Survival of *Penicillium* spp. conidia during deep-frying and baking steps of frozen chicken nuggets processing

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**ABSTRACT**

This study aimed at determining whether *Penicillium* spp. strains could survive through the heat treatment applied during the processing of frozen chicken nuggets. Firstly, it was found that the conidia of *Penicillium* were not able to survive the heat shock in phosphate buffer at pH 7.2 in thermal death tubes (TDT) at 80 °C/30 min. Subsequently, each *Penicillium* strain was inoculated in frozen chicken nuggets, which were subjected to the following treatments: i) only deep frying (frying oil at 195–200 °C), ii) only baking (120–130 °C until the internal temperature reached 70 °C) and iii) deep frying followed by baking (frying oil temperature of 195–200 °C and baking temperature of 120–130 °C, until the internal temperature reached 70 °C). The results indicated that *Penicillium polonicum* NGT 23/12, *Penicillium commune* NGT 16/12, *Penicillium solitum* NGT 30/12 and *Penicillium crustosum* NGT 51/12 were able to survive after the combined treatment (deep frying followed by baking) when inoculated in chicken nuggets. *P. polonicum* NGT 23/12 was the most resistant strain to the combined treatment (deep frying and baking), as its population was reduced by 3 log cycles CFU/g, when the internal temperature reached 78 °C after 10 min and 30 s of baking. The present data show that if *Penicillium* spp. is present in high numbers in raw materials, such as breading flours, it will survive the thermal processing applied during chicken nuggets production.

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**1. Introduction**

Microbial contamination of foods is usually caused by failures in hygiene and/or implementation of quality programs (Fondo and Bartz, 2011). Microbial contamination of foods can lead to their spoilage by specific microorganisms according to the food composition, packaging and storage conditions. As such, microbial growth control is a major challenge in the manufacture of food products.

In general, bacteria are responsible for the spoilage of meat products at optimum pH, water activity and temperature conditions (Jay, 2005). Under these conditions, fungi are at a natural competitive disadvantage because of higher generation time and for being bad competitors (Pitt and Hocking, 2009). However, filamentous fungi are more versatile than bacteria in overcoming environmental barriers, such as pH, temperature, and water activity, which are often used by industry for controlling microbial growth. Therefore, although fungal spoilage is not very common in poultry products, it occurs more easily when bacteria and yeast are less competitive. This type of spoilage occurs mainly in post-processing stages, especially during the storage period (Jay, 2005), when the fungal growth can lead to changes in appearance and color as well as to the development of off-flavors and mycotoxins (Samson et al., 2002).

Several studies have reported the occurrence of filamentous fungi in meat products (Asefa et al., 2010; Castellari et al., 2010; Comi et al., 2004; Iacumin et al., 2009; López-Díaz et al., 2001; Papagianni et al., 2007; Sonjak et al., 2011). In the specific case of frozen chicken nuggets, estimates indicate that approximately 1.0–1.5% industrial loss occurs because of fungal spoilage (Wigmann et al., 2015). In a previous study, Wigmann et al. (2015) have found that *Penicillium glabrum, Penicillium polonicum, Penicillium commune, Penicillium solitum* and *Penicillium crustosum* were the main species associated with spoilage (visible mycelia) of...
frozen chicken nuggets. In addition, these fungi have been isolated from raw materials used in the manufacture of frozen chicken nuggets, such as pre dust and breader flours (Wigmann, 2015). For example, mean counts of 10^4 CFU/g of P. polonicum and a prevalence of 40% were found in pre dust flour (Wigmann, 2015). It is known that psychrophilic strains of P. glabrum, P. polonicum, P. commune, P. solitum and P. crustosum may be isolated from foods (Samson et al., 2002). The spoilage of chicken nuggets by psychrophilic Penicillium is noticeable during the storage period and is likely caused due to the exposure of frozen chicken nuggets to temperatures higher than −18 °C for short or long periods.

Although freezing is used to extend the shelf life of meats, it should be clear that microorganisms are only inhibited (not destroyed) (Zhou et al., 2010). Even though the recommended storage temperature of frozen products is −18 °C (Codex Alimentarius, 1976), fluctuating temperature or temperature abuse during commercialization and consumer steps may be experienced. In some cases, average temperature recorded in freezing equipment may be >10 °C above the recommended temperature (−18 °C), while the maximum temperature can reach up to 5 °C (Mürrmann et al., 2005). It is known that some Penicillium species can grow and spoil foods when temperature is >7 °C (Pitt and Hocking, 1987; Adams and Moss, 2000). In fact, spoilage of frozen chicken nuggets by Penicillium species, such as P. glabrum (29/12 NGT) and P. polonicum (33/12 NGT) has been already reported. These fungi were found to be able to grow and form visible colonies on the surface of frozen chicken nuggets stored between 0 and −5 °C, respectively (Saccomori et al., 2015). The presence of Penicillium species in these products may be because of the use of raw materials with a high fungal load (that will survive and grow in the product if temperature abuse or fluctuation takes place) or post—thermal processing recontamination.

During the manufacture of chicken nuggets, heat treatments such as deep-frying and baking are applied mainly aiming to improve the sensory characteristics (color and texture) of these products (Barbut, 2012). However, studies reporting the impact of heat treatment during chicken nuggets processing (deep-frying and baking) on the inactivation of psychrophilic fungi, such as Penicillium spp. strains, are scarce. As these fungi can contaminate raw materials (i.e., bread flour) used in the production of chicken nuggets, it is fundamental to assess their inactivation during thermal processing of these products.

Such data can be further used to guide actions in industries aiming to control psychrophilic fungi in chicken nuggets. Thus, this study aimed to evaluate the impact of deep-frying and baking applied during frozen chicken nuggets processing on the inactivation of P. polonicum (NGT 23/12 e NGT 33/12), P. glabrum (NGT 29/12 and NGT 35/12), P. commune (NGT 16/12), P. solitum (NGT 30/12) and P. crustosum (NGT 51/12).

2. Material and methods

2.1. Strains of filamentous fungi and preparation of conidia suspensions

P. glabrum (29/12 NGT and 35/12 NGT), P. polonicum (23/12 NGT and 33/12 NGT), P. commune (16/12 NGT), P. solitum (30/12 NGT) and P. crustosum (51/12 NGT) were used in the present study. All these fungi were responsible for the spoilage of frozen chicken nuggets that was characterized by mycelial growth on chicken nuggets surfaces exposed to temperature above −10 °C (Wigmann et al., 2015).

For the preparation of conidia suspensions, plates containing malt extract agar (MEA) [malt extract, 20 g (Neogen, Lansing, USA); glucose, 20 g (Labsynth, Diadema, Brazil)]; agar, 20 g (Kasvi, Curitiba, Brazil); casein peptone, 1 g (Kasvi, Curitiba, Brazil); distilled water, 1 L] were inoculated with each fungal strain, and incubated at 25 °C for 7 days. Conidia were collected by scraping the mycelium of each plate using sterile distilled water and 0.1% of Tween 80 (Labsynth, Diadema, Brazil) when slides observed under optical microscope were covered by more than 90% of conidia. Further, filtration was carried out with sterile gauze to retain the mycelium and hyphal fragments. Then, centrifugation was performed at 11,962.6 × g (Sorvall RC-5C, Hampton, USA) for three consecutive times at 5 °C for 15 min, interspersing washing with sterile distilled water (Delgado et al., 2012a, 2012b; Silva et al., 2010). The concentration of conidia in each fungi suspension was standardized at 10^6 conidia per mL using Neubauer chamber and counting in MEA (25 °C/5 days) (Delgado et al., 2012a, 2012b; Sant’Ana et al., 2009). Standardized suspensions were further stored at 2 °C. Spore suspensions were stored for up to 4 months, and periodically checked through microscopy and enumeration on MEA agar to ensure their concentration remained stable.

2.2. Survival of Penicillium spp. conidia under after shock in phosphate buffer pH 7.2

One hypothesis for fungi spoilage of chicken nuggets was the survival of their conidia to the heat treatment applied during deep-frying and baking. For this purpose, the first trial was to ascertain whether conidia of different strains could survive heat shock at pH 7.2 phosphate buffer. Herein, 1.8 mL of phosphate buffer [potassium phosphate, 34 g (Inlab, São Paulo, Brazil); distilled water, 500 mL]; final pH was adjusted to 7.2 with 1 N NaOH solution, Synth, Diadema, Brazil) and 0.2 mL of the conidia suspension of each Penicillium strain was mixed into thermal death tubes (TDT) (8 mm outer diameter, 6 mm inner diameter, and 1 mm thickness) (Delgado et al., 2012a; Sant’Ana et al., 2009). The TDTs tubes were sealed and placed in a water bath (TE 057, Tecnal, Piracicaba, Brazil) at 80 °C for 10 and 30 min, which included the come-up time. The time and temperature binomial were chosen according to the method for the enumeration of heat resistance fungi in foods (Beuchat and Pitt, 2001). After conducting the heat shock treatment, the TDTs were removed from the water bath and immediately cooled. Then, TDTs were opened, and the content was subjected to decimal dilutions in 0.1% peptone water and further spread plated onto MEA supplemented with chloramphenicol (50 mg/L; Inlab, São Paulo, Brazil), following incubation at 25 °C for 7 days. After the incubation period, the absence of fungal colonies on the plates indicated the inactivation of Penicillium conidia. The experiments were replicated independently in two different days with five repetitions each.

2.3. Effect of deep-frying and baking on survival of Penicillium spp. in chicken nuggets

2.3.1. Inoculation of chicken nuggets with Penicillium spp. conidia

The chicken nuggets made of minced chicken meat were provided by a Brazilian food industry. The basic steps used for processing the chicken nuggets included: i) frozen chicken meat was ground and further homogenized with ingredients such as salt and additives (antioxidant, etc.) (Ordóñez, 2005); ii) further this mixture was placed in molds to acquire a specific format (Sams, 2001). This step was performed at −2 to −4 °C to allow proper molding, and then iii) a layer of pre dust, batter and bread flour (coating systems) were applied (Barbut, 2002). Pre dust and bread flour are commonly composed of wheat flour, corn flour, spices, starches and gums (Dill et al., 2009). Then, iv) pre-frying took place at 195–200 °C for approximately 6 s, and the chicken nuggets were baked at 120–130 °C until 70 °C was reached at the cold spot of the
product (Dill et al., 2009). The final steps v) included the freezing at −18 °C, packaging and storage. In food companies, this process can be partially or fully automated.

In the current study, semi-ready frozen chicken nuggets were collected prior to deep-frying and baking process. Then, they were transported and maintained frozen (−18 °C) until used for experiments in the laboratory. Frozen chicken nuggets samples were spot-inoculated (of conidia suspensions approx. 20 droplets distributed on the surface of the product) to achieve a final concentration of 10^6 conidia/g.

2.3.2. Effect of deep-frying and baking on the inactivation and survival of Penicillium spp. in chicken nuggets

After inoculation, the frozen chicken nuggets were divided into the following groups: i) nuggets subjected only to the deep-frying process, ii) nuggets subjected only to the baking process and iii) nuggets subjected to the complete process (deep-frying and baking). In parallel, three control groups were prepared: i) non-inoculated chicken nuggets that were neither fried nor baked (in order to assess the initial load of fungi), ii) non-inoculated chicken nuggets subjected to the complete process (deep-frying and baking) (in order to assess potential survivors naturally present in the chicken nuggets) and iii) inoculated chicken nuggets that were neither fried nor baked (in order to assess the initial conidia load inoculated in the chicken nuggets).

The deep-frying and baking conditions tested here aimed to simulate the industrial process. It should be highlighted that frozen chicken nuggets are semi-ready products and, therefore, they must be heated before consumption. Heating conditions for preparation may vary according to the manufacturer and processing method (baking at oven, microwave oven, frying).

For deep-frying, frozen chicken nuggets (initial temperature 0 °C) were immersed in 500 mL of soybean oil (Soya Bunge, São Paulo, Brazil) for 6 s at 195–200 °C. A total of four nuggets per batch were fried: i) nuggets subjected only to the deep-frying process, ii) nuggets subjected to the complete process (deep-frying and baking and iii) non-inoculated nugget (control) and iv) nugget used for temperature record. After frying, the frozen chicken nuggets were allowed to drain in a stainless steel strainer for 1 min. Then, nuggets were baked or cooled for enumeration of fungi (as described in Section 2.3.3).

The samples subjected to baking process (initial temperature 0 °C) were baked at a temperature of 120–130 °C (Industrial furnace, 8–4000 W, Imequi, São Paulo, Brazil). The samples subjected to the combined treatment (deep-frying and baking) were baked for 6 s at 195–200 °C. Then, frozen chicken nuggets were further baked at a temperature of 120–130 °C until they reached 70 °C at the cold spot of the product, which took about 10 min. Similar time and temperature conditions were used in all treatments.

The temperature data were collected at the beginning and at the end of each trial with the aid of type T thermocouples (flexible copper-constantan type T thermocouple, RSA, BR) and temperature recorder (Hydra series II, Fluke, Everett, USA). The experiments were replicated independently in two different days with two repetitions each (i.e., two chicken nuggets for each condition studied done in two different days).

2.3.3. Enumeration of fungi in the chicken nuggets subjected to deep-frying and baking

Ten grams of chicken nuggets were cut into small pieces and further homogenized with 90 mL of 0.1% peptone water (Stomacher 400, Seward Lab System, USA) for 1 min. Subsequently, aliquots were plated on MEA supplemented with chloramphenicol (50 mg/L; Inlab, São Paulo, Brazil), and incubated at 25 °C for 7 days. After

the incubation period, the colonies were enumerated and the results expressed as colony forming units per gram of product (CFU/g). The number of decimal reductions (γ) achieved for each unit operation (deep-frying, baking, and deep-frying followed by baking) was determined, considering the initial (N0) and final fungal populations (Nf) inoculated in the chicken nuggets.

2.3.4. Inactivation of P. polonicum (strain 23/12 NGT) during thermal processing of chicken nuggets

The most heat resistant strain to the treatments reported in Section 2.3.2 was used in this step of the study. The frozen chicken nuggets were inoculated with P. polonicum (23/12 NGT) at a concentration of 10^5 conidia per g. Then, the frozen chicken nuggets were subjected to the complete thermal processing, i.e., consisting of deep-frying in soybean oil at 195–200 °C for 6 s, followed by baking in an oven at 120–130 °C. During baking, frozen chicken nugget samples were collected at different time intervals until the maximum time used by the industry to reach an internal temperature of 70 °C at cold spot of the nugget was reached (10–12 min). The enumeration of P. polonicum (23/12 NGT) processing frozen chicken nuggets samples throughout the thermal processing was performed as described in Section 2.3.3. These experiments were replicated independently in two different days with two repetitions each.

2.4. Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by the Scott–Knott test version 7.6 (Campana Grande, Brazil) (Silva and Azevedo, 2002) with a significance level of p ≤ 0.05 (Granato et al., 2014; Nunes et al., 2015).

3. Results and discussion

P. glabrum, P. polonicum, P. commune, P. solitum and P. crustosum were recently isolated from spoiled frozen chicken nuggets (Wigmann et al., 2015). Thus, concerns have emerged about whether these fungi could survive deep-frying and baking treatments applied during processing of frozen chicken nuggets. To date, no studies on the survival and inactivation of psychrophilic strains of Penicillium sp. in frozen chicken nuggets subjected to deep-frying and baking processes were found in the literature. Thus, this study was performed using strains isolated from spoiled frozen chicken nuggets (Wigmann et al., 2015) and simulated the industrial deep-frying and baking conditions applied in the manufacture process.

Firstly, Penicillium sp. strains were subjected to a heat shock at 80 °C/30 min, aiming to confirm the hypothesis that a potential survival of these strains in frozen chicken nuggets would be related to the matrix effect. In fact, all the strains studied (P. polonicum (NGT 23/12 and NGT 33/12), P. glabrum (NGT 29/12 and NGT 35/12), P. commune (NGT 16/12), P. solitum (NGT 30/12) and P. crustosum (NGT 51/12) were inactivated after heat shock at 80 °C/30 min in phosphate buffer pH 7.2 in TDT tubes (data not shown). These results can be explained by the fact that these fungi do not produce structures of resistance, such as ascospores, which are common in Byssochlamys spp., Eupenicillium spp., Talaromyces spp. and Neurosartorya spp. (Pitt and Hocking, 2009). Even among those fungi that produce conidia, such as Eurotium amstelodami, E. chevalieri, Aspergillus candidus and Wallemia sebi, the inactivation occurs after exposure to 70 °C/10 min (Pitt and Christian, 1970). Thus, in buffer solutions, which do not provide protection to conidia, such fungi are easily inactivated when exposed at 70 °C for a few minutes.

Considering the above-mentioned facts and aiming to verify whether fungi would be protected when inoculated in the meat matrix (in this case due to the low water activity of the bread flour:}
0.42–0.54), fungal conidia were inoculated in chicken nuggets (water activity of 0.98). Then, deep-frying and baking processes were simulated separately (only deep-frying and only baking) or combined (deep-frying followed by baking). Although the industrial process includes deep-frying followed by baking, the assessment of fungi survival was also carried out in separate processes, aiming to determine their lethal effects. This information was deemed relevant in order to determine which thermal process best contribute to fungal inactivation when the complete process (deep-frying followed by baking) is applied. Fig. 1 shows the internal temperature variation (°C) of frozen chicken nuggets during deep-frying and baking until the cold spot of the product reached 70 °C. A variability in terms of time to reach the target temperature (between 7 and 8 min) was observed and could be attributable to some specific factors, such as the number of chicken nuggets fried at the same time and the position of the chicken nuggets in the oven, among others (Fig. 1). Nonetheless, one should take into account that this variability is also observed in practice of thermal processing. The temperature of 70 °C is used as a reference value by the industry, and when reached, indicates that the food was properly cooked and, therefore, the risk of survival of pathogens, such as Salmonella, is reduced (Bucher et al., 2008). In this sense, Fig. 1 is presented to highlight that in all experiments the target temperature at the cold spot (70 °C) was reached in 10–12 min, meaning that the minimal thermal processing requirement to produce a safe product was delivered. This condition should also be sufficient to inactivate other microorganisms, such as filamentous fungi (Pitt and Christopher, 1970) and, therefore, the contamination of chicken nuggets is not due to internal contamination (meat batter) from an external contamination (bread flour).

The mean fungal count of the control samples (non-inoculated and non-thermally processed frozen chicken nuggets) used in this study was 2.6 log CFU/g. This result indicates high fungal loads (with predominance of Penicillium spp.) in raw materials, such as the flour used for breading of chicken nuggets. Nevertheless, when subjected to complete treatment (deep-frying followed by baking), a reduction on fungal counts (>2.6 cycles log CFU/g) was observed in the control samples (data not shown). Thus, it can be stated that the fungi enumerated in the samples subjected to thermal processes corresponded to the intentionally inoculated strains. Table 1 shows the number of decimal reductions (γ) caused by deep-frying, baking and complete treatment (deep-frying + baking) for different Penicillium species. It can be seen that the initial count (N0) ranged from 3.3 to 5.8 log CFU/g of product. Despite the variation, the initial counts inoculated into the chicken nuggets allowed to properly assess the impact of deep-frying and baking steps on the strains studied. The data indicate that deep-frying and baking, applied separately, are responsible for 1–2 and 0–0.9 decimal reductions, respectively (Table 1). The major number of decimal reductions in fungal populations observed in the deep-frying process, as compared to baking, can be explained by the fact that the fungal contamination of frozen chicken nuggets seems to be mainly superficial. This microbial load is likely originated from the flour used for breading (Saccomori et al., 2015). However, regardless of the treatment (deep-frying or baking), no significant differences (p > 0.05) were observed in the number of decimal reductions for the different strains studied. When the impact of the combined treatment (deep-frying followed by baking) on the different strains was tested, a significant number of decimal reductions was found (p < 0.05). The fact that the combined treatment (deep-frying followed by baking) led to a greater number of decimal reductions as compared to the sum of the treatments applied separately (baking and frying) could be explained by a more continuous and effective heat transfer in the former than in the later treatment. For example, in the combined treatment (deep-frying followed by baking), the frozen chicken nuggets (0 °C) are immersed for 6 s in soybean oil at 195–200 °C and immediately transferred to an oven at 120–130 °C until the temperature at the cold spot reaches 70 °C.

The number of decimal reductions of P. commune NGT 16/12, P. polonicum NGT 23/12, P. glabrum NGT 29/12, P. crustosum NGT 51/12 and P. soltum NGT 30/12 in the combined treatment (deep-frying + baking) was significant lower (p < 0.05) when compared to decimal reductions of P. polonicum NGT 33/12 and P. glabrum 35/12 strains (Table 1). The combined treatment (deep-frying followed by baking) caused greater number of decimal reductions in P. polonicum NGT 33/12 (approximately 5 decimal reductions), while the lowest number of decimal reductions was observed for P. polonicum NGT 23/12 (Table 1) (p < 0.05). Thus, P. polonicum NGT 23/12 was considered the most heat-resistant strain isolated from the spoiled frozen chicken nuggets. The higher resistance of this strain to deep-frying and baking processes may be associated with several genetic and evolution factors (Samson et al., 2002). Given this, the estimation of the variability in thermal resistance of different fungal strains potentially present in raw materials is of foremost importance not only for the proper design of thermal processes but also to ensure microbiologically shelf-stable products.

As P. polonicum (NGT 23/12) was the most heat resistant strain in the combined treatment (deep-frying followed by baking), this strain was selected for further experiments (Fig. 2). Although a drop of one log cycle was observed at the beginning of the baking step (Fig. 2), the populations of this fungus remained unchanged up to approximately 6 min and 30 s of this step. This period was characterized by the presence of a shoulder in the inactivation curve of P. polonicum (NGT 23/12), and it was observed an increase of the temperature at the cold spot from 0 °C to about 60 °C (Fig. 2). However, when the temperature of the nuggets raised from 60 to 78 °C after 6 min of treatment, a reduction of fungal counts of approximately 2 log cycles CFU/g was observed. The thermal treatment comprising deep-frying followed by baking led to a reduction of three log cycles in the population of P. polonicum (GTN 23/12). Therefore, although deep-frying has a great importance on reducing the fungal load in frozen chicken nuggets (as these contaminants will mainly be found at surface of these products), it is evident that the baking procedure ensures greater fungal inactivation. According to our hypothesis, the thermal processing applied
to frozen chicken nuggets will be able to cause a reduction com-
prehended between 2 and 5 log, depending on the fungal strain
present in the breading flours and external portion of the chicken
nuggets. In this case, the increased apparent heat-resistance of
filamentous fungi can be explained by the fact that under low water
activities, the heat transfer is impaired, resulting in increased D-
values (Podolak et al., 2010).

Even though chicken nuggets may be contaminated by several
microorganisms, only those able to grow at low temperatures
will be able to spoil this frozen product. At lower temperatures,
fungal lag time and growth rate will be extended, which will
likely lead to detection of spoilage by consumers. As alterations
of frozen foods, but most importantly, the use of raw materials
that are hygrophilic and have a high mycological quality (which means very low
fungal counts). If properly applied, these measures will reduce
the risk of frozen chicken nuggets spoilage by psychrophilic fungi
during their storage and commercialization, mainly if it is carried
out at temperature around -6 °C. Furthermore, increasing target
temperature for thermal processing at the cold spot of the
product from 70 °C to about 78 °C may be another alternative to
ensure the microbiological stability of these products during
shelf life.

### Table 1

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Strain</th>
<th>Initial inoculum (N_0) (log CFU/g)</th>
<th>Decimal reductions (D) (log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Frying</td>
</tr>
<tr>
<td>P. commune</td>
<td>NGT 16/12</td>
<td>5.85 ± 0.00</td>
<td>1.02 ± 0.05a</td>
</tr>
<tr>
<td>P. polonicum</td>
<td>NGT 23/12</td>
<td>5.62 ± 0.10</td>
<td>1.00 ± 0.15a</td>
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<td>P. glabrum</td>
<td>NGT 29/12</td>
<td>3.34 ± 0.06</td>
<td>1.25 ± 0.21a</td>
</tr>
<tr>
<td>P. solitum</td>
<td>NGT 30/12</td>
<td>4.78 ± 0.12</td>
<td>1.01 ± 0.03a</td>
</tr>
<tr>
<td>P. polonicum</td>
<td>NGT 33/12</td>
<td>5.23 ± 0.06</td>
<td>1.66 ± 0.49a</td>
</tr>
<tr>
<td>P. glabrum</td>
<td>NGT 35/12</td>
<td>4.85 ± 0.10</td>
<td>1.76 ± 0.24a</td>
</tr>
<tr>
<td>P. crustosum</td>
<td>NGT 51/12</td>
<td>5.64 ± 0.70</td>
<td>1.92 ± 0.32a</td>
</tr>
</tbody>
</table>

In the column of decimal reductions (for each treatment), means followed by the same letter do not differ significantly according to the Scott–Knott test at 5% significance level.

Fig. 2. Time (minutes), mean fungal counts (log CFU per g), and temperature (°C) at the cold spot of frozen chicken nuggets inoculated with Penicillium polonicum NGT 23/12 for both heat treatments (deep-frying and baking).

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