al. (2013).

On the other hand, PPO generates numerous oxidation products, such as *o*-quinones, which may lead by polymerization to the formation of brown pigments. This implies that PPO activity is directly related to colour changes in the mushrooms (Devece et al. 1999). However, colour changes are more dependent on the residual enzyme activity evolution than on its final value. Therefore, in order to estimate the relationship between PPO activity and colour loss, integrated residual PPO activity was calculated by Eq. 12:

$$IAR_{sur} = \int_0^{t_p} \left(\frac{A_t}{A_0} \right) dt \quad (12)$$

2.6. Statistical analysis

All treatments were performed in duplicate, and all of the parameters studied were also determined per triplicate for each treatment. Statistical analysis was done to determine the significance of the effect of ultrasound on shrinkage, texture and colour. All experimental data were statistically analyzed using analysis of variance (ANOVA) from software STATGRAPHICS Plus 4.0. (Manugistics Inc., USA). The difference between mean values was analyzed by Tukey's test ($p<0.05$). The parameters of the shrinkage kinetics (K_0 and Ea) were estimated by a linear regression analysis using the OriginPro software (version 8; Origin Lab Corp., Northampton, MA). Results were expressed as mean ± standard deviation (SD).

3. Results and Discussion

3.1. Size variation

Blanching produced the shrinkage of mushroom samples. The results showed (Fig. 2) two stages: a rapid size reduction at the first stages of heating which becomes slower at higher process times. Konanayakam

and Sastry (1998) explained this mushroom shrinkage pattern through the concept of water-holding capacity. During the first stage, the mushroom ability to hold immobilized water weakened (perhaps due to protein denaturation) and the rapid loss of mass produced the product shrinkage. In this sense, Jasinski et al. (1984), in a study on the effect of thermal processing on the structure of mushrooms, found that the heat caused the coagulation of cytoplasmic material and the disruptions of intracellular membranes, which resulted in the loss of water holding capacity of the tissue. However, the loss of semipermeability of membrane tissue and the loss of intracellular water makes that the rapid shrinkage phase ended and the slow shrinkage began to appear. This phase could be attributed to loss of some bound water.

As can be seen in Fig. 2, the variation of the dimensionless size was dependent on the different processing temperatures and the application of ultrasound. Thus, the higher the blanching temperatures applied, the greater the observed shrinkage rate was. In this regard, Biekman et al. (1997) found that temperature increase during blanching of mushrooms is directly related with sample shrinking. It is known that the higher temperatures increase the protein denaturation that decrease the water holding capacity and increase the shrinkage. In this sense, the longer time of treatment increases the temperature effects.

With regard to the application of ultrasound, in the range of temperatures from 50 to 80 ºC, the shrinkage rate of UB experiments was higher than CB ones. This fact can be attributed to several effects produced by ultrasound. The successive compressions and expansions of mushrooms induced by high intensity acoustic waves, mechanism known as “sponge effect” (Gallego-Juárez et al. 2007), could accelerate the movement of water outside the solid and enhance the degassing of the immersed mushroom. Simal et al. (1998) suggested that the degassing effect observed under sonication may be similar to that observed under vacuum treatment. According to Biekman et al. (1997), during the shrinkage process, up to 50 % of the fluid within the tissue is lost in an internal movement of water towards the surface of the mushroom that also contribute to the increase of heat transport. The asymmetric collapse of cavitation bubbles close to mushroom surface can generate microjets in the direction of the surface that enhance heat and mass transfer. In this regard, Sastry et al. (1989) found that the natural convective heat transfer coefficient can be approximately doubled when ultrasound is applied. Moreover, Jambrak et al. (2007a) showed that ultrasound disturbs the cell walls of mushrooms and thereby facilitates the removal of the cell contents. These authors concluded that disrupts in biological membranes could be caused by a combination of the cavitation phenomena and the associated shear disruption, localized heating, and the free radical formation.

Shrinkage difference between CB and UB was higher at the lowest temperatures tested, decreasing with the increase of temperature. For instance, at 60 °C and after 10 min of blanching, the shrinkage obtained in UB was 1.4 times higher than the one obtained in CB, and at 80 °C (10 min) was only 1.1 times higher. At the highest temperature tested, 90 °C, non-significant differences ($p > 0.05$) were observed between both treatments, CB and UB. This fact could be attributed to at these conditions, the effects of ultrasound can be masked by those produced by the temperature self. Moreover, the increase of vapor pressure of water at higher temperatures which makes the collapse of cavitation bubbles less violent (Sala et al. 1995).

On the other hand, after the maximum processing time considered, 30 min (a conventional industrial process include the immersion in water at 80-90 °C for 8-9 min, Devece et al. 1999), the treatments carried out at 50 ºC only reached the 4.5 % and the 10.4 % of shrinkage for CB and UB, respectively, far from the target set shrinkage (18 %). For this reason, this treatment temperature was not considered in the following sections of this work.

Fig. 2 Variation of dimensionless size during the tested processes of blanching: Conventional (×) 50 °C, (▲) 60 ºC, (♦) 70 ºC, (●) 80 ºC, (■) 90 ºC and Ultrasonic assisted (×) 50 °C, (▲)60 ºC, (♦) 70 ºC, (●) 80 ºC, (■) 90 ºC. Values predicted by the first-order kinetics model are shown through continuous lines. Bars represent mean ± standard deviation

As can be seen in Fig. 2, the dependence of the dimensionless size variation with time was adequately fitted ($R^2 > 0.98$) by the first-order kinetics model (Eq. 1). From modelling it was possible to quantify the effects of both, temperature and ultrasound application on samples size variation (Table 1). Calculated values of K_m showed an increase with water bath temperature for both treatments, CB and UB, which means an augmentation of the contraction rate with temperature. For instance, K_m obtained at 90 °C was 8.4 times higher than at 60 °C for CB treatments. Regarding UB, as it is shown in Table 1, ultrasound application enhanced the shrinkage rate of mushrooms compared to CB, particularly at low temperatures. For instance, the identified shrinkage rate in UB experiments at 60 °C was 4.6 times higher than CB experiments carried out at the same temperature. These differences between rates decreased when the medium temperature increased from 60 to 90 ºC.

The influence of temperature on the increase of the shrinkage rate of both types of experiments, CB and UB, was well described by an Arrhenius type equation. As can be observed in Table 1 the R^2 of the fitting was above 0.9. In general, the activation energy values obtained for CB experiments was higher than

those found for UB experiments indicating that the shrinkage in these experiments was less sensitive to temperature when ultrasound was applied.

From modelling, it was also possible to estimate the processing time necessary to achieve a size reduction of 18 % at the different conditions tested (Fig. 3). As expected, the results obtained showed that the application of ultrasound significantly reduced the blanching time in the range of temperatures studied (except for 90 ºC), being this reduction greater as temperature considered was lower. Thus, the reduction in process time was 39.1, 46.0 and 30.7 % for blanching temperatures of 60, 70 and 80 ºC, respectively.

Fig. 3 Estimated process time to achieve a size reduction of 18 % for conventional and ultrasonic assisted blanching at different temperatures. Bars represent mean ± standard deviation

3.2. Implementation of the simulation model

The heat transfer model developed was numerically solved to simulate the evolution and distribution of mushrooms temperatures during the application of the different blanching processes tested. For the correct implementation of the model, experimental measurements of heat transfer coefficients were employed (Lespinard et al. 2009). The values of heat transfer coefficients for the CB and UB and the relative difference percentage between both processes are shown in Table 2. The values obtained for CB increased with the bath temperature and resulted similar to those found by Lespinard et al. 2009, for the same conditions. Results presented in Table 2 also indicate that in all cases, the ultrasound application significantly ($p<0.05$) increased the convective heat transfer coefficient from 205 % at 90 ºC to 599 % at 60 ºC.

The extent of ultrasonic enhancement was found to be dependent on the processing temperature. The influence of ultrasound application was higher at the lowest temperatures, decreasing with the increase of temperature. These results are in agreement with those presented by Lima and Sastry (1990) who found that the convective heat transfer coefficient was increased (260 %) from 562 to 2028 W/m^2°C by assisting blanching with ultrasound.

The simulation model was validated successfully since $E_{r\ ave}$ and R^2 between predicted and experimental temperatures of the thermal center of the mushroom were lower than 5 % and greater than 0.98, respectively, for all the tests carried out.

3.3. Texture

In all cases studied, a decrease of the treated sample hardness was observed compared to that of the unprocessed samples (Fig. 4). According to Zivanovic and Buescher (2004) these results can be attributed to losses of cell wall integrity due to processing temperatures. These authors reported that loss of mushroom toughness after blanching agreed with the solubilization of cell wall polymers. In the present work, texture losses (i.e., hardness reduction) in the range of 74.6-77.7 % were observed after the conventional blanching processes tested (to achieve 18 % shrinkage), showing no significant differences ($p>0.05$) among the different temperatures studied (Fig. 4). This similar hardness decrease can be explained by the fact that equivalent heat treatments were applied: low temperatures involved long processing times (33.6 min at 60 °C) and high temperatures reduced the heat treatment time (3.9 min at 90 °C).

On the other hand, the hardness of the mushrooms was larger when blanching at temperatures of 60 and 70 °C was assisted by ultrasound. Then, at 60 ºC the reduction in hardness loss in UB compared to CB was of 40.8 % and the processing time reduction of 39.8 %. In the same way, the UB at 70 °C reached a reduction of 25.2 % and 46.5 % in hardness loss and processing time, respectively. However, UB carried out at 80 and 90 ºC showed similar reduction hardness values than CB. This fact could be explained because at these blanching temperatures the reductions in the processing times were lower. Other authors (McArdle et al. 1974; Jasinski et al. 1984; Konanayakam and Sastry 1988) reported that high temperatures during blanching probably caused protein denaturation, membrane disruption, and loss of weight and volume of mushroom tissue. Moreover, it is possible that blanching at temperatures close to the boiling point of water disrupted hydrogen and other noncovalent bonds between cell wall polymers (Finley, 1985), loosened the strength of the wall network, and resulted in loss of toughness. On the other hand, blanching at low temperature (55-75 °C) activates pectin-methyl-esterase (PME) and improves textural properties as a result of PME action on the cell wall. PME acts on the cell wall pectic substances causing demethoxylation and produces free carboxyl groups. The formation of free carboxyl groups increases the possibilities and the strength of calcium and magnesium links between pectin polymers, hence increasing firmness (Sanjuán et al. 2005). Consequently, lower processing temperatures are desirable to minimize changes in texture during blanching of mushrooms. However, as reported in the present work, if these low temperatures are maintained for long process times, equivalent decrease in mushroom hardness are obtained than those found for high temperatures. Therefore, a reduction in the

process time, by the application of high power ultrasound at low blanching temperatures, can result into a reduction in mushroom hardness losses.

Fig. 4 Relative percentage of hardness loss for (■) conventional and (■) ultrasonic assisted blanching at different temperatures. Proposed combined treatments: (○) CT-15 and (Δ) CT-30. Bars represent mean ± standard deviation.

3.4. Colour

The colour parameter *L** (lightness) decreased during blanching showing the darkening of samples (Fig. 5). This darkening could be attributed to the fact that, at temperatures above 45ºC, a damage of cellular membrane of mushrooms occurs favoring the contact between PPO and its substrate and producing the sample browning (Biekman et al. 1997). Furthermore, enzymes as PPO may be active from the beginning of blanching treatment until the temperature in the tissue increased above the inactivation temperature of the enzyme. Thus, the lightness (*L**) reduction linearly decreased as the water bath temperature increased. This behavior was in agreement with that reported by Gouzi et al. (2012) who concluded that, in the context of browning inhibition in *Agaricus bisporus*, high temperature and short time should be preferred to long heating time at lower temperatures, to achieve efficient deactivation of PPO.

On the other hand, the influence of the ultrasound application on the lightness retention was significant ($p<0.05$) at the lowest temperatures tested, 60 and 70 ºC reducing the lightness decrease by 13.8 and 16.8 %, respectively, compared to CB. This fact could be attributed to the lower processing time in UB (39.7 and 46.5 % lower for 60 and 70 °C, respectively). However, the highest lightness retention was achieved when the blanching was carried out at 90 °C, being in this case the *L** relative decrease similar between conventionally and ultrasonically assisted blanching.

Fig. 5 Relative percent decrease of lightness after (■) conventional and (■) ultrasonic assisted blanching at different temperatures. Proposed combined treatments: (○) CT-15 and (Δ) CT-30. Bars represent mean ± standard deviation.

3.5. Cooking value

The C_{ave} obtained for CB experiments at 60, 70 and 80 ºC was quite similar varying around a mean value of 0.54 min (Fig. 6). On the contrary, the C_{ave} obtained for UB experiments, for the same temperatures (60, 70 and 80 ºC), increased as the bath temperature rose. Practically, no difference was found between the C_{ave} values identified for the 80 and 90 ºC ultrasonically assisted treatments. The textural changes in

the blanched mushrooms shown in section 3.3 should be related to the $C_{ave.}$ In this regard, a significant linear relationship (HL(%)=223.11C_{ave}-16.54) (R^2=0.93) between hardness losses (HL) and cooking values (C_{ave}) were found for UB in the range of C_{ave} from 0.27 to 0.39 min. However, no significant relationship (p>0.05) was found between the hardness losses and the C_{ave} for CB in the range of C_{ave} from 0.42 to 0.60 min. This fact could indicate that there is a threshold value of C_{ave} (close to 0.4) above which no difference of hardness losses is found, being the average hardness loss of 75 % when the cooking value exceeds that value. Therefore, the results obtained indicate that cooking values of UB samples at temperatures of 60, 70 and 80 ºC are lower than those of CB, which explains the lower hardness changes found in UB mushrooms at those temperatures. Moreover, it is possible to use the C_{ave} for the estimation of hardness changes in mushrooms during blanching when its value is lower than 0.4 min.

Fig. 6 Average cooking values (C_{ave}), simulated for (■) conventional and (■) ultrasonic assisted blanching at different temperatures. Proposed combined treatments: (○) CT-15 and (Δ) CT-30

3.6. Enzymatic activity of polyphenoloxidase

The final residual activity of mushroom PPO after CB and UB treatments (at the estimated process time to achieve a size contraction of 18 %), is presented in Table 3. As can be observed, mushroom PPO was inactivated in treatments carried out at 70, 80 and 90 ºC (reduction higher than 99 %) but not completely inactivated at 60 ºC. Therefore, the PPO activity reduction appears to be the limiting factor controlling the needed processing time only at blanching temperatures below 70 °C. On the other hand, for blanching temperatures from 70 °C to 90 °C the time needed to achieve a size contraction of 18 % was enough as to inactivate the enzymatic activity of mushrooms.

The inactivation kinetics curves of mushroom PPO for conventional and ultrasonically assisted treatments are shown in Fig. 7. It can be seen that the inactivation rate of mushroom PPO increased dramatically with temperature. For instance, to achieve a residual mushroom PPO activity of 32.2 % a treatment time of 15 min was needed for a conventional process at 60 °C, whereas only 0.9 min was needed for the complete inactivation of the enzyme at 90 ºC.

On the other hand, the ultrasound application during blanching enhanced the inactivation, of PPO in mushrooms compared with conventional thermal treatment. This effect is appreciated even at high temperatures. For instance, a residual mushroom PPO activity of 35.0 and 0.16 % were achieved after 0.4 min at 90 °C for CB and UB, respectively.

Fig. 7. Variation of surface residual PPO activity estimated from modelling during different blanching conditions: Conventional (Δ) 60 ºC, (◊) 70ºC, (○) 80 ºC, (□) 90 ºC, and Ultrasonic assisted (▲)60 ºC, (♦) 70 ºC, (●) 80 ºC, (■) 90 ºC. A zoom of the dotted area is shown.

The final enzyme activity obtained (Table 3) did not correspond with the observed lightness changes (Fig. 5). However, the values obtained for IAR_{sur} (Fig. 8) showed the same effect of temperature and ultrasound than colour (Fig. 5). These results confirm that the colour of mushrooms after processing is strongly influenced by the PPO activity evolution. In this regard, Rodríguez-López et al. (1999) indicated that browning reactions in mushrooms are directly related to the inactivation rate of PPO and pointed to the importance of rapid PPO inactivation to reduce browning of mushroom. Moreover, non-lineal relationships (R^2>0.99) between lightness losses (LL) and integrated residual PPO activity (IAR_{sur}) were obtained for CB (LL(%) = 3.65·ln(IAR_{sur}) + 9.94) and UB (LL(%)=2.23·ln(IAR_{sur}) + 10.97). Therefore, the prediction of the integrated residual activity through the heat transfer model resulted to be a useful parameter to estimate changes in colour during blanching of mushrooms.

Fig. 8 Integrated residual PPO activity (IAR_{sur}) after (■) conventional and (■) ultrasonic assisted blanching at different temperatures. Proposed combined treatments: (○) CT-15 and (Δ) CT-30

3.7. Combined treatment

From the results obtained, it can be stated that the blanching treatment that provided the best retention of texture was the UB treatment at 60 ºC, while to maintain the colour of mushrooms it was the CB and UB at 90 ºC (Figs. 4 and 5). These opposed values of temperature makes it difficult to optimize the process in order to maximize simultaneously both the texture and colour retention. For this reason, an interesting alternative could be the application of a two stage combined treatment (CT) which consists of a first stage of immersing the mushrooms in water at 90 °C for a short period of time and then a second stage applying an ultrasonically assisted blanching at 60 °C. The goal was to take advantage of the benefits of both treatments, obtaining a fast inactivation of the enzyme PPO that minimize the colour changes in the first stage (conventional blanching at 90 ºC) and reaching the desired shrinkage in the second stage by an ultrasonic assisted blanching at 60 ºC which would maximize the texture retention.

For that purpose, two processing experiments were carried out varying the treatment time for the first stage, 15 and 30 s, (CT-15 and CT-30 respectively) and maintain a fixed time of 19.9 min for the second stage (estimated time to reach 18 % shrinkage, Fig. 3). As can be seen in Fig. 4, the introduction of a first stage of conventional treatment at 90 ºC in the ultrasonically assisted blanching at 60 ºC, produced a

small increase of the hardness loss. The difference between measured hardness in CT-15 and CT-30 experiments was not significant ($p>0.05$). On the other hand, the combined treatment CT-15 reduced significantly the decrease of L^* compared to UB at 60 °C. This reduction was even greater, and equivalent to that obtained by treatment at 90 °C (Fig. 5), when CT-30 was applied. This could be explained by the fact that the residual activity of PPO obtained after the first stage of treatment was very different between both treatments (69.6 and 18.3 % for CT-15 and CT-30, respectively). Moreover, the PPO activity during the entire combined treatments, quantified by integrated residual activities (IAR_{sur}), reached values of 8.2 and 2.0 min (Fig. 8) which were in agreement with lightness losses (13.0 and 6.5 % for CT-15 and CT-30, respectively, Fig. 5). To sum up, the best conditions in terms of simultaneous texture and colour retention were obtained for the CT-30 treatment.

4. Conclusions

Kinetics of mushroom shrinkage was developed and coupled to a heat transfer model for describing conventional and ultrasonic assisted blanching. This model was employed to predict temperature, quality parameters and PPO activity evolution for both types of processes. Hardness and lightness changes were related with the cooking value and the integrated residual activity of PPO, respectively, determined by means of temperature predictions using the heat transfer model. The application of ultrasound reduced the blanching time and enhanced hardness and lightness retention compared to conventional heat treatment, particularly at low temperatures. A two-stage ultrasonically assisted blanching was found to simultaneously maximize hardness and lightness retention. Finally we can conclude that the present findings will help to optimise the design of mushrooms blanching conditions with heat and ultrasound.

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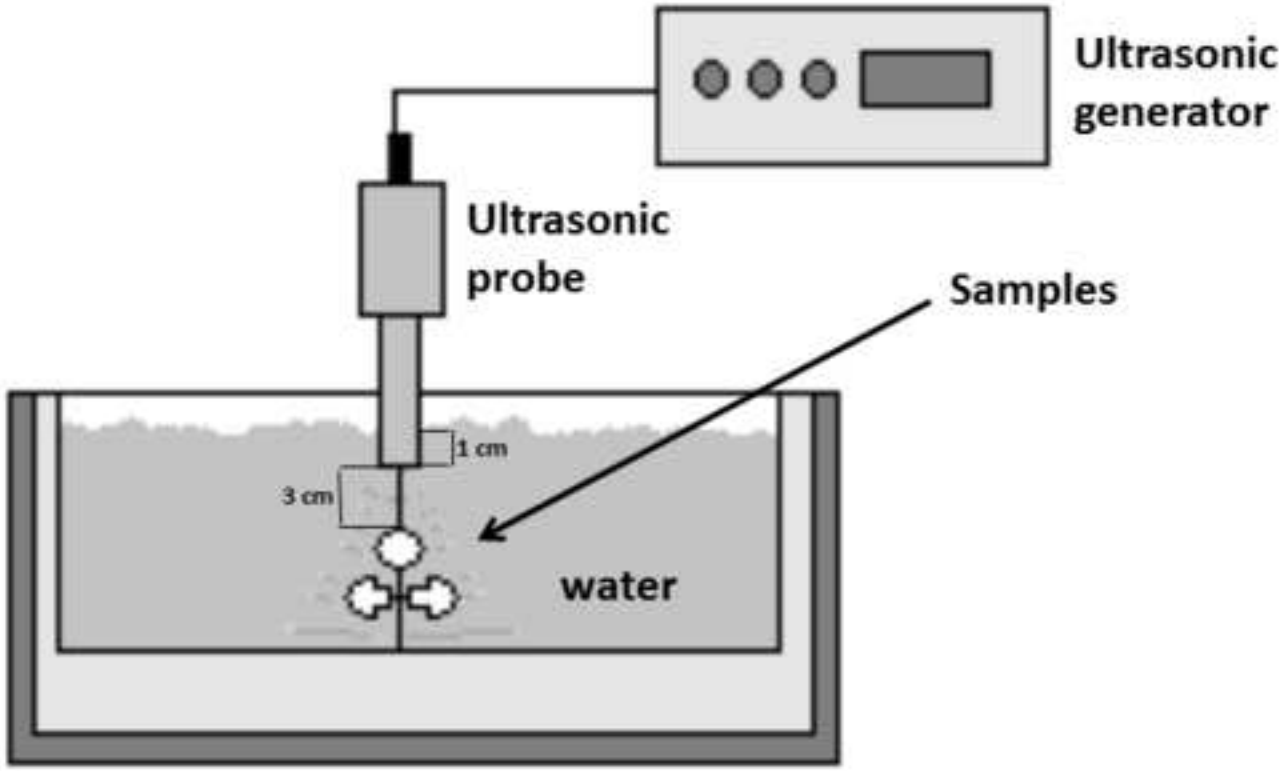

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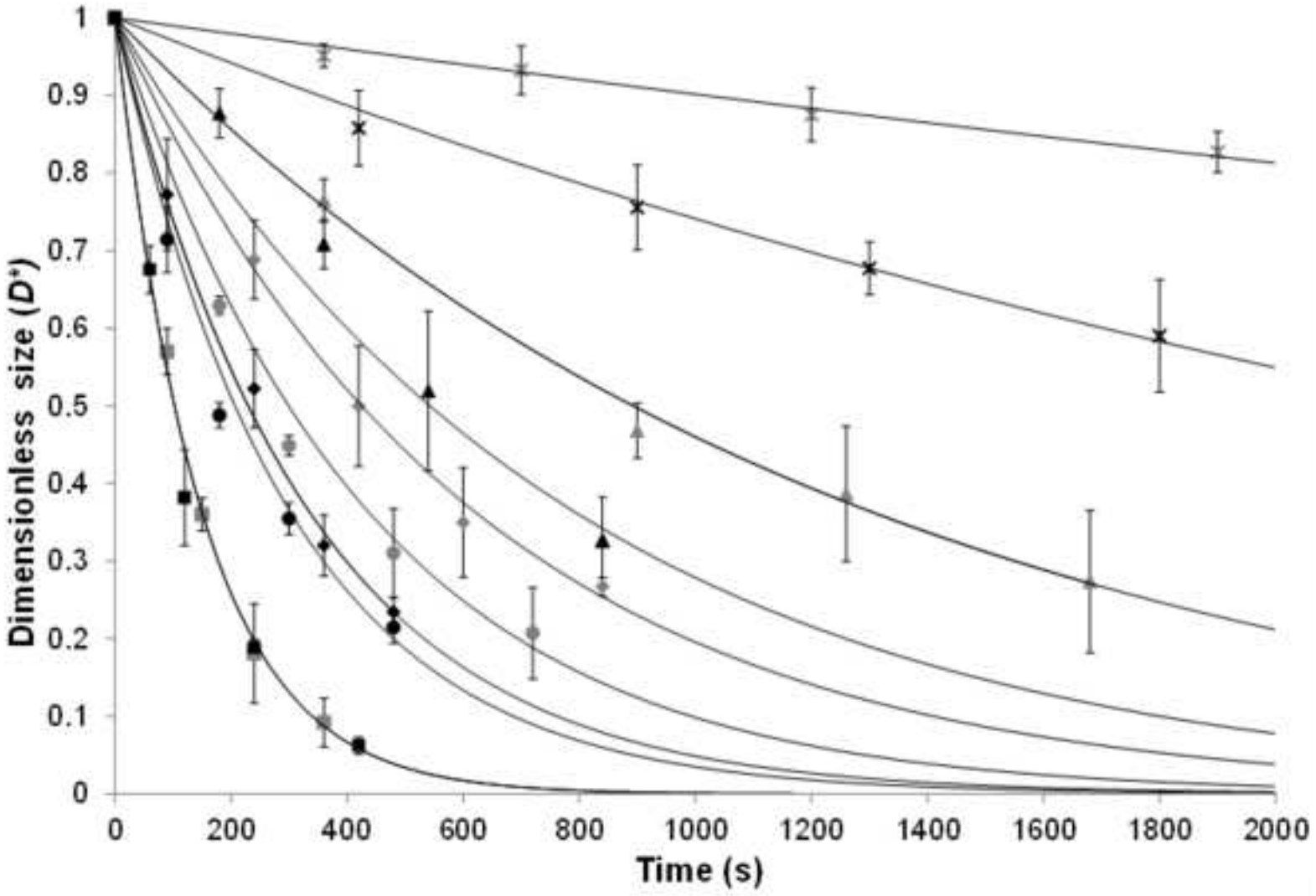

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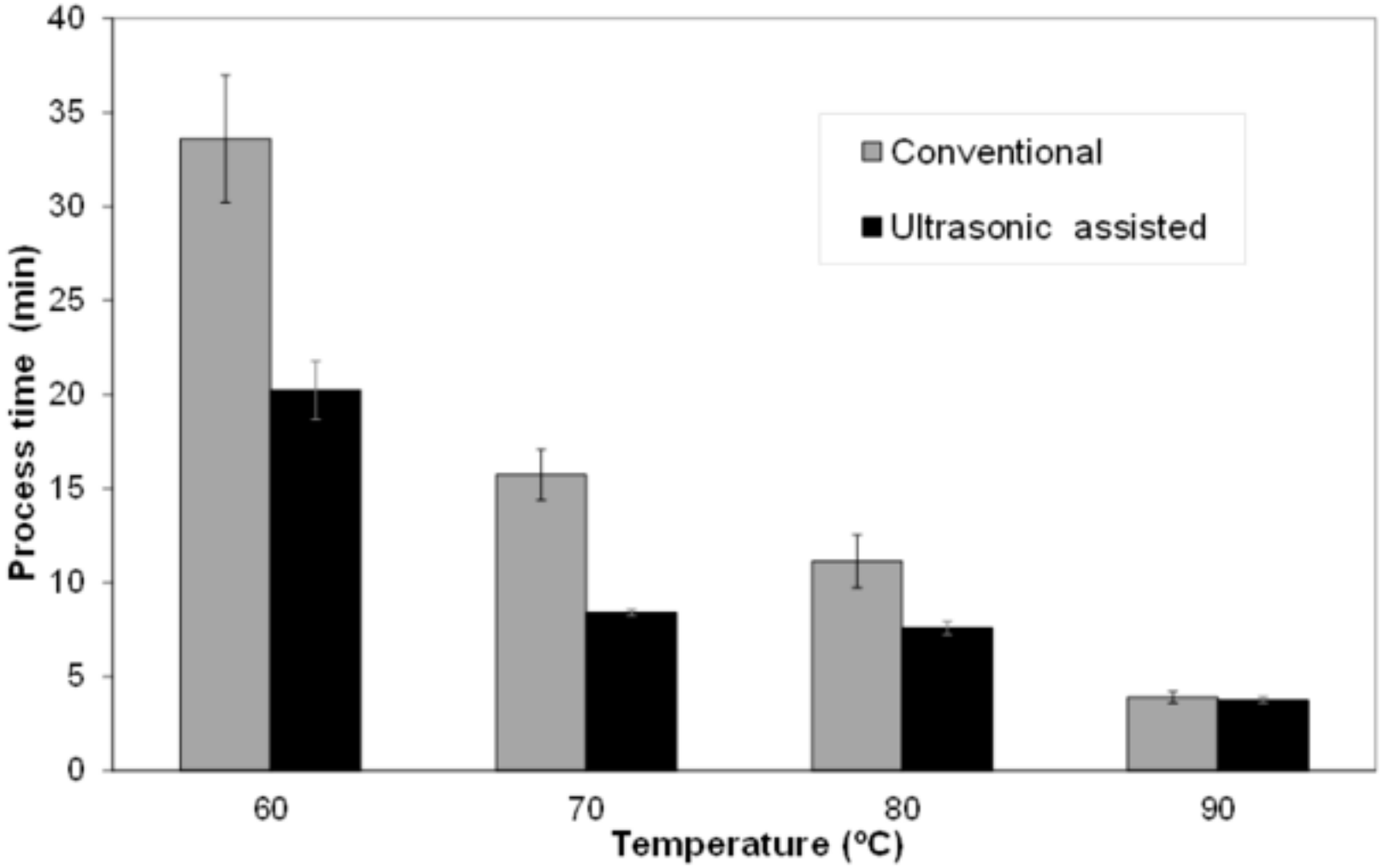

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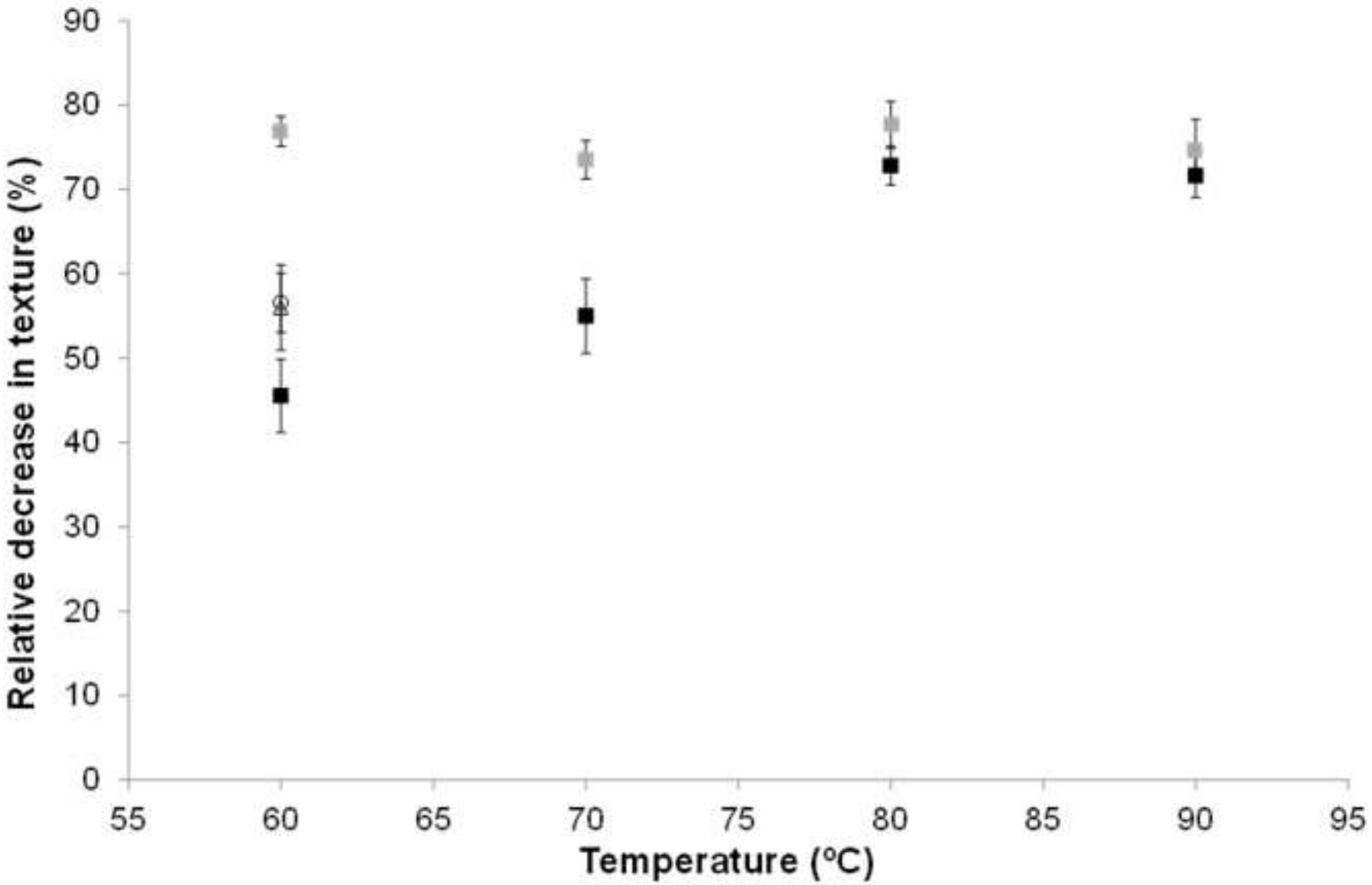

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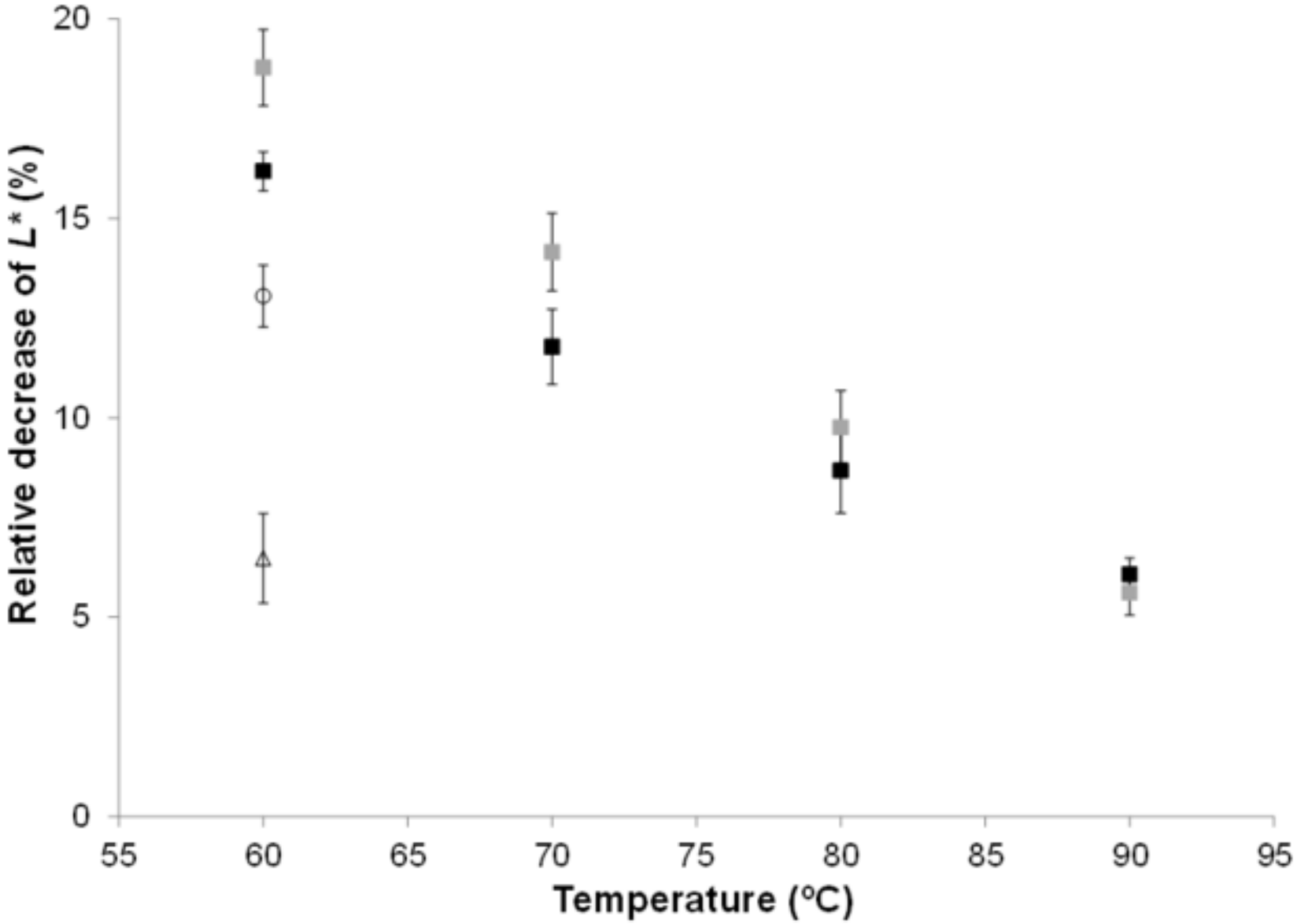

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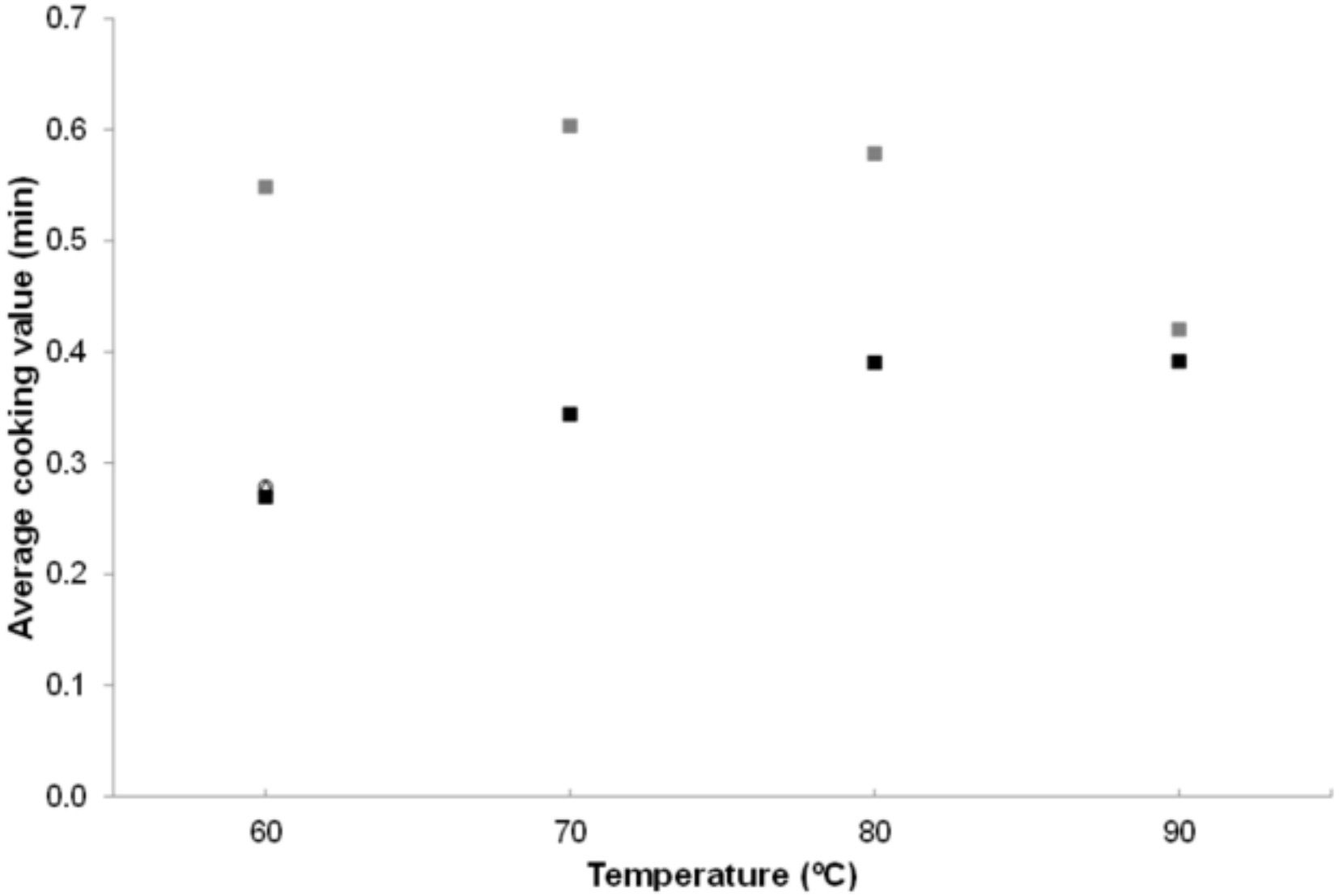

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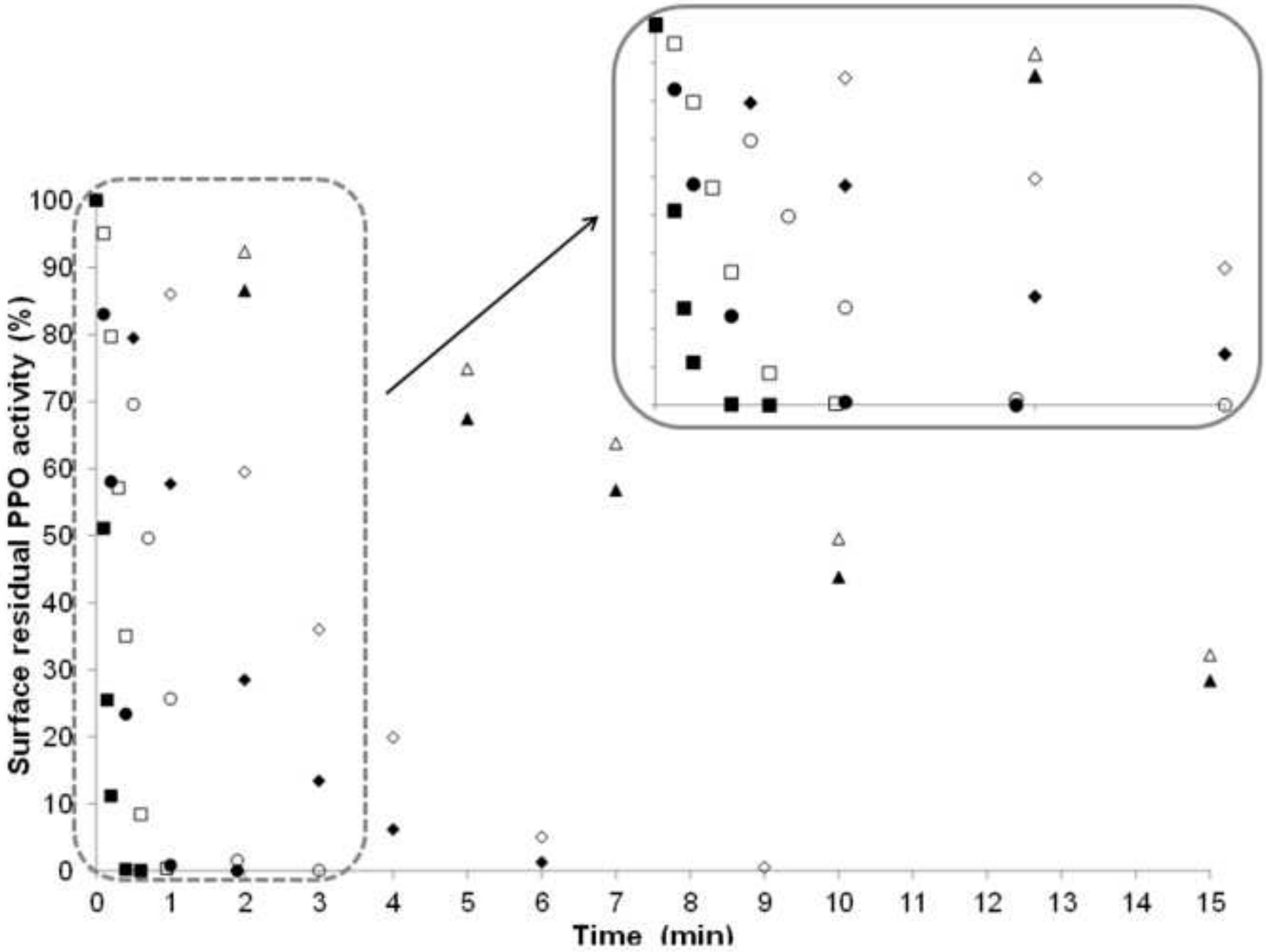

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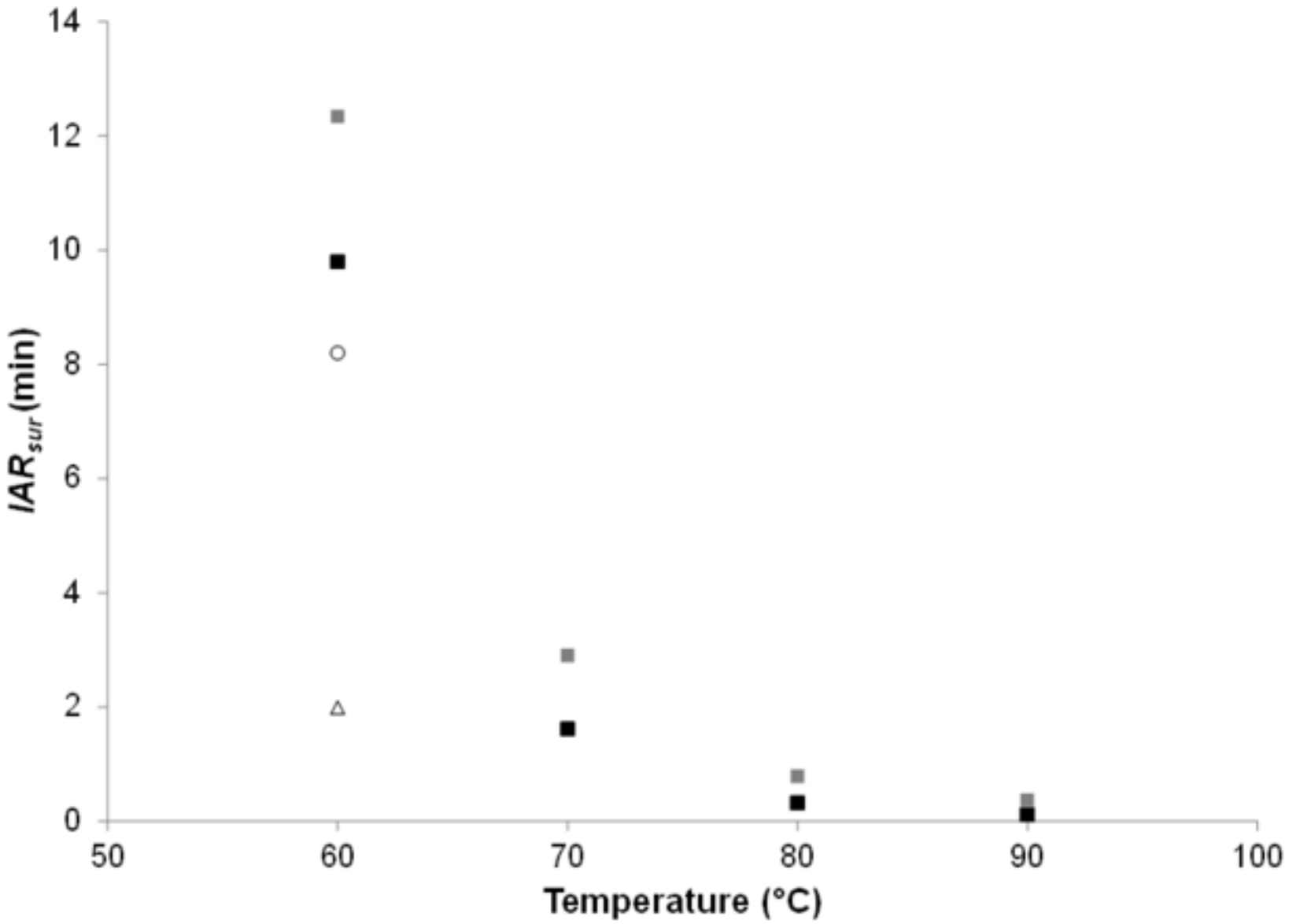

Table 1
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Table 1 Kinetic constants for the modelling of mushrooms shrinkage during blanching.

	Conventional blanching	***Ultrasonic assisted blanching***
Temperature (ºC)	K_m (s^{-1}) x 10^3	K_m (s^{-1}) x 10^3
50	$0.10\pm0.01^{a,A}$	$0.30\pm0.03^{a,B}$
60	$0.78\pm0.17^{b,A}$	$1.28\pm0.22^{b,B}$
70	$1.62\pm0.13^{c,A}$	$3.02\pm0.06^{c,B}$
80	$2.31\pm0.29^{d,A}$	$3.35\pm0.16^{c,B}$
90	$6.53\pm0.51^{e,A}$	$6.77\pm0.31^{d,A}$
E_a (kJ mol^{-1})	90.79	70.71
K_0 (s^{-1})	$8.36\ 10^{10}$	$1.19\ 10^{8}$
R^2	0.935	0.922

[a-e] Mean values within the column followed by the same lowercase letter are not significantly different ($p<0.05$). [A-B] Mean values within the row followed by the same capital letter are not significantly different ($p<0.05$).

Table 2
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Table 2 Convective heat transfer coefficients between the mushroom and the heating medium.

Temperature (ºC)	***Conventional blanching*** h (W m^{-2} K^{-1})	***Ultrasonic assisted blanching*** h (W m^{-2} K^{-1})	***Relative difference*** *(%)*
60	579.49±20.43^{a,A}	4044.79±161.53^{a,B}	599
70	650.63±16.01^{b,A}	3743.39±175.61^{b,B}	475
80	862.97±42.60^{c,A}	3438.12±145.66^{c,B}	298
90	968.97±50.64^{d,A}	2960.62±96.09^{d,B}	205

$^{a-d}$ Mean values within the column followed by the same lowercase letter are not significantly different (p<0.05). $^{A-B}$ Mean values within the row followed by the same capital letter are not significantly different (p<0.05).

Table 3
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Table 3 Calculated surface enzymatic activity retention of PPO (AR_{sur}).

	Conventional blanching	***US assisted blanching***
Temperature (ºC)	AR_{sur} (%)	AR_{sur} (%)
60	6.9	18.5
70	<1.0	<1.0
80	<1.0	<1.0
90	<1.0	<1.0
	Combined treatment	
	AR_{sur} (%)	
CT-15	12.0	
CT-30	3.1	

Abstract

The main aim of this work was to assess the influence of the application of power ultrasound during blanching of mushrooms (60-90ºC) on the shrinkage, heat transfer, and quality parameters. Kinetics of mushroom shrinkage was modelled and coupled to a heat transfer model for conventional (CB) and ultrasonic assisted blanching (UB). Cooking value and the integrated residual enzymatic activity were obtained through predicted temperatures and related to the hardness and colour variations of mushrooms, respectively. The application of ultrasound led to an increase of shrinkage and heat transfer rates, being this increase more intense at low process temperatures. Consequently, processing time was decreased (30.7-46.0%) and a reduction in hardness (25.2-40.8%) and lightness (13.8-16.8%) losses were obtained. The best retention of hardness was obtained by the UB at 60 ºC, while to maintain the lightness it was the CB and UB at 90 ºC. For enhancing both quality parameters simultaneously, a combined treatment (CT), which consisted of a CB 0.5 min at 90°C and then an UB 19.9 min at 60°C, was designed. In this manner, compared to the conventional treatment at 60°C, reductions of 39.1, 27.2 and 65.5% for the process time, hardness and lightness losses were achieved, respectively. These results suggest that the CT could be considered as an interesting alternative to CB in order to reduce the processing time and improve the overall quality of blanched mushrooms.

Keywords: Mushroom; Blanching; Ultrasound; Shrinkage; Quality Parameters; Polyphenoloxidase